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Systematic or Meta-analysis Studies

# Liquid biopsy after resection of pancreatic adenocarcinoma and its relation to oncological outcomes. Systematic review and meta-analysis☆

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ABSTRACT

*Background:* It has been hypothesised that manipulation during surgery releases tumoral components into circulation. We investigate the effect of surgery on plasma-borne DNA biomarkers and the oncological outcomes in resectable pancreatic ductal adenocarcinoma (PDAC). We also compare non-touch isolation techniques (NTIT) with standard techniques.

*Materials and methods:* We performed a systematic review and a meta-analysis of studies analysing liquid biopsy as circulating tumour DNA (ctDNA), circulating tumour cells (CTCs), and messenger RNA (mRNA) in resectable PDAC patients who underwent surgery and its association with overall survival (OS) and disease-free survival (DFS). Research in EMBASE, Web of Science and PubMed was performed. The ctDNA shift negative-to-positive (ctDNA −/+) or ctDNA shift positive-to-negative (ctDNA +/−) before and after surgery was evaluated.

*Results:* Twelve studies comprising 413 patients were included. Shorter OS and DFS were identified in patients with positive ctDNA status before (HR = 2.28, $p = 0.005$ and HR = 2.16, $p = 0.006$) or after surgery (HR = 3.88, $p < 0.0001$ and HR = 3.81, $p = 0.03$), respectively. Surgical resection increased the rate of ctDNA +/−. There were no differences in OS or DFS in the ctDNA +/− group compared with ctDNA +/+ or ctDNA −/+. However, there was a trend to shorter OS in the ctDNA −/+ group (HR = 5.00, $p = 0.09$). No differences between NTIT and standard techniques on liquid biopsy status were found.

*Conclusion:* Positive ctDNA in the perioperative period is associated with a worse prognosis. Surgical resection has a role in the negativisation of liquid biopsy status. More studies are needed to assess the potential of minimally invasive techniques on ctDNA dynamics.

## Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the seventh cause of cancer-related death worldwide and third place in Europe [1,2]. The actual 5-year survival rate is 12 % [3]. This low survival rate is mainly due to late diagnosis, aggressive biology and resistance to current chemotherapy regimens [4–7]. Only 20 % are resectable at diagnosis; despite surgical resection, approximately 80 % recurred. Furthermore, several factors may contribute to the recurrence of PDAC after resection, such as R1 status (<1mm), tumour size, lymph node involvement, and an incomplete chemotherapy regimen, among others [8,9]. Additionally, inaccurate staging and hidden micrometastasis during the preoperative period are reasons for early recurrence. Metastatic capacity at the early stages of the disease, even in precancerous lesions (PanIN), has also been described [10].

Circulating tumour cells (CTCs) and circulating tumour (ct) nucleic acids, such as ctDNA and ctRNA, have been recognised in PDAC patients' blood, being released from the primary tumour and or metastatic site. The circulating capacity of PDAC cells is linked to the transition from epithelial to mesenchymal (EMT) phenotype, acquiring survival

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and self-renewal properties that confer cells the ability to metastasise. Pancreatic cancer cells have also shown anoikis resistance, a defence cell mechanism that induces apoptosis when the cell is detached and in the absence of an extracellular matrix. Anoikis resistance confers higher migratory and invasive properties to pancreatic cancer cells [11,12]. The detection rate of CTCs in PDAC is relatively low; however, the advances achieved in ctDNA have improved the detection rate of circulating tumoral components and have therefore become the most used liquid biopsy technique[13]. The incidence of ctDNA detection in pancreatic cancer is 26 %–62 %, being higher in advanced stages. For resectable disease, rates up to 50 % have been reported [14–18]. Detectable ctDNA has been associated with shorter disease-free survival (DFS) and overall survival (OS) after pancreatic cancer resection [14,15,19].

In that line, it has been reported that tumour manipulation during surgery releases cancerous cells into blood circulation, showing an increase in ctDNA and CTCs detection after surgery which could hypothetically increase the risk of recurrence [15,20]. This phenomenon could equally increase the risk of metastasis. Based on this observation, non-touch isolation techniques (NTIT) or vessel-first approaches have emerged as alternative techniques that could decrease the release of cancerous cells into the bloodstream. Some authors reported decreases in CTCs levels using NTIT compared to the standard technique in colorectal cancer [21–27]. However, strong evidence is still needed to support the oncological superiority of NTIT against standard techniques, especially in pancreatic cancer surgery.

Previous systematic reviews analysing ctDNA status and its impact on OS and DFS in PDAC included unresectable and metastatic stages [28–30]. Additionally, there is little evidence addressing the real effect of surgical resection on releasing tumoral components and its effect on DFS and OS in PDAC patients.

We systematically reviewed circulating tumoral cell components (CTCs, ctDNA, ctRNA) in patients who underwent pancreatic cancer resection. We aimed to investigate if surgical resection impacts tumoral component release by favouring shift dynamics in liquid biopsy status and if the type of surgical technique plays a role. Besides, we intend to demonstrate the association of liquid biopsy status with survival and recurrence in resectable pancreatic cancer patients.

## Methods

This systematic review has been done according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [31].

For this study, different types of liquid biopsies were analysed, such as ctDNA, CTCs and ctRNA.

### *Study selection and eligibility criteria*

A search in EMBASE, Web of Science and PubMed was performed between November and December 2021. We used the following Medical Subject Heading (MeSH) keywords:

- *"Pancreatic cancer" AND "tumour manipulation" OR "Non-Touch" OR "circulating DNA" OR "circulating tumour DNA" OR "ctDNA" OR "ctRNA" OR "Circulating tumour cells" OR "CTC".*
- *"Pancreatic adenocarcinoma" AND "tumour manipulation" OR "Non-Touch" OR "circulating DNA" OR "circulating tumour DNA" OR "ctDNA" OR "ctRNA" OR "Circulating tumour cells" OR "CTC".*
- *"Pancreatic tumour" AND "tumour manipulation" OR "Non-Touch" OR "circulating DNA" OR "circulating tumour DNA" OR "ctDNA" OR "ctRNA" OR "Circulating tumour cells" OR "CTC".*
- *"Pancreatic cancer surgery" AND "tumour manipulation" OR "Non-Touch" OR "circulating DNA" OR "circulating tumour DNA" OR "ctDNA" OR "ctRNA" OR "Circulating tumour cells" OR "CTC".*

We defined the main question of the study according to the PICO framework (population, intervention/exposure, comparator and outcome) [32]. Studies were selected if they met the following eligibility criteria:

- (P) Study population included adults $\geq$ 18 years with potentially resectable pancreatic cancer undergoing surgery.
- (I) All patients had preoperative and postoperative liquid biopsy samples.
- (C) Two analyses were performed: 1) Time-point liquid biopsy status (preoperative and postoperative) as positive versus negative and 2) the shift occurrence after surgery in every individual as a shift to positive versus shift to negative.
  - ctDNA −/+ w*as* defined as a positivisation of liquid biopsy in the postoperative period in a patient with preoperatively negative liquid biopsy.
  - ctDNA +/− was defined as a negativisation of liquid biopsy in the postoperative period in a patient with preoperatively positive liquid biopsy.
- (O) Compared outcomes were OS, DFS, mortality rate and recurrence rate.

A subanalysis also based in the PICO framework was performed in (P) potentially resectable pancreatic cancer patients (I) treated with a non-touch surgery (C) compared to the standard technique evaluating (O) ctDNA −/+ and ctDNA +/−. All studies selected were available in English.

### *Selection of reviews, extraction, and management of information*

All studies in this search were included in an Excel database, and repeated articles were excluded. Two reviewers (L.V.P and E.P.R) separately assessed publication titles, abstracts, and full-text selection based on eligibility criteria. If disagreement was found in a study, it was solved in consensus between reviewers J.B.V. and A.V.P. Some references were searched and selected manually from the reference list of previously included studies. First author's name, year and country of publication, the number of included subjects, TNM, type of surgery, margin status, neoadjuvant chemotherapy, origin and time of sample, liquid biopsy detection method, marker, tissue sample, follow-up time and outcomes were extracted by each reviewer independently. Outcomes were collected as Hazard Ratio (HR) with a corresponding 95 % confidence interval or as Risk Ratio (RR) or Odds Ratio (OR).

In some studies that included metastatic stages, locally advanced non-resectable and resectable pancreatic cancer patients, only the potentially resectable pancreatic cancer patients that underwent surgery were considered for this meta-analysis. If this stratification was not possible, the article was excluded from the meta-analysis.

Information was obtained from articles' tables, graphics and texts. Supplementary tables and graphics were also used. If data was not available, the IP of each study was contacted.

### *Risk of bias*

The quality and risk of bias of all studies included in the meta-analysis were assessed independently by L.V.P and E.P.R. using the Quality In Prognosis Studies (QUIPS) tool [33], and inconsistencies were solved by a third reviewer, J.B.V and A.V.P. For each quantitative analysis, publication bias was assessed using a Funnel plot.

### *Data analysis*

This meta-analysis data was analysed by one reviewer L.V.P and supervised by E.P.R. The compared groups have been established according to positive and negative liquid biopsy status in each study. We set a subject as positive or negative according to the detectable

concentration threshold of each molecular test defined in each study methodology.

Liquid biopsies were performed at the following time points: pre-operative and postoperative. Scenarios were evaluated separately, comparing mortality, OS, recurrence and DFS in positive and negative liquid biopsy groups. Additionally, we analysed if negativisation (ctDNA +/−) or positivisation (ctDNA −/+) occurred after surgery and assessed its association with survival. The secondary sub-analysis compared liquid biopsy status preoperative and postoperative between NTIT and standard technique in pancreatic cancer surgery.

OS, DFS, mortality, and recurrence rates were analysed and exported directly from tables as HR with 95 % CI or RR/OR with 95 % CI. If RR and OR were not available, it was determined from individual subject information from supplementary data. If HR was unavailable, we extracted individual subject information from the supplementary data and calculated it by using a univariate Cox regression analysis on IBM® SPSS ® 27.

All quantitative analyses were performed on RevMan 5.4.1 ®. We used the Der-Simonian and Laird random effects model to estimate the pooled effect size in our meta-analysis [34]. The chi-squared heterogeneity test was used to assess the statistical heterogeneity between the trials, and the $I^2$ statistic was used to assess the proportion of variability attributable to between-study heterogeneity. For each analysis, a Forest plot and Funnel Plot were obtained.

## Results

### *Study selection*

Following a systematic search, 4157 studies were retrieved, of which 818 duplicates were removed. The titles and abstracts of the remaining 3339 were assessed, excluding 3261 because they included animal subjects, other biliopancreatic tumours, non-original articles, absence of surgical treatment, or missing blood samples from before or after surgery (Fig. 1).

A total of 78 articles were entirely read, and 67 were excluded due to the absence of preoperative or postoperative samples or due to the inclusion of metastatic patients. One additional study which was extracted from the references list before the quantitative data analysis was included. Finally, 12 articles were included in the meta-analysis, including 413 patients with potentially resectable PDAC. The study inclusion period was from 1998 to 2021.

Patients were included in the quantitative analysis according to the intention-to-treat principle. Of note, 17 (4,1 %) patients were finally classified as stage IV due to the positivity of distant lymph nodes after the analysis of resected specimens, false negative CT or unexpected

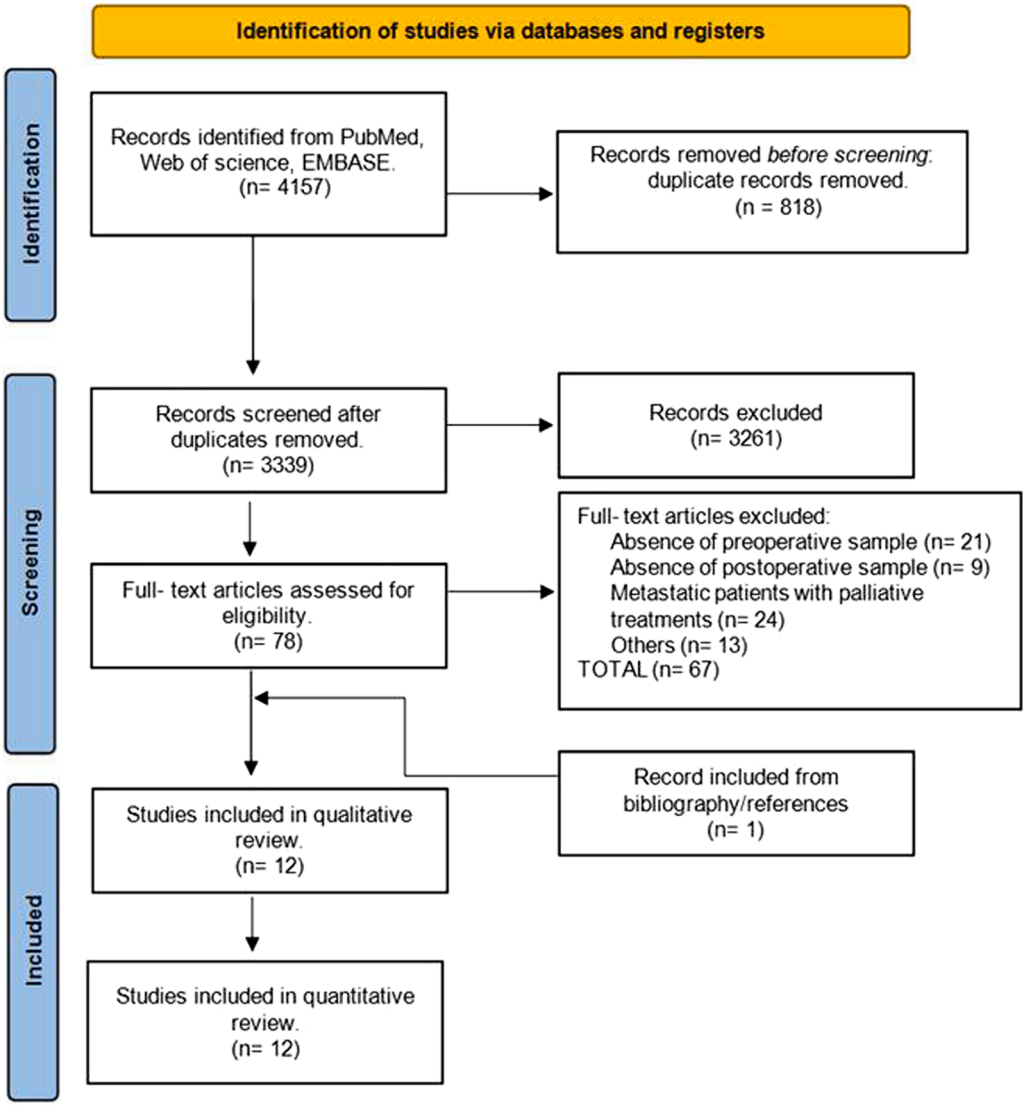


**Fig. 1.** Flow diagram of the systematic review Cochrane [35].

findings during surgery. A consensual decision was made to include these stage IV patients in the meta-analysis to resemble a real clinical scenario and to perform an intention-to-treat analysis.

*Study characteristics*

Table 1 summarises the characteristics of the studies included. Most studies were prospective, except for one retrospective [39] and one randomised controlled trial [26]. The studies were performed in Japan [27,38,40–44], China [37,45], England [26], the USA [36] and Australia [39]. The AJCC TNM classification system was the most used, followed by the Japanese Pancreas Society Classification; two articles did not mention the classification used [37,39]. Two publications compared different techniques, NTIT versus standard technique [26,27].

In all studies, blood samples were taken preoperatively, postoperatively, and in some cases, during surgery. Time of liquid biopsy is detailed in Table 1. Preoperative samples are mostly taken during the 24 h before surgery or prior tumour manipulation. Postoperative samples are mainly collected between specimen removal and the 10th postoperative day. The majority of the studies used Ethylenediaminetetraacetic (EDTA) preservation tubes (Supplementary material, Table S1). The most widely used liquid biopsy was ctDNA based on *KRAS* mutation detection in plasma. Some authors matched tumour mutation in peripheral blood and tissue [37,39,41–45]. CEA mRNA and CTCs in whole blood were used as liquid biopsies by *Hirota et al. and Gall et al.* and were the two studies included in the sub-analysis comparing NTIT techniques. Overall survival was the commonest endpoint among the studies.

*Risk of bias*

The results of the risk of bias assessment are shown in Fig. 2 (Supplementary material, Fig. S1).

**Mortality and recurrence risk analysis according to liquid biopsy status after surgery**

The mortality risk was analysed in eight studies, including 328 patients. The analysis showed a trend of an increased risk for death among patients with ctDNA+ after surgery (RR = 2.67, CI95 %:0.88–8.08, $p$ = 0.08), although it did not achieve statistical significance (Supplementary material, Figs. S2–S3).

Sensitivity assessment by repeated quantitative analysis identified that the *Yamaguchi et al.* study increased heterogeneity. An extended follow-up period (2–132 months) yielded a higher incidence of events in all groups.Publication bias was confirmed in the Funnel Plot. When excluding this publication, statistically significant results were achieved (RR = 2.67, CI95 %:1.90–3.76, p < 0.00001) (Supplementary material, Figs. S4–S5).

The recurrence rate was analysed in 328 patients from 8 studies. There was no publication bias. Recurrence was higher in patients with ctDNA+ after surgery (RR = 1.42, CI95 %:1.08–1.87, $p$ = 0.01, Supplementary material, Figs. S6–S7).

*Effect of surgery ctDNA on liquid biopsy dynamics*

Seven studies, including 282 patients, analysed the shift status after surgery, ctDNA +/− and ctDNA −/+. There was no publication bias in the Funnel Plot. (Supplementary material, Fig. S8).This comparison revealed a higher rate of patients with ctDNA +/− (RR of 0.34, CI95 %:0.17–0.70, $p$ = 0.003, Fig. 3A).

*Survival analysis*

*Survival analysis according to liquid biopsy status before surgery*

Seven studies were included for OS analysis. CtDNA was the biomarker reported in all cases. There was no publication bias, according to the Funnel Plot. (Supplementary material, Fig. S9). The analysis showed a decrease in OS in patients with ctDNA+ before surgery compared with those with ctDNA - (HR = 2.28, CI95 %:1.28–4.08, $p$ = 0.005, Fig. 3B).

Six studies analysed DFS according to preoperative ctDNA liquid biopsy status. There was no publication bias (Supplementary material, Fig. S10). Patients with ctDNA+ before surgery had decreased DFS compared to patients with ctDNA - (HR = 2.16, CI95 %: 1.25–3.72, $p$ = 0.006, Fig. 3C).

*Survival analysis according to liquid biopsy status after surgery*

OS according to postoperative ctDNA status was analysed in 8 studies. There was no publication bias, according to Funnel Plot (Supplementary material, Fig. S11). The analysis showed a decrease in OS in patients with ctDNA+ after surgery compared to patients with ctDNA - (HR = 3.88, CI95 %:2.02–7.46, $p$ < 0.0001, Fig. 3D).

Six studies analyse DFS according to postoperative ctDNA status. There was no publication bias (Supplementary material, Fig. S12). Patients with ctDNA+ after surgeryhad decreased DFS compared to patients with ctDNA - (HR = 3.81, CI95 %:2.03–7.14, $p$ = 0.03, Fig. 3E).

*Survival analysis according to the shift dynamics after surgery*

*Shift to negative analysis (ctDNA +/−).* Three studies compare OS and DFS between patients with ctDNA +/− versus those patients ctDNA +/+ and ctDNA −/+. No publication bias was found in the Funnel plot. (Supplementary material, Fig. S13). The analyses revealed no differences in OS between groups (HR = 1.39, CI95 %:0.54—3.54, p = 0.50, Fig. 3F).

For DFS analysis no publication bias was found (Supplementary material, Fig. S14). The analyses revealed no differences in DFS between groups (HR = 1.62, CI95 %:0.50–5.23, p = 0.42, Fig. 3G).

*Shift to positive analysis (ctDNA −/+).* Three studies evaluate OS between patients with ctDNA −/+ versus those patients ctDNA −/− and ctDNA +/−. No publication bias was found (Supplementary material, Fig. S15). The analyses revealed no differences in OS between groups. Although statistical significance was not reached, a trend to a decreased OS in patients ctDNA −/+ versus ctDNA −/− and ctDNA +/− was found (HR of 5.0, CI95 %:0.79—31.75, p = 0.09, Fig. 3H).

Two studies evaluate DFS in patients with ctDNA −/+ versus those patients ctDNA −/− and ctDNA +/−. No publication bias was found. No differences in DFS between groups were found in this analysis (HR = 1.25, CI95 %:0.73–2.12, p = 0.42, Supplementary material, Figs. S16–S17).

*Comparison between non-touch techniques (NTIT) versus standard technique*

A subanalysis was performed to investigate if NTIT were associated with liquid biopsy negativisation after surgery. Liquid biopsies used were CTC and CEA mRNA. The small number of patients and studies included confers a bias. No differences in negativisation rates after surgery between techniques were found (HR = 1.69, CI95 %0.25–11.28, p = 0.59, Supplementary material, Figs. S18–S19).

*Sensitivity analysis*

A sub-analysis excluding patients with stage IV diagnosed intraoperatively, we did not obtain any changes in the primary outcomes analysed concerning liquid biopsy status (Supplementary material, Figs. S20–S24).

In a sub-analysis after excluding studies from the nineties, no differences were found in the primary outcomes (Supplementary material, Figs. S25–S29).

**Table 1**
General characteristics of the included articles.

| Study | Year | Country | Sample size | Stage TNM | Type of surgery | Margin status (R0) | Neoadjuvant chemotherapy | Sample | Time of sample pre | Time of sample post | Detection method | Technology/Assay | Marker | Targets | Endpoint | Follow up (months) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gall et al. [26] | 2014 | England | 12 | II (12) | sPD (6), nPD (6) | 3/6 (50 %), 3/6 (50 %) | 0/12(0 %) | Blood | Before tumor manipulation | After specimen removal | Fluorescence microscopy & scanning | Cell Search System (Veridex) | CTCs | counts | OS, DFS | 14.6 (10.5–27.9) |
| Groot et al. [36] | 2019 | USA | 59 | I-II (43); III (16) | PD (39), DP (13), TP (7) | 46/59 (78 %) | 24/59 (40.7 %) | Plasma | Before incision | Prior to discharge | Digital PCR | RainDrop Digital PCR system (RainDance Technologies) | ctDNA | KRASm (G12V, G12D, G12R, Q61H) | OS, DFS | 16 (13–19) |
| Hirota et al. [27] | 2005 | Japan | 18 | I (2), II (1), III (7), IV (8) | sDP (4), sPD (6), nDP (3), nPD (5) | Not reported | 0/18 (0 %) | Blood (PV) | Before tumor manipulation | After specimen removal | Real-time RT-PCR | LightCycler (Roche Biochemicals) | ctRNA | CEA | OS, DFS | 42 |
| Jiang et al. [37] | 2020 | China | 27 | I (13), II (9), IV (5) | Not defined | Not reported | Not reported | Plasma | Before incision | POD 7 | NGS | Custom hybrid-capture panel | ctDNA | 1017 cancer susceptibility genes | DFS | 18.6 (12.4–28.9) |
| Kitahata et al. [38] | 2021 | Japan | 27 | I (3), II-III (24) | PD (22), DP (4), TP (1) | 26/27 (96.3 %) | 27/27 (100 %) | Plasma | Pre NAC - Post NAC | 4–8 weeks PO | Digital PCR | QX200 Droplet Digital PCR system (BioRad) | ctDNA | KRAS multiplex assays (G12A, G12C, G12D, G12R, G12S, G12V, G13D) | OS, DFS | 14.5 (9.5—38.4) |
| Lee et al. [39] | 2019 | Australia, New Zeland, Singapore | 35 | II (3), III (32) | Not defined | 16/35 (45.7 %) | 0/35 (0 %) | Plasma | Diagnosis | 4–8 weeks PO | NGS | SafeSeqS (Illumina) | ctDNA | KRAS (G12, G13, G61) | OS, DFS | 38.4 |
| Nakano et al. [40] | 2018 | Japan | 45 | I (2), II (8), IIB (35) | PD (25), DP (16), TP (4) | 36/45 (80 %) | 11/45 (24.4 %) | Serum | Before incision | Discharge | Real-time PCR | Peptide nucleic acid (PNA)-directed PCR clamping | ctDNA | KRAS (codon 12 and 13) | DFS, OS | 43 |
| Nomoto et al. [41] | 1998 | Japan | 10 | I (1), II (4), III (4), IV (1) | Not defined | Not reported | 0/10 (0 %) | Blood | Before incision | Intraoperative and POD 1–7 | PCR/RFLP & Sanger sequencing | Amplification KRAS mutant and WT and selective enzym digestion WT | ctDNA | KRAS (codon 12) | OS | 19 |
| Watanabe et al. [42] | 2019 | Japan | 39 | I (3) II (34) III (1), IV (1) | DP (19), PD (15), TP (5) | Not reported | 7/39 (18 %) | Plasma | Before incision | 1–12 weeks PO | Digital PCR | QX200 Droplet Digital PCR system (BioRad) | ctDNA | KRAS (G12V, G12D, G12R, Q61H) | OS | 16.2 |
| Yamada et al. [43] | 1998 | Japan | 9 | I (2), II (3), III (2), IV (2) | PD (5), DP (4) | 5/9 (55.6 %) | 0/9 (0 %) | Plasma | Before incision | 4–8 weeks PO | Real-time PCR | Mutant allele-specific amplification (MASA-PCR) | ctDNA | KRAS (1st & 2nd nucleotide in codon 12) | OS | 12.2 (1.3–43.5) |
| Yamaguchi et al. [44] | 2021 | Japan | 97 | I (4), II (93) | PD (97) | 71/97 (73.2 %) | 30/97 (30.9 %) | Plasma | Before incision | POD 3 | Digital PCR | QX200 Droplet Digital PCR system (BioRad) | ctDNA | KRAS (G12V, G12R, G12D) | OS, DFS | 29 (2–132) |
| Yang et al. [45] | 2018 | China | 35 | I (3), II (29), III (3) | PD (35) | 23/35 (65.7 %) | 0/35 (0 %) | Plasma | Day before surgery | POD 10 | Digital PCR | QuantStudio™ 3D Digital PCR System (Thermo Fisher Scientific) | ctDNA | KRAS (G12V, G12D, G12R) | PFS, OS | 12.4 (6.1–17.2) |
| TOTAL | | | 413 | | | | | | | | | | | | | |

*Abbreviations: DFS, disease-free survival; OS, overall survival; PFS, progression-free survival; PD, pancreatoduodenectomy; DP, distal pancreatectomy; TP, total pancreatectomy; sDP, standard distal pancreatectomy; sPD, standard pancreatoduodenectomy; nDP, non touch distal pancreatectomy; nPD, non touch pancreatoduodenectomy; NTIT, non touch isolation technique; RT, reverse transcriptasa; PV, portal vein; PA, peripherial artery; NGS, next generation sequencing; SVC, superior vena cava; NAC, neoadjuvancy. POD: postoperative day; PO: postoperative.

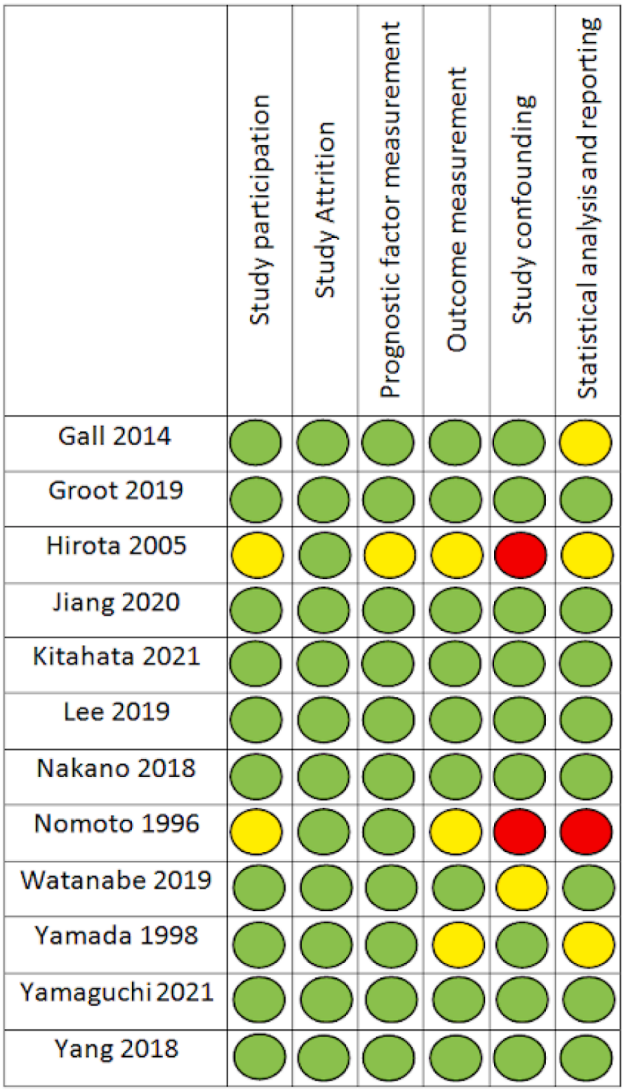


**Fig. 2.** Individual assessment of the risk of bias for each article with the QUIPS tool.

## Discussion

To our knowledge, this is the first meta-analysis in the literature that focuses on studying the interplay between surgical resection in PDAC patients and liquid biopsy status, including only resected patients. Our study demonstrates that surgical resection has a role in ctDNA negativisation in PDAC resectable patients (HR = 0.34, $p = 0.003$). Nevertheless, regarding OS and DFS in patients with ctDNA +/− after surgery, no impact was found in our analysis. However, a tendency to a decreased OS was observed in patients with ctDNA −/+. Patients with ctDNA −/+ had worse OS (HR = 3.88, p < 0.0001, Fig. 3D) and DFS (HR = 3.81, p = 0.03, Fig. 3E).

Biomarkers, more precisely ctDNA, are a revolutionary tool used in the diagnostic, prognostic, and guiding treatment of malignant diseases [46–49]. Over the last few years, publications about ctDNA and pancreatic cancer have increased substantially. *Milin-Lazovic et al.* concluded in their meta-analysis that a positive liquid biopsy significantly negatively impacts OS and progression-free survival (PFS) in PDAC [29]. This association was also found by *Bounduc et al.* [30]. Both meta-analyses included different tumour stages, including metastatic patients, chemotherapy and surgery treatments with curative and palliative intention in the quantitative analysis.

*Role of surgical resection on liquid biopsy status*

This systematic review answers the hypothesis that surgical resection has a role in circulating tumoral component status. Despite our initial hypothesis of increased release of tumour components to blood flow after surgical manipulation, our results demonstrate ctDNA negativisation after surgery (HR = 0.34, p = 0.003). A potential explanation for these results is that most studies in our meta-analysis reported post-operative ctDNA determination longer than 24 h after surgical specimen removal and before the fourth week after surgery [26,27,36,37,40,41,44,45]. The half-life of ctDNA has a wide variability from minutes to hours. Consequently, the later the ctDNA is determined after surgery, the more probability of finding a negative ctDNA. Additionally, these results corroborate that a blood negativisation is expected when the primary source of ctDNA is removed (the primary tumour).

Cell free DNA (cfDNA) levels increase and persist for up to two to four weeks following surgery, possibly masking persistent ctDNA in relapse patients, this phenomenon could be explained by the concept of trauma-induced cell free DNA (cfDNA) [20,50]. To decrease the likelihood of false negatives, performing the liquid biopsy between a window period of 2–4 weeks after surgery has to be considered [50].

Interestingly, studies focused on NTIT which performed ctDNA determinations during and immediately after specimen removal found positivisation of ctDNA, in agreement with the short half-life of ctDNA. *Nomoto et al.* reported a progressive positivisation of liquid biopsy during surgical manipulation in up to 100 % of cases after tumour removal [41]. Nonetheless, it is uncertain whether tumoral cells released immediately after tumour manipulation could resist anoikis and lately develop micrometastatic disease and recurrence weeks after surgery.

On the other hand, in patients where ctDNA remains positive after surgical resection, a tumour source that continues releasing ctDNA, such as a missing micrometastasis or a lack of local control after surgery (positive lymph nodes, neural infiltration), could be hypothesised. The contribution of surgery to increase ctDNA concentrations in cases with permanently positive ctDNA remains a matter to clarify in future studies.

*Impact of liquid biopsy status on survival*

Resectable PDAC patients with ctDNA -/~~+~~ were associated with longer OS (HR = 2.28) and DFS (HR = 2.16) compared to patients with ctDNA +/~~+~~. Likewise, the time point liquid biopsy status revealed that ctDNA ~~+~~/+negatively impacted OS (HR = 3.88) and DFS (HR = 3.81).

It is essential to analyse the dynamics shift during the intervention in each individual instead of the liquid biopsy status at a given time point in all the cohorts. When we analysed shift after surgical resection, we found that surgery increased negativisation of liquid biopsy status, eventually influencing OS and DFS.

However, this meta-analysis could not demonstrate the association between ctDNA +/− and improved OS and DFS. One of the explanations could be the small number of studies providing exact information on liquid biopsy change of status in each individual. Additionally, sensibility of ctDNA in post-operative PDAC may be low, also the outcome of PDAC is overall so dismal that it may be complicated to find the cured population. Further explanations are that other factors such as fragility, advanced age, postoperative complications and adjuvant chemotherapy could influence DFS and other rescue treatments after recurrence could impact OS. In that line, it has been demonstrated that certain adjuvant chemotherapies, such as FOLFIRINOX, yield more favourable OS and DFS in resectable PDAC patients when compared to Gemcitabine [51]. This meta-analysis did not allow for stratification based on adjuvant therapy.

Regarding NTIT, this meta-analysis failed to prove less release of tumoral components after surgical manipulation, nor better OS and DFS in NTIT compared to the standard technique. These results may be explained due to; the low number of studies published comparing NTIT

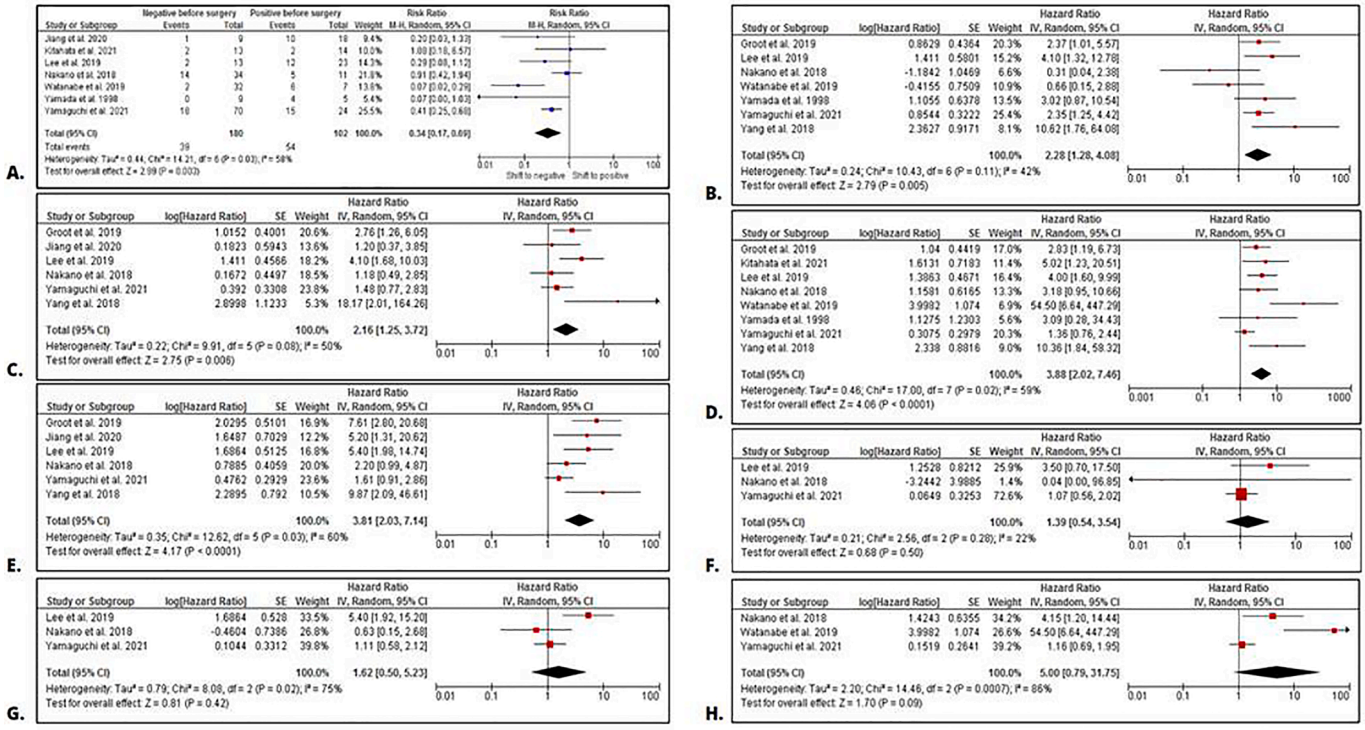


**Fig. 3.** **A**, Forest plot of articles analysed comparing ctDNA shift after surgery in patients with resectable PDAC. **B**, Forest plot of OS comparing positive versus negative ctDNA status before surgery in patients with resectable PDAC. **C**,Forest plot of DFS comparing positive versus negative ctDNA status before surgery in patients with resectable PDAC. **D**, Forest plot of OS comparing positive versus negative ctDNA status after surgery in patients with resectable PDAC. **E**, Forest plot of DFS comparing positive versus negative ctDNA status after surgery in patients with resectable PDAC. **F**, Forest plot of OS in patients with ctDNA +/− versus those patients with ctDNA +/+ and ctDNA −/+. **G**, Forest plot of DFS in patients with ctDNA +/− versus those patients with ctDNA +/+ and ctDNA −/+. **H**, Forest plot of OS between patients with ctDNA −/+ versus those patients with ctDNA −/− and ctDNA +/−.

versus standard technique in PDAC, the reduced number of patients included (total n = 30) and the heterogeneity of results presentation.

*Hirota et al.* described decreased detection of CEA-mRNA and low recurrence rate after NTIT compared to the standard technique [27]. NTIT includes vessels and lymphatic first approach by ligating the gastrocolic trunk at its communication with the superior mesenteric vein, followed by ligation of the gastroduodenal artery, right gastric artery, inferior pancreatic arteries, all pancreaticoduodenal branches from the portal vein and ligation and section of the bile duct and lymphatic vessels before manipulation of tumour. *Gall et al.* have demonstrated a significantly reduced number of CTCs in the portal vein in NTIT compared to the standard technique but failed to achieve survival differences [26].

Fortunately, the mere presence of tumoral components in the blood circulation is insufficient to guarantee metastasis development. Metastatic implantation is a complex phenomenon which implies many biological pathways. Tumoral cells from the primary tumour or premalignant lesions can create a pre-metastatic niche (PMN) in the liver before even starting dissemination. They secrete soluble factors and extracellular vesicles that enhance vascular permeability and docking, change the extracellular matrix, and gather immunosuppressive inflammatory cells into the liver, conferring a supportive niche to the cancerous cells [52,53]. As previously mentioned, these cells need anoikis resistance and the capacity to transition from epithelial to mesenchymal phenotype to travel through the blood circulation and attach to the PMN or travel in groups of cells [10–12]. Some of the fundamentals of neoadjuvant therapy are based on those pathways [53]. Nevertheless, our results showed that patients with positive tumoral components after surgery have worse DFS and OS, suggesting that the above-mentioned pathways occur.

## Limitations and strengths

This meta-analysis has certain limitations. As an intention-to-treat analysis, we included a small number of patients (4.1 %) re-staged as metastatic disease intraoperatively. However, a sub-analysis excluded those patients without obtaining changes in the primary outcomes analysed concerning liquid biopsy status (Supplementary material, Figs. S30–S34).

The sensitivity and specificity of liquid biopsy are also a limitation since ctDNA is only detected in 50 % of resectable early-stage patients [28–32]. Some mutations are not detected in standardised diagnostic panels, that mainly include KRAS (G12, G13, G61), TP53, and SMAD4. In 7 of the 12 studies (225 patients), a matching tissue strategy between primary tumour and liquid biopsy was performed in order to increase ctDNA detection. In addition, the fact that different detection methods were used, such as ddPCR, NGS or qPCR, could influence detection rate. Reports included in the meta-analysis only determine patients as positive or negative considering the threshold concentration of ctDNA of each test without reporting the exact ctDNA concentration (copies/ml$^3$), being not possible to determine tumour burden.

Even though precise ctDNA mutant allelic fraction is not specified in the articles, the limit of detection (LoD) of the tests utilized is between 1/1000–1/10,000 (mutant templates/normal templates). In general, ctDNA reported fractions in the literature are >1/1000, since tests are also limited by the genome equivalents present in plasma samples. Hence, the results obtained in the different studies are comparable (Supplementary material, Table S1).

Although different preserving tubes were used, no differences are expected when samples are processed within 6 h in EDTA tubes. CellSave has demonstrated to stabilize ctDNA and cfDNA better than EDTA tube after 48 h, but none of the included studies delay sample processing more than 6 h (Supplementary material, Table S1) [55,56].

Additionally, this meta-analysis could not assess the role of neoadjuvant treatment, and the radicality status effect over liquid biopsy status. In our meta-analysis, 99 patients (24 %) received neoadjuvant treatment, all included in publications from 2018 forward.

The publication year range of this meta-analysis goes from 1998 to 2021. Significant improvements have been made during these last decades, including better diagnosis accuracy, TNM classification modifications, more effective chemotherapies, enhanced surgical techniques and increased knowledge of liquid biopsy [54,57,58]. Regardless, the meta-analysis includes eight studies published in the last five years that use similar KRAS mutation (ctDNA) detection tests. When those studies are analysed separately no significant differences are found (Supplementary material, Figs. S30–S34).

This meta-analysis is focused exclusively on potentially resectable patients, therefore a likely curable population, trying to understand the role of surgical resection on liquid biopsy status. Our results are encouraging; thus, a role of negativisation of the ctDNA is related to surgery. These results may open new research lines to optimise the reduction of tumoral components after resection using new promising surgical techniques and molecular biology advances. Minimally invasive surgical procedures allow accurate and minimal manipulation of tumours during surgery, aligning with this proposal [59].

This report also corroborates biomarkers, more precisely ctDNA, as a reliable prognostic factor in resectable PDAC patients. According to this, ctDNA may have a role in PDAC follow-up and could be used to track down patients with higher relapse probabilities after surgery. Our results encourage that ctDNA may have the potential to determine which patients with resectable PDAC may benefit from neoadjuvant chemotherapy. Despite our efforts, we have identified some publication bias, suggesting that our findings should be interpreted cautiously.

Future efforts must determine if minimally invasive surgery could contribute to a greater negativisation of ctDNA after surgery in resectable PDAC patients conferring better oncological outcomes.

## Conclusion

In resectable PDAC patients, surgical resection has a role in the negativisation of liquid biopsy status. Detectable ctDNA is a prognostic factor for surveillance and relapse in resectable PDAC patients. NTIT did not demonstrate increased liquid biopsy negativisation compared to the standard technique, although more studies are needed to obtain more robust conclusions.

## CRediT authorship contribution statement

**Laura Vidal:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing – original draft, Visualization, Funding acquisition. **Elizabeth Pando:** Conceptualization, Methodology, Project administration, Investigation, Writing – review & editing, Supervision, Funding acquisition. **Laia Blanco:** Writing – review & editing. **Carles Fabregat-Franco:** Writing – review & editing. **Florian Castet:** Writing – review & editing. **Alexandre Sierra:** Writing – review & editing. **Teresa Macarulla:** Writing – review & editing. **Joaquim Balsells:** Supervision, Writing – review & editing. **Ramón Charco:** Writing – review & editing. **Ana Vivancos:** Supervision, Writing – review & editing.

## Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Laura Vidal reports article publishing charges was provided by Autonomous University of Barcelona.

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### *Author contribution*

LVP: project development, data collection and analysis, manuscript writing and editing. EPR project development, data collection and analysis, manuscript writing and editing. JBV and AVP contribute to solving data disagreements. AVP, TMM, JBV and RCH contributed to the final version of the manuscript. All authors reviewed the manuscript.

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## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ctrv.2023.102604.

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## Glossary

*ctDNA:* circulating tumour DNA.
*CTCs:* circulating tumour cells.
*mRNA:* messenger RNA.
*PDAC:* pancreatic ductal adenocarcinoma.
*Ct:* circulating tumour.
*EMT:* epithelial to mesenchymal transition.
*DFS:* disease-free survival.
*OS:* overall survival.
*NTIT:* non-touch isolation techniques.
*ctRNA:* circulating tumour RNA.
*MeSH:* medical Subject Heading.
*HR:* Hazar Ratio.
*RR:* Risk Ratio.
*OR:* Odds Ratio.
*QUIPS tool:* Quality In Prognosis Studies tool.
*AJCC:* American Joint Committee of Cancer.
*PFS:* Progression-free survival.
*PMN:* pre-metastatic niche.

*ddPCR:* droplet digital PCR.
*NGS:* next generation sequencing.
*MASA-PCR:* Mutant allele specific amplification PCR.
*PD:* pancreatoduodenectomy.
*DP:* distal pancreatectomy.
*TP:* total pancreatectomy.
*sDP:* standard distal pancreatectomy.
*sPD:* standard pancreatoduodenectomy.
*nDP:* Non touch isolation technique distal pancreatectomy.
*nPD:* Non touch isolation technique pancreatoduodenectomy.
*RT:* reverse transcriptase.
*PV:* portal vein.
*PO:* postoperative.
*POD:* postoperative day.
*NAC:* neoadjuvancy.
*LoD:* Limit of detection.
*EDTA:* ethylenediaminetetraacetic acid.
*cfDNA:* cell free DNA.