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Prognostic role of cell-free DNA biomarkers in pancreatic adenocarcinoma: A systematic review and meta–analysis

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ABSTRACT

This systematic review and meta-analysis evaluated the prognostic role of cell-free DNA (cfDNA) in pancreatic ductal adenocarcinoma (PDAC). Eligible studies reported differences in overall (OS) and progression-free survival (PFS) by cfDNA status. The random effect model yielded the pooled hazard ratios (HRs) and 95 % confidence intervals (CI). Detection of circulant-tumor DNA (ctDNA), KRAS mutations and other cfDNA alterations constitute detectable cfDNA biomarkers. Altogether, 38 studies (3,318 patients) were eligible. Progression-free and overall survival were decreased with detectable ctDNA (HR = 1.92, 95 %CI:(1.29,2.86); HR = 2.25, 95 % CI:(1.73,2.92)) and KRAS mutations (HR = 1.88, CI:1.22,2.92,); HR = 1.52, 95 %CI:(1.22,1.90)) respectively, across various stages. In unresectable cases, ctDNA (HR = 2.50, 95 %CI:(1.94,3.23)), but not KRAS mutations (HR = 1.16, 95 %CI:(0.46,2.94)) signaled risk for progression. Detectable cfDNA biomarkers correlated with worse prognosis in resectable cases and if detected during treatment. In conclusion, cfDNA biomarkers indicate accelerated progression and decreased survival in PDAC. Significance of KRAS mutations detection in unresectable cases is to be determined.

1. Introduction

Pancreatic cancer (PC) is the 7th most common cause of cancer-related death worldwide, and its incidence is increasing (Tempero et al., 2017; Silvestris et al., 2020; Winter et al., 2019; GLOBOCAN, 2020). The 5-year overall survival rate is around 9 %, and it has only marginally improved during the last decades (Hussung et al., 2021; Qi et al., 2018). The poor prognosis results from the low resectability rate at

Abbreviations: BEAMing, beads Emulsion Amplification Magnetics; CA 19-9, carbohydrate antigen 19-9; CI, confidence interval; CT, computer tomography; CTC, circulating tumor cells; cfDNA, cell-free deoxyribonucleic acid; ctDNA, circulant tumor deoxyribonucleic acid; dPCR, digital polymerase chain reaction; ddPCR, droplet digital polymerase chain reaction; DSS, disease-specific survival; FDA, Food and Drug Administration; Fig., figure; Fig., s supplementary figure; FOLFIRINOX, Folinic Acid, Fluorouracil, Irinotecan, and Oxaliplatin; HR, hazard ratio; KRAS, Kirsten rat sarcoma viral oncogene homolog; N/A, not available; NGS, next-generation sequencing; OS, overall survival; PC, pancreatic cancer; PCR, polymerase chain reaction; PDAC, pancreatic ductal adenocarcinoma; PFS, progression-free survival; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; UHR, univariate (unadjusted) pooled hazard ratio; AHR, multivariate (adjusted) pooled hazard ratio.

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diagnosis, surgery being the only potentially curative treatment, and the high resistance rate to chemo, radio and immunotherapy (Sivapalan et al., 2021). The methods currently in use for disease monitoring are contrast-enhanced computer tomography (CT), with dual-phase pancreatic protocol, and the serum level of carbohydrate antigen 19−9 (CA19-9) (Tempero et al., 2017). Tumor size and attenuation, alongside contact with vessels, are the main criteria analyzed during CT surveillance, although with an overall low accuracy for assessing treatment response (Elbanna et al., 2020). Similarly, CA19-9 is not tumor-specific and has a sensitivity of 73 % in identifying relapse (Groot et al., 2019; Daamen et al., 2018). Improvement of methods for disease monitoring may allow a timely adjustment of treatment and amelioration of prognosis.

Liquid biopsy is a tool of precision medicine that has gained momentum in oncology (Sivapalan et al., 2021). It consists of analyzing, among others, circulating tumor cells, exosomes, or cell-free nucleic acids isolated from different body fluids such as blood, saliva, urine, etc. (Qi et al., 2018). It provides information on the molecular and genetic background of the tumor and facilitates repeated sampling for a longitudinal follow-up (Qi et al., 2018).

Circulating tumor DNA (ctDNA) is the most broadly studied type of liquid biopsy in PDAC (Sivapalan et al., 2021). It consists of short chains of nucleic acids released by the tumor cells mainly during necrosis or apoptosis and carries highly tumor-specific genetic and epigenetic changes (Botrus et al., 2021). It represents up to 1 % of the circulating cell-free DNA (cfDNA) pool (Botrus et al., 2021). There is growing evidence of the association of cfDNA biomarkers with tumor burden, grading and an overall poor prognosis in PDAC (Hammel et al., 2020; Sausen et al., 2015; Hadano et al., 2016; Pietrasz et al., 2017). Moreover, detection of ctDNA is thought to be associated with the presence of micrometastases and could predict disease relapse before it becomes detectable by imaging, therefore showing great potential as a method for disease monitoring (Qi et al., 2018; Guo et al., 2020).

In this systematic review and meta-analysis, we aimed to investigate the association of cfDNA biomarkers with overall (OS) and progression-free survival (PFS) in PDAC irrespective of disease stage. Furthermore, we performed subgroup analysis for resectable and unresectable stages, detection of cfDNA biomarkers during treatment, according to the cfDNA biomarkers types and method of detection.

2. Methods

We reported our findings according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement (PRISMA checklist) (Moher et al., 2009). Our meta-analysis protocol was previously registered on the International prospective register of systematic reviews (PROSPERO registration number CRD42021223891). We adhered to the study protocol. Besides, were able to perform additional subgroup analyses according to the biomarkers detection method and detection of cfDNA biomarkers during treatment. Also, we included in our analysis results from two randomized control trials (RCTs) (Bachet et al., 2020; Van Laethem et al., 2017).

2.1. Literature search and eligibility

We performed the systematic search on the 21st of October 2020 in five medical databases – MEDLINE (via PubMed), Embase, Web of Science, Cochrane Central Register of Controlled Trials (CENTRAL), and Scopus without applying any search restrictions. The same search key was used for each database (detailed in Appendix 1). The reference list of the included studies was manually screened for additional eligible articles.

We formulated our question using the PECO framework (populations, exposure, comparator, outcomes). The eligibility criteria for the studies included in our review were as follows: study population (P) comprised adults (age ≥18 years) with pancreatic ductal adenocarcinoma in whom peripheral blood cfDNA samples were analyzed. The cases in which cfDNA biomarkers (E) as defined in each study were detected were compared to the cases in which the biomarkers were not detected (C). The analyzed outcomes (O) were progression-free survival (PFS) and overall survival (OS). The associations between liquid biopsy results and the outcomes were assessed by unadjusted or adjusted Cox regression analysis and reported as hazard ratios (HRs) and 95 % confidence intervals (CI).

2.2. Study selection and data extraction

The Cochrane Handbook recommendations were followed (Higgins and Green, 2015) for study selection and data extraction. Articles were imported to the reference management program EndNote X9 (Clarivate Analytics, Philadelphia, PA, USA). After duplicate removal, the selection was performed by two independent investigators by title, abstract, and full-text contents, according to previously established inclusion and exclusion criteria. The rate of agreement was calculated using Cohen's Kappa coefficient at each selection step, and any disagreements were resolved by third-party arbitration (McHugh, 2012). The exclusions made at the full-text selection step were documented. In the case of overlapping populations, we selected the studies with a higher number of participants. If multiple biomarkers were assessed in the same study, we included in the meta-analytical calculations the results for the biomarker detected in the greater number of patients.

Data were extracted by two independent investigators and recorded in a standardized collection form. Any disagreement was resolved by third-party arbitration. The following information was collected: first author, publication year, digital object identifier, study site, study type, demographic characteristics of the analyzed population, technique of cfDNA analysis, definition for detection of the cfDNA biomarkers, time of sample collection, tumor stage, number of exposed patients, number of unexposed patients and unadjusted and adjusted HRs (UHR and AHR) with 95 %CIs.

2.3. Data synthesis

The meta-analytical calculations were performed by one biostatistician using Stata 15.1 data analysis and statistical software (Stata Corp LLC, College Station, TX, USA) and Comprehensive Meta-Analysis (version 3, Biostat Inc., Englewood, NJ, USA).

The pooled HRs and 95 %CIs were calculated with the DerSimonien-Laird estimation, random effect model (Dersimonian and Laird, 1986). Statistical heterogeneity was analyzed using the I^2 ($I^2 = 100\ \% \times (Q - df)/Q$), that represents the percentage of the total variability across studies (30 %–60 %—moderate, 50 %–90 %—substantial and 75 %–100 %—considerable degree of heterogeneity) (Grant and Hunter, 2006). Cochrane's Q^2 test was used to gain probability-values, and $p < 0.1$ was defined to indicate significant heterogeneity (Grant and Hunter, 2006).

For subgroup analysis the biomarkers were categorized as follows: detection of KRAS mutations, detection of ctDNA – by other means than based solely on KRAS mutations – e.g. by targeted sequencing using a custom gene panel (Bachet et al., 2020), according to a certain cut-off for the concentration of cfDNA (Lapin et al., 2018) and others, and detection of cfDNA biomarkers that included all categories. Some studies reported on disease-specific survival (DSS) (Adamo et al., 2017), considered here as OS, and relapse-free survival (RFS) (Guo et al., 2020; Lee et al., 2019), disease-free survival (DFS) (Jiang et al., 2020; Nakano et al., 2018; Okada et al., 2020) or time to progression (TTP) (Chen et al., 2017), considered as PFS. Metastatic cases were deemed unresectable (Tempero et al., 2017).

We analyzed the association between OS and PFS and the status of cfDNA biomarkers isolated from peripheral blood in PDAC patients. We performed subgroup analysis according to —disease stage —for the resectable and unresectable cases, exposure type —for each category of biomarkers, according to the method used for biomarker detection – for

next generation sequencing (NGS) and droplet digital PCR (ddPCR), and for detection of cfDNA biomarkers during treatment including cases in which longitudinal monitoring was performed. The analyses included non-overlapping populations.

2.4. Risk of bias assessment

Two independent investigators applied the Quality in Prognosis Studies (QUIPS) tool to assess the risk of bias in the individual eligible studies (Hayden et al., 2006). Disagreements were solved by consensus. The assessed domains were: statistical analysis reporting, study confounding, outcome measurement, prognostic factor measurement, study attrition and study participation. The definitions were as follows: overall low risk of bias if all domains associated a low risk, or if for one domain the risk of bias was moderate, overall high risk of bias if one domain associated high risk of bias, or if 3 or more domains had a moderate risk, and overall moderate risk of bias corresponding to all in between cases (detailed in Appendix 2).

Publication bias was evaluated by visual inspection of funnel plots end Egger's test for all the hypotheses verified in at least 8 studies (Higgins and Green, 2015).

3. Results

3.1. Search and selection

The search and selection processes are described in Fig. 1. Altogether, 1,978 records were identified through database searching. After duplicate removal, we screened 1,133 articles, of which 38 were eligible for qualitative and quantitative synthesis.

3.2. Characteristics of the included studies

The main characteristics of the included studies are summarized in Table 1. The 38 articles counted 3,318 patients with PDAC. Besides cohort studies, we included in our analysis two randomized control trials (Bachet et al., 2020; Van Laethem et al., 2017). KRAS mutations were the most frequently assessed biomarkers and among others, next-generation sequencing (NGS) and droplet digital polymerase chain reaction (ddPCR) were the main molecular techniques used for cfDNA biomarkers detection. The results of our analyses are summarized Table 2.

3.3. The association between detection of ctDNA and survival in PDAC

First, we analyzed the association with survival of ctDNA detection across various PDAC stages, irrespective of detection method. Our results showed that ctDNA was associated with increased risk for mortality (UHR = 2.25, 95 %CI:1.73–2.92, Fig. 2), and progression (UHR = 1.92, 95 %CI: 1.29–2.86, Fig. S1A). The difference in progression free survival did not hold significant in multivariate analysis (AHR = 1.97, 95 % CI:1.00–3.87, Fig. S1B). Subsequently we performed subgroup analysis by detection method. Samples were plasmatic, collected at baseline and NGS was used for ctDNA detection. Overall survival was decreased in positive cases (UHR = 2.39, 95 %CI: 1.80–3.16, Fig. S2).

Performing subgroup analysis for unresectable disease stages revealed an increased risk for progression (UHR = 2.50, 95 % CI:1.94–3.23 Fig. 3A) and mortality (UHR = 2.30, 95 %CI: 1.86–2.85, Fig. S3) in cases where ctDNA was detected. Various methods of detection were used.

3.4. The association between detection of KRAS mutations and survival in PDAC

We first assessed the impact of KRAS mutations on survival across various PDAC stages. A decreased OS (UHR = 1.52, 95 %CI: 1.22–1.90, Fig. S4a) and PFS (UHR = 1.99, 95 %CI: 1.36–2.92, Fig. S5a) was revealed in cases that were tested positive.

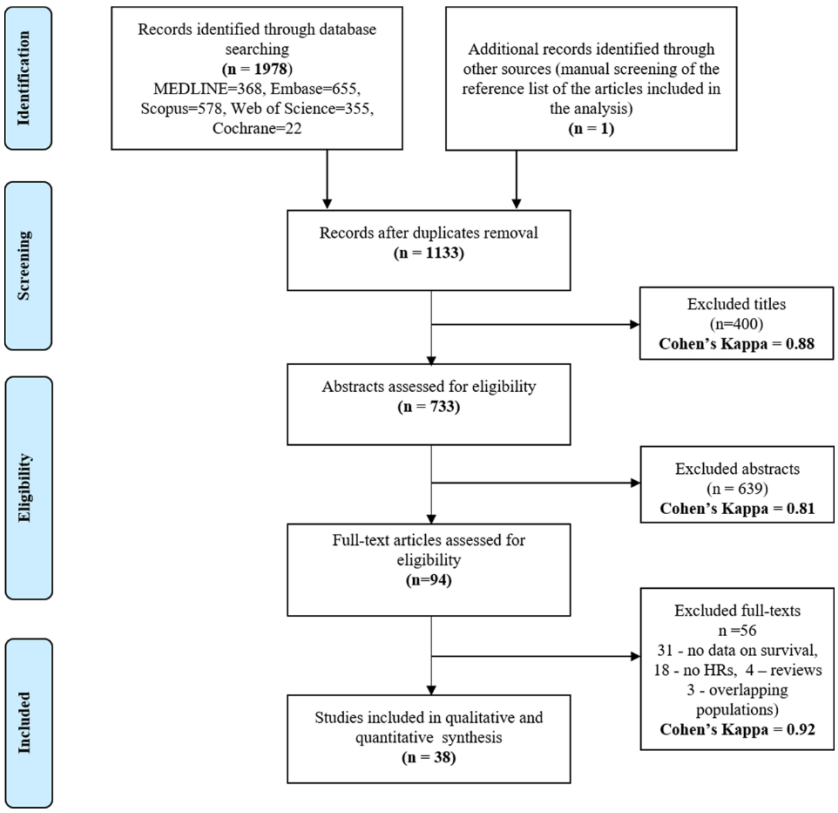

Fig. 1. PRISMA flow chart.

Table 1
Characteristics of the included studies.

Study	Country	Sample size (female%)	Disease stage	Marker	Detection method[e]	Time of sample collection	Outcomes
(Adamo et al., 2017)	UK	28 (38)	I-IV TNM	cfDNA, KRAS	tNGS	Baseline	DSS
(Bachet et al., 2020)	France	113 (41.7)	IV, TNM	ctDNA	NGS	Baseline &during treatment	OS, PFS
(Bernard et al., 2019)[a]	US	104 (43)	Metastatic	KRAS	ddPCR	Baseline &during treatment	OS, PFS
(Chen et al., 2017)	Denmark	189 (49.7)	III-IV, AJCC	KRAS	Mutation enrichment PCR + NGS	Baseline	OS, TTP
(Cheng et al., 2017)	China	188 (34.04)	Metastatic	cfDNA	NGS, ddPCR	Baseline &during treatment	OS, PFS
(Cheng et al., 2020)	China	210 (37.10)	III-IV AJCC 2018	KRAS	ddPCR	Baseline	OS
(Earl et al., 2015)	Spain	31 (46.67)	All stages[b]	KRAS	ddPCR	Baseline /during treatment	OS
(Groot et al., 2019)	USA	59 (47)	Localized	ctDNA, KRAS	ddPCR	Baseline &during treatment	OS, RFS
(Guo et al., 2020)	China	113 (38.9)	I-IV AJCC 8th ed.	ctDNA, KRAS	NGS, ddPCR	Baseline	OS, RFS
(Hadano et al., 2016)	Japan	105 (48)	I-IV UICC	KRAS, ctDNA	NGS ddPCR	Baseline	OS
(Henriksen et al., 2017)	Denmark	95 (43)	I-IV AJCC 7th ed.	Methylation	PCR	Baseline	OS
(Jiang et al., 2020)	China	27 (37.04)	I, II, IV TNM	ctDNA	NGS	Baseline &during treatment	DFS
(Kim et al., 2018)	Korea	106 (36.80)	All stages[b]	cfDNA, KRAS	Qubit; ddPCR	Baseline	OS, PFS
(Kinugasa et al., 2015) [d]	Japan	75 (28)	I-IV JPS	KRAS	ddPCR	Baseline	MST
(Kruger et al., 2018)	Germany	54 (43)	Unresectable[a]	KRAS	BEAMing	Baseline &during treatment	OS, PFS
(Lapin et al., 2018)	Norway	61 (43)	III-IV TNM	cfDNA	Agilent2100 bioanalyzer	Baseline	OS, PFS
(Lee et al., 2019)	International	37 (37.84)	Resectable[c]	KRAS	SafeSeqS	Baseline &during treatment	OS, RFS
(Li et al., 2018)	China	65 (48)	I-IV UICC	ctDNA, KRAS	ddPCR	Baseline	OS
(Liu et al., 2019)	China	74 (n.r.)	I-IV TNM	cfDNA, KRAS	NGS	Baseline	OS
(Mohan et al., 2019)[a]	UK	55 (49,09)	Unresectable[a]	cfDNA, KRAS	tNGS, ddPCR	Baseline	OS
(Moriyama et al., 2002)	Japan	20 (45 %)	I-IV TNM	MSI, AI	PCR	Baseline &during treatment	OS
(Nakano et al., 2018)	Japan	45 (n.r.)	I-IV UICC 7th ed.	cfDNA	PNA clamp PCR	Baseline &during treatment	OS, DFS
(Okada et al., 2020)	Japan	96 (56.25)	0-III UICC 8th ed.	cfDNA, KRAS, GNAS	dPCR	Pre-treatment	OS, DFS
(Patel et al., 2019)	USA	94 (n.r.)	All stages[b]; recurrent	ctDNA, KRAS	NGS	Baseline &during treatment	OS
(Pietrasz et al., 2017)	France	104 (n.r.)	All stages[b]	ctDNA, KRAS	NGS, ddPCR	Baseline &during treatment	OS
(Sausen et al., 2015)	USA	101 (37.62)	II, TNM	ctDNA, Chromatin Modifying Genes	NGS, ddPCR	Baseline	OS
(Sefrioui et al., 2017)	France	56 (n.r.)	All stages[b]	cfDNA, KRAS	ddPCR	Baseline	OS
(Singh et al., 2015)	India	127 (30,7)	All stages[b]	cfDNA, KRAS	PCR	Baseline	OS
(Strijker et al., 2020)	Netherlands	58 (46.6)	Metastatic	ctDNA	NGS, ddPCR	Baseline &during treatment	OS
(Sugimori et al., 2020)	Japan	44 (n.r.)	II-IV UICC 7th ed.	ctDNA, KRAS, NRAS	dPCR	Baseline &during treatment	PFS
(Takada et al., 2020)[d]	Japan	35 (n.r.)	I-IV UICC 7th ed.	ctDNA, KRAS	NGS	n.r.	OS
(Takai et al., 2015)	Japan	259 (38.2)	I-IV UICC 7th ed.	cfDNA, KRAS	tNGS, ddPCR	Baseline	OS
(Toledano-Fonseca et al., 2020)	Spain	61 (44.3)	Metastatic	cfDNA, RAS	dPCR	Baseline &during treatment	OS
(Uesato et al., 2020)	Japan	104 (35.6)	Unresectable[a]	cfDNA	NGS	Baseline /during treatment	OS
(Van Laethem et al., 2017)	Belgium	60 (n.r.)	Unresectable[a]	KRAS	BEAMing	During treatment	OS
(Wang et al., 2021)	China	149 (34.2)	n.r.	ctDNA, KRAS	ddPCR	Baseline	OS
(Watanabe et al., 2019)	Japan	78 (51.28)	All stages[b]; recurrent	KRAS	ddPCR	Baseline &during treatment	OS, PFS
(Wei et al., 2019)	China	38 (28.95)	III-IV AJCC	cfDNA	NGS	Baseline &during treatment	OS

***Abbreviations**: BEAMing – Beads Emulsion Amplification Magnetics, cfDNA – cell-free deoxyribonucleic acid, ctDNA – circulant-tumor deoxyribonucleic acid, (d)dPCR – (droplet) digital Polymerase Chain Reaction, DFS – disease free survival, DSS -disease specific survival, JPS – Japan Pancreas Society, n.r. – not reported, (t)NGS – (targeted)Next Generation Sequencing, OS - overall survival, PCR – polymerase Chain Reaction, PFS – progression-free survival, RFS – relapse-free survival, TNM – Tumor Node Metastasis; KRAS - Kirsten rat sarcoma viral oncogene homolog mutations; MSI – microsatellite instability; allelic imbalance; TTP – time to progression; RFS – recurrence-free survival; MST – median survival time; UICC – Union for International Cancer Control; PNA – peptide nucleic acid; ed. – edition; AJCC - American Joint Committee on Cancer.

[a] locally advanced, metastatic.
[b] resectable, locally advanced, metastatic.
[c] as diagnosed by multidisciplinary team.
[d] serum sample types; all other samples were plasmatic.
[e] detailed in Appendix 3.

Table 2
Summary of results for each subgroup analysis.

PDAC	BIOMARKER	OUTCOME	DETECTION METHOD	UNADJUSTED ANALYSIS		ADJUSTED ANALYSIS	
				HR (95 %CI)	I^2(%), p value	HR (95 %CI)	I^2 (%), p value
all stages	ctDNA	OS	Various	2.25 (1.73, 2.92)	52.1, 0.03	2.39 (1.79, 3.19)	44.2, 0.084
			NGS	2.39 (1.80, 3.16)	0, 0.789	NA	
		PFS	Various	1.92 (1.29, 2.86)	68.6, 0.004	1.97 (1.00, 3.87)	77.3, 0.012
	KRAS	OS	Various	1.52 (1.22, 1.90)	88.36, <0.001	1.71 (1.31, 2.24)	86.6, <0.001
			NGS	2.39 (1.68, 3.40)	91, <0.001	1.55 (1.03, 2.32)	83.7, <0.001
			ddPCR	1.25 (0.80, 1.96)	80.3, <0.001	1.88 (1.25, 2.81)	79.4, <0.001
		PFS	Various	1.99 (1.36, 2.92)	81.8, <0.001	1.99 (1.32, 3.00)	86.6, <0.001
			NGS	2.68 (1.75, 4.09)	78.9, <0.001	2.03 (1.27, 3.22)	90.6, <0.001
			ddPCR	1.37 (0.63,2.99)	89.2, <0.001	NA	
	cfDNA_bmk[a]	OS	Various	1.78 (1.43, 2.21)	78, <0.001	1.99 (1.58, 2.50)	88.2, <0.001
			NGS	2.70 (1.93, 3.76)	74, <0.001	2.63 (1.78, 3.89)	65.5, 0.005
			ddPCR	1.29 (0.84, 1.98)	78, <0.001	1.99 (1.36, 2.91)	78.9, <0.001
		PFS	Various	2.04 (1.51, 2.74)	78.3, <0.001	2.20 (1.49, 3.26)	88.2, <0.001
			NGS	2.46 (1.48, 4.07)	81.4, <0.001	2.03 (1.27, 3.22)	90.6, <0.001
			ddPCR	1.56 (0.87, 2.81)	84.9, <0.001	NA	
unresectable	ctDNA	OS	Various	2.30 (1.86, 2.85)	0, 0.949	2.20 (1.49, 3.26)	88.2, <0.001
		PFS	Various	2.50 (1.94, 3.23)	0, 0.527	2.39 (1.79, 3.19)	44.2, 0.084
	KRAS	OS	Various	1.20 (0.64, 2.25)	87.5, <0.001	1.54, (1.07,2.21)	53.1, 0.119
			ddPCR	0.97 (0.47, 1.99)	87.7, <0.001		
		PFS	Various	1.16 (0.46, 2.94)	90.3, <0.001	NA	
			ddPCR	0.8 (0.32, 1.99)	87.7, <0.001		
	cfDNA_bmk[a]	OS	NGS	2.39 (1.68, 3.40)	91, <0.001	1.46 (1.19, 1.78)	0, 0.48
			ddPCR	1.46 (1.19, 1.78)	0, 0.489	1.16 (0.64, 2.10)	86.7, <0.001
		PFS	NGS	2.46 (1.48, 4.07)	81.4, <0.001	2.03 (1.27, 3.22)	90.6, <0.001
resectable	cfDNA_bmk	PFS	Various	3.57 (2.42, 5.28)	0, 0.380		
during treatment	cfDNA_bmk	OS	Various	3.39 (1.48, 7.80)	77.6, <0.001	NA	
			NGS	2.11 (1.63, 2.72)	0, 0.545		
		PFS	Various	1.96 (1.05, 3.65)	73.6, 0.002		

Abbreviations: ctDNA – circulant tumor deoxyribonucleic acid, KRAS – Kirsten rat sarcoma viral oncogene homolog mutations, cfDNA – cell-free DNA, cfDNA_bmk – cell,free DNA biomarkers, OS – overall survival, PFS – progression-free survival, HR – hazard ratio, N/A – not available, no. – number; NGS – next generation sequencing; ddPCR – droplet digital PCR.

[a] any kind of cfDNA alterations, including KRAS mutations and detection of ctDNA, as defined in each study, were referred to as cfDNA biomarkers.

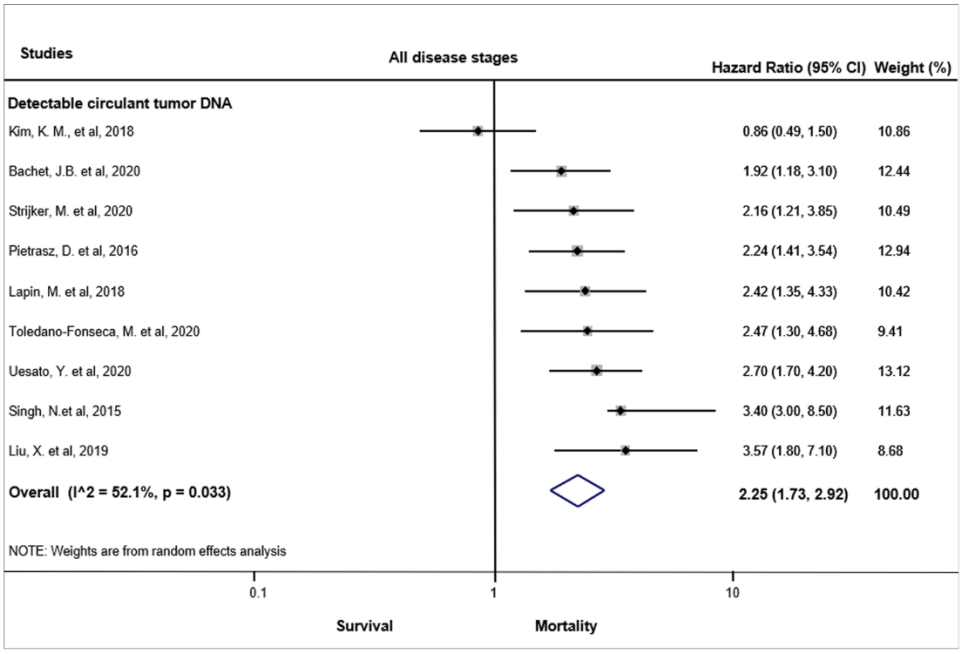

Fig. 2. Unadjusted analysis of association between detection of circulant tumor DNA and overall survival in pancreatic ductal adenocarcinoma – all disease stages.

Furthermore we performed subgroup analysis for unresectable disease stages. Neither PFS (UHR = 1.16, 95 %CI: 0.46–2.94, Fig. 3B) nor OS (UHR = 1.20, 95 %CI: 0.64–2.28, Fig. S6A) were decreased in cases with detectable KRAS mutations. The adjusted analysis for OS in this subgroup showed contradictory results (AHR = 1.54, 95 %CI:1.07–2.21, Fig. S6B). We performed further subgroup analysis by detection method. Samples were taken at baseline and analyzed by ddPCR. KRAS mutations did not indicate a decreased overall and progression free survival in positive cases (UHR = 0.97, 95 %CI:0.47–0.99; UHR = 0.8, 95 % CI:0.32–1.99, Fig. S7).

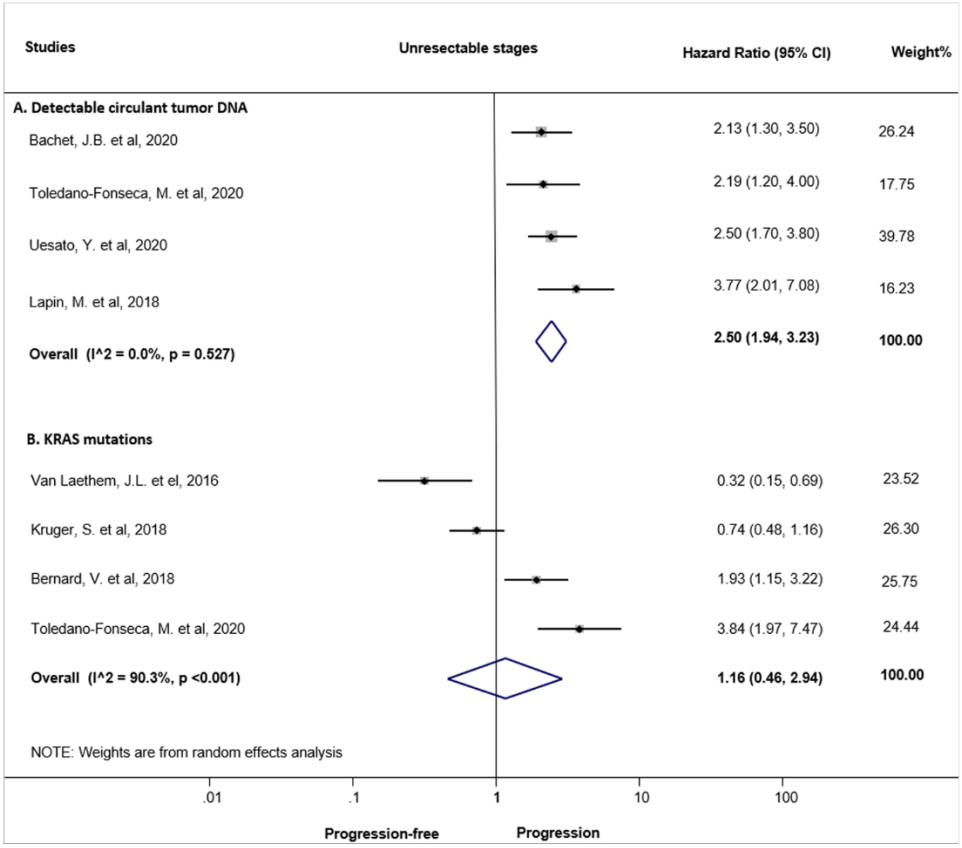

Fig. 3. Unadjusted analysis of association between progression-free survival in unresectable pancreatic ductal adenocarcinoma and detection of A. circulant tumor DNA, B. KRAS mutations.

3.5. *The association between cfDNA biomarkers and survival in PDAC*

We also evaluated the association of cfDNA biomarkers detected by various methods across multiple disease stages. Cases that tested positive had an increased risk for progression (UHR = 2.04, 95 % CI:1.51–2.74, Fig. S8A) and mortality (UHR = 1.78, 95 %CI:1.43–2.21, Fig S9A). Subgroup analysis by detection method was performed. All samples were plasmatic and collected at baseline. Detection of cfDNA biomarkers using NGS indicated decreased overall and progression-free survival (UHR = 2.70, 95 %CI:1.93–3.76, Fig. S10A, UHR = 2.46, 95 % CI:1.48–4.07, Fig. S11A) respectively. Still for the biomarkers detected by ddPCR the decrease in survival in cases that were tested positive did not hold significant (Table 2, Figs. S12 and S13).

We were able to perform subgroup analysis for resectable disease stages. All samples were plasmatic and collected at baseline. Detection of cfDNA biomarkers through various methods indicated decreased PFS (UHR = 3.57, 95 %CI:2.42–5.28, Fig. 4).

We also assessed the impact on prognosis of cfDNA biomarkers detection during treatment. In this subgroup 2 studies reported on resectable cases and samples were taken at 7 days or more after surgery or before discharge (Lee et al., 2019; Jiang et al., 2020; Nakano et al., 2018; Watanabe et al., 2019). Not all the studies reporting on unresectable cases undergoing chemotherapy stated clearly the moment of blood sampling for cfDNA isolation – nevertheless in most of the cases it was done 4–8 weeks after chemotherapy (Bachet et al., 2020; Lapin et al., 2018; Sugimori et al., 2020; Watanabe et al., 2019). Cell-free DNA biomarkers detected by various methods indicated decreased OS (UHR = 3.39, 95 %CI:1.48–7.80, Fig. S14A) and PFS (UHR = 1.96, 95 %

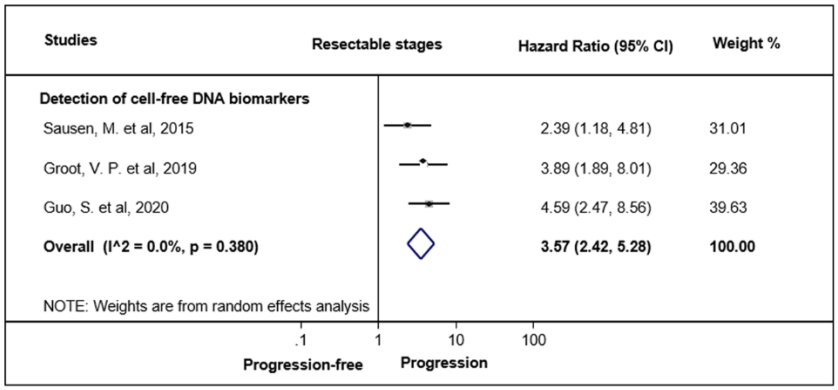

Fig. 4. Unadjusted analysis of association between detection of cell-free DNA biomarkers and progression-free survival in resectable pancreatic ductal adenocarcinoma.

CI:1.05–3.65, Fig. 14B). The association held significant when further analysis was performed by detection method. Detection by NGS of cfDNA biomarkers isolated from plasma during treatment, indicated decreased OS across various PDAC stages (UHR = 2.11, 95 % CI:1.63–2.72, Table 2, Fig. S15).

3.6. Risk of bias assessment

The QUIPS tool revealed a low overall risk of bias in the included studies for statistical analysis reporting, study confounding, prognostic factor measurement, and study participation and moderate risk of bias for study attrition and outcome measurement (Fig. S23).

Regarding publication bias, all funnel plots we could analyze were asymmetric and the results of Egger's test was $p < 0.05$ for each, except one – the adjusted analysis of association between KRAS mutation and OS across various PDAC stages ($p = 0.2$) (Figs. S24–s32).

4. Discussion

The dismal prognosis of PC has led to extensive research aiming to optimize disease management. The most widely used biomarker for PDAC monitoring is CA19-9, which although associated with prognosis, is not tumor-specific, and may be undetectable in Lewis antigen-negative patients (Tempero et al., 2017). Cell-free DNA based tests have recently been approved as a companion diagnostics in various malignancies (Anon., 2021). The emerging data about cfDNA biomarkers applicability in PDAC show great potential, yet there is no consensus on their use to guide disease management (Tempero et al., 2017; Zhu et al., 2020). We performed a systematic review and meta-analysis which revealed that cfDNA biomarkers indicate a decreased overall and progression free survival in PDAC both in resectable and unresectable stages, and when detected during treatment.

Chen L. et al. published in 2018 a meta-analysis on the same topic, comprising 18 studies and 1,243 patients with overall similar results (Chen et al., 2018a). Nevertheless, we overcame some of the limitations they reported regarding the paucity and retrospective nature of data. Since then, the publications on this subject have doubled in number, prospective studies were published, and we could perform additional clinically relevant subgroup analyses.

Lack of standardization for cfDNA biomarkers' assessment is one major limitation for their use in clinical practice (Qi et al., 2018). We therefore focused on two categories of cfDNA biomarkers — KRAS mutations and ctDNA as detected by assessing cfDNA alterations different than solely KRAS mutations. Detection of ctDNA was proved to indicate a poor prognosis across various malignancies (Takai et al., 2015; Hegyi et al., 2020, 2021). Our results in PDAC showed an association with shorter survival, earlier relapse in resectable cases and earlier progression in the unresectable ones. The correlation of ctDNA with tumor burden in PDAC has been reported in the literature (Bachet et al., 2020) and our results indicate a tendency for more aggressive tumor behavior also when detected in early stages. The concentration of ctDNA in the plasma/serum is variably associated with prognosis in PDAC, although it showed an increasing trend in metastatic cases (Sausen et al., 2015; Lapin et al., 2018; Adamo et al., 2017). The status of ctDNA is also positively correlated with tumor grade (Bachet et al., 2020) and therefore could complement the rationale for deciding therapeutic approach.

KRAS mutations are the most common genetic alteration in PDAC, occurring in around 90 % of cases (Lapin et al., 2018; Toledano-Fonseca et al., 2020). Nevertheless, their detection in cfDNA varies widely from 20.5 % up to 93.7 % of cases (Guo et al., 2020; Chen et al., 2018a), with higher frequencies being reported in more advanced stages (Earl et al., 2015; Chen et al., 2018a). Our findings revealed an association with poorer prognosis when detected across various disease stages. In the subgroup analysis for the unresectable cases, the association did not stand significant. We could not assess in meta-analysis the association of KRAS mutations and prognosis in resectable PDAC stages. In a cohort of 59 cases that underwent curative intent surgery, detection cfDNA KRAS mutations both at baseline, and during follow-up indicated increased risk for recurrence and progression respectively (Groot et al., 2019). Assessment of KRAS mutations in cfDNA was proved superior to CA19-9 as a disease monitoring tool in PDAC (Hussung et al., 2021; Sefrioui et al., 2017). Our results signal potential limitations of this approach in the unresectable cases, albeit based on limited amount of data which do not allow drawing strong conclusions. Nevertheless, assessment of ctDNA by performing targeted sequencing on a gene panel customed for pancreatic cancer, rather than a single gene, could bring more clinically relevant information especially if targetable alterations are included. Studies comparing the two approaches could further clarify the superior reliability of either.

The subgroup analysis on resectable cases comprised three studies (Groot et al., 2019; Guo et al., 2020; Zhu et al., 2020). It revealed a 3.5 times higher risk for relapse if cfDNA biomarkers were detectable before surgery. Moreover, one of the studies showed that patients undergoing neoadjuvant treatment were more likely to have undetectable cfDNA biomarkers preoperatively (Groot et al., 2019). Cell-free DNA biomarkers could therefore be a tool for selection of resectable PDAC cases eligible for neoadjuvant chemotherapy as this indication is still under debate (Tempero et al., 2017; Silvestris et al., 2020). Nevertheless, only prospective trials reporting on cfDNA–based decisions can confirm their utility in this clinical scenario (Groot et al., 2019; Lee et al., 2019).

According to our results, detection of cfDNA biomarkers during treatment indicates decreased overall and progression-free survival. In the study of Lee B. et al., relapse occurred in all PDAC patients with detectable postoperative ctDNA and in only 45 % of those with undetectable post-operative ctDNA (Lee et al., 2019). The patients received gemcitabine-based chemotherapy, the samples were taken 4–8 weeks after resection, and the median follow-up time was 38.4 months (Lee et al., 2019). Also, in a group of unresectable cases treated with FOLFIRINOX—Folinic Acid, Fluorouracil, Irinotecan, and Oxaliplatin, undetectable cfDNA biomarkers during chemotherapy were associated with partial response or stable disease (Wei et al., 2019). In PDAC management CA19-9 is measured at baseline and throughout disease course as it correlates with risk of relapse after surgery, risk of progression during chemotherapy and overall survival in all disease stages (Tempero et al., 2017). Still, when compared with cfDNA biomarkers, changes in tumor markers levels are less pronounced and have a higher latency to indicate radiological response (Kruger et al., 2018). In one study, cell-free DNA biomarkers could signal disease relapse 6.5 months before detection by imaging, while CT and CA19-9 proved only moderate accuracy for detecting disease recurrence (Qi et al., 2018; Elbanna et al., 2020; Groot et al., 2019).

4.1. Implication for practice and research

Regarding implications for practice, closer monitoring is justified for patients with detectable cfDNA biomarkers at baseline, as they predict a poor prognosis. For resectable cases, they might indicate benefit from neoadjuvant therapy, though biomarker-guided studies are needed for confirmation. Simultaneously, detection during follow-up should lead to assessment of treatment response by the currently approved methods. Further research is necessary for the standardization of detection methods and establishing threshold values for different cfDNA biomarkers. Also, prospective trials on cfDNA-based therapeutic decisions are needed to confirm their clinical applicability. Lastly, despite a decreasing trend of costs in the last decade for such tests, they are still expensive and require access to advanced technologies. Development of strategies to increase their accessibility and cost-effectiveness analyses will bring them closer to current use.

4.2. Strengths and limitations

The strengths of our meta-analysis are: 1) the rigorous methodology for data synthesis and reporting, 2) the comprehensiveness of the study – comprising more than double the amount of articles included in a previous publication on the same topic (Chen et al., 2018b), and 3) performing subgroup analyses for clinically relevant scenarios like the use of cfDNA biomarkers in resectable disease stages and for longitudinal monitoring.

However, limitations must be pointed out: 1) the high heterogeneity of our results that is derived from the variability of methods and design across the included studies. The definitions for detectable cfDNA biomarkers and the molecular techniques used for their assessment were different. Subgroup analysis by detection method did not change our results. Furthermore, although only in a few cases, it resolved heterogeneity, which further adds to the relevance of our findings. The disease stage, treatment type and follow-up period of the enrolled patients were diverse which can also account for heterogeneity; 2) some of the studies had moderate-high risk of bias; 3) significant publication bias was detected. However this could also be due to artifacts in the Egger's test and Funnel plots results that have limitations in case of prognostic meta-analyses.

5. Conclusion

Cell-free DNA biomarkers are significant predictors of survival in PDAC and their positivity indicates the need for closer patient monitoring. They reveal more aggressive tumor behavior, and possible disease progression if detected during follow-up. In resectable cases they could help the decision on giving neoadjuvant therapy. Lack of standard methods for detection and inaccessibility prevent them from yet entering clinical routine.

Data statement

The data that support the findings of this study are available from the corresponding author, [PH], upon reasonable request.

Ethical approval

No ethical approval was required for this review, as all data were already published in peer-reviewed journals. No patients were involved in the design, conduction, or interpretation of our review. The datasets used in this study can be found in the full-text articles used in the systematic review and meta-analysis.

Data availability

No data was used for the research described in the article.

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Declaration of Competing Interest

No conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.critrevonc.2021.103548.

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