REVIEW ARTICLE

# Exosomes as prognostic biomarkers in pancreatic ductal adenocarcinoma—a systematic review and meta-analysis

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**Extensive research is focused on the role of liquid biopsy in pancreatic cancer since reliable diagnostic and follow-up biomarkers represent an unmet need for this highly lethal malignancy. We performed a systematic review and meta-analysis on the prognostic value of exosomal biomarkers in pancreatic ductal adenocarcinoma (PDAC). MEDLINE, Embase, Scopus, Web of Science, and CENTRAL were systematically searched on the 18th of January, 2021 for studies reporting on the differences in overall (OS) and progression-free survival (PFS) in PDAC patients with positive vs negative exosomal biomarkers isolated from blood. The random-effects model estimated pooled multivariate-adjusted (AHR) and univariate hazard ratios (UHRs) with 95% confidence intervals (CIs). Eleven studies comprising 634 patients were eligible for meta-analysis. Detection of positive exosomal biomarkers indicated increased risk of mortality (UHR = 2.81, CI:1.31−6,00, $I^2$ = 88.7%, $P < 0.001$), and progression (UHR = 3.33, CI: 2.33−4.77, $I^2$ = 0, $P$ = 0.879) across various disease stages. Positive exosomal biomarkers identified preoperatively revealed a higher risk of mortality in resectable stages (UHR = 5.55, CI: 3.24−9.49, $I^2$ = 0, $P$ = 0.898). The risk of mortality in unresectable stages was not significantly increased with positive exosomal biomarkers (UHR = 2.51, CI: 0.55−11.43, $I^2$ = 90.3%, $P < 0.001$). Detectable exosomal micro ribonucleic acids were associated with a decreased OS (UHR = 4.08, CI: 2.16−7.69, $I^2$ = 46.9%, $P$ = 0.152) across various stages. Our results reflect the potential of exosomal biomarkers for prognosis evaluation in PDAC. The associated heterogeneity reflects the variability of study methods and need for their uniformization before transition to clinical use. (Translational Research 2022; 244:126−136)**

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**Abbreviations:** 95%CI = 95% confidence interval; AHR = multivariate-adjusted hazard ratios; CA19-9 = carbohydrate antigen 19-9; CT = contrast-enhanced computer tomography; CTCs = circulating tumor cells; ctDNA = circulating tumor DNA; ddPCR = droplet digital polymerase chain reaction; DFS = disease−free survival; DNA = deoxyribonucleic acid; ECOG = Eastern Cooperative Oncology Group; ELISA = enzyme−linked immuno−sorbent assay; EpCAM = epithelial cells adhesion molecule; ExoDNA KRASmut = KRAS mutations of exosomal DNA; ExmiRs = Exosomal micro ribonucleic acids; exoEpCAM = exosomal epithelial cell adhesion molecule; EpCAM+ExmiR = EpCAM positive exosomal micro RNA; Exo cric−PDE8A = circular ribonucleic acid phosphodiesterase; exoCXCR4 = exosomal chemokine receptor 4; exoCD = exosomal cluster of differentiation; %+exo bmk = percentage of patients with positive exosomal biomarkers; -exo bmk = negative exosomal biomarkers; ExlncRNA-UCA1 = exosomal long non-coding RNA urothelial carcinoma-associated 1; EV = extracellular vesicle; FACS = flow cytometry analysis; FF-nPES = far-field nanoplasmon-enhanced scattering; GPC-1 = glypican-1; GPC1+crExo = concentration of glypican−1 positive circulating exosome; HRs = hazard ratios; IG−TEM = Immunogold Transmission Electron Microscopy; lnc−Sox2ot = long non-coding RNA SOX2 overlapping transcript; MAF = mutation allele frequency; miRNAs = micro ribonucleic acids; MRM−MS = multiple reaction monitoring mass spectrometry; NTA = nanoparticle tracking analysis; OS = overall survival; P = population; C = comparison group; E = exposure group; O = outcome; PB = peripheral blood; PC = pancreatic cancer; PDAC = pancreatic ductal adenocarcinoma; PD = L1 Programmed death-ligand 1; PE34:1 = phosphatidylethanolamine; PFS = progression-free survival; PVB = portal venous blood; PRISMA = Preferred Reporting Items for Systematic Reviews and Meta-Analyses; PROSPERO = International Prospective Register of Systematic Reviews; Pre = treatment before treatment; Post = treatment after treatment; SEC = size exclusion chromatography; sEV−EZR = small extracellular vesical Ezrin; qRT−PCR = quantitative real−time polymerase chain reaction; QUIPS = Quality in Prognosis Studies tool; RT−qRT−PCR = real−time quantitative reverse−transcription polymerase chain reaction; SEM = scanning electron microscopy; TNM = tumor node metastasis; UC = ultracentrifugation; UHRs = univariate hazard ratios; UICC = Union for International Cancer Control

## INTRODUCTION

By 2040, the incidence of pancreatic cancer (PC) is expected to almost double in Asia and Africa, to rise by 30% in Europe and up to 50% in North America.[1] Lack of specific symptoms in the early disease phases and of screening methods lead to detection in unresectable stages in more than 80% of cases with a 5-year overall survival (OS) rate of less than 5%.[2,3]

A substantial research effort is directed towards the development of early diagnostic strategies and optimization of disease management. The methods currently approved for PDAC monitoring are the serum level of carbohydrate antigen 19-9 (CA19-9) and contrast-enhanced computer tomography (CT).[4] Despite being accessible, they lack specificity and signal disease progression with delay.[4−6] More reliable biomarkers for assessing treatment response are necessary to allow its timely adjustment.

Liquid biopsy is increasingly used in clinical oncology. The minimally invasive sampling methods enable real-time disease monitoring. Exosomes are nanosized (30−150 nm), physiologically released extracellular vesicles of endosomal origin. They can act on target cells either distally, traveling through different body fluids or by paracrine and autocrine mechanisms and activate specific signaling pathways.[7,8] For this purpose, they carry mainly nucleic acids, lipids, and proteins protected from degradation in the extracellular environment by a lipid bilayer.[7,9] In PC, exosomes are involved in processes like the epithelial−to−mesenchymal transition, cell proliferation, angiogenesis, premetastatic niche formation, hence favoring tumor development and spread.[9,10] Although the isolation of exosomes is laborious, they can accurately reflect the tumoral heterogeneity by the variety of their molecular contents and the stability in the extracellular space.[11,12]

The available data on the clinical applications of exosomes in PDAC derives from small observational cohorts. To our knowledge, this is the first systematic review and meta-analysis that evaluates the association between exosomal biomarkers and survival outcomes in PDAC.

## MATERIALS AND METHODS

We followed the Cochrane recommendations for study methodology and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 Statement for reporting our results (details in Supplementary Table1).[13,14] The protocol for our study has been previously submitted to the International prospective register of systematic reviews (CRD42021237390) and implemented without deviations.

The systematic search was performed without filters until the 18th of January, 2021 in five medical databases − MEDLINE (via PubMed), Embase, Web of Science, Cochrane Central Register of Controlled

Trials (CENTRAL), and Scopus using the search key detailed in Annex 1a. Furthermore, we manually screened the reference lists of the included studies for additional eligible articles.

We included all the studies that met the following eligibility criteria: population (P)—adult patients (above 18 years of age) diagnosed with pancreatic adenocarcinoma; Exposure (E) —positive exosomal biomarkers as defined in each study; comparison group (C) —patients with negative exosomal biomarkers. The assessed outcomes (O) were overall (OS) and progression-free survival (PFS). For inclusion in the quantitative synthesis, the prognostic ability of the biomarkers should be either analyzed by Cox regression yielding hazard ratios (HRs) and 95% confidence intervals (CI), or raw data that allows the calculation of HRs should be reported. Case reports and case series were excluded from our review. We selected only studies analyzing exosomal biomarkers isolated from blood.

The selection was performed with the reference management program EndNote X9 (Clarivate Analytics, Philadelphia, PA, USA). After automatic and manual duplicate removal, two independent investigators manually selected the articles in a stepwise manner by title and abstract and full−text contents, adhering to the predefined eligibility criteria. The Cohen's kappa coefficient was calculated at each selection step to quantify the agreement between assessors.[15] Disagreements were settled by third-party arbitration. In case of overlapping populations, the studies with a higher number of participants were selected.

The data in each article was extracted manually by two independent researchers. To ensure quality, the two investigators crosschecked each other's data pool after extraction. Disagreements were solved by consensus. The information was summarized in a standardized data collection form (details in Annex 1b).

If multiple biomarkers were analyzed, those with a higher positivity rate were considered more representative and were selected for meta-analytical calculations. One biostatistician performed the statistical analyses using the Stata 15.1 software (Stata Corp LLC, College Station, TX, USA) and Comprehensive Meta-Analysis (version 3, Biostat Inc., Englewood, NJ, USA). The pooled unadjusted and adjusted HRs (UHRs and AHR) with 95% confidence intervals (CIs) as yielded by the random-effects model (DerSimonian−Laird estimation) revealed the differences in OS and PFS between patients with positive and negative exosomal biomarkers, respectively.[16] Statistical heterogeneity was assessed by the $I^2$ and $Q^2$ statistics (< 30% − low, 30%−60% − moderate, 50%−90% − substantial and 75%−100% − considerable degree of heterogeneity).[14] A $P$-value < 0.1 indicated $Q^2$ results statistical significance.[14] We performed subgroup analysis for the associations of positive exosomal micro ribonucleic acids (ExmiRs) and OS irrespective of disease stage and positive exosomal biomarkers and OS for the resectable and unresectable cases respectively to explore causes of heterogeneity. Disease-free survival, as reported by some studies was counted as PFS.[17,18] Also, metastatic cases were categorized as unresectable.[4]

The Quality in Prognosis Studies (QUIPS) tool was applied by two independent investigators to assess the methodological quality for each of the included studies (detailed in Annex2).[19] The disagreements were solved by consensus.

To assess publication bias by visual inspection of funnel plots and Egger's test minimum 10 studies should be available for the evaluated outcome.[14]

No ethical approval was required for this review. All included studies recruited patients that provided informed consent before enrolment.

## RESULTS

**Study selection.** The results of the search and selection processes are summarized in Fig 1. Cohen's kappa indices for the title and abstract and full-text selection were 0.98, and 0.88 respectively. Our search key identified 2224 records. Of the 18 articles eligible for qualitative synthesis,[3,11,17,18,20−33] 11 were suitable for quantitative synthesis.[3,11,17,18,20−26] The 904 patients included in our review comprise only non-overlapping populations. No additional articles were found by screening the reference lists of the included papers. The conference abstracts we identified as eligible—Bittoni et al. and Kim et al. were excluded, as the results were also reported in the studies of Giampieri and Bernard, respectively.[3,22,34,35] All included articles are available in full-text and were published in peer-reviewed journals.

**Study characteristics.** Table 1 summarizes the main characteristics of the included studies. Positive exosomal biomarkers were variably defined. The molecular techniques used for detection varied according to the biomarker type. The exosomal biomarkers were isolated mainly from peripheral blood, with one exception where portal venous blood (PVB) was also sampled.[11]

**The association between detectable exosomal biomarkers and survival in PDAC.** All pooled hazard ratios are collected in Supplementary Table 2. A higher risk for mortality (UHR = 2.81, CI:1.31−6,00, $I^2$ = 88.7, $P$< 0.001 Fig 2(A); AHR = 2.45, CI:1.28−4.68, $I^2$ = 81.7, $P$< 0.001, supplementary Fig 1, Fig s1) and progression (UHR = 3.33, CI:2.33−4.77,

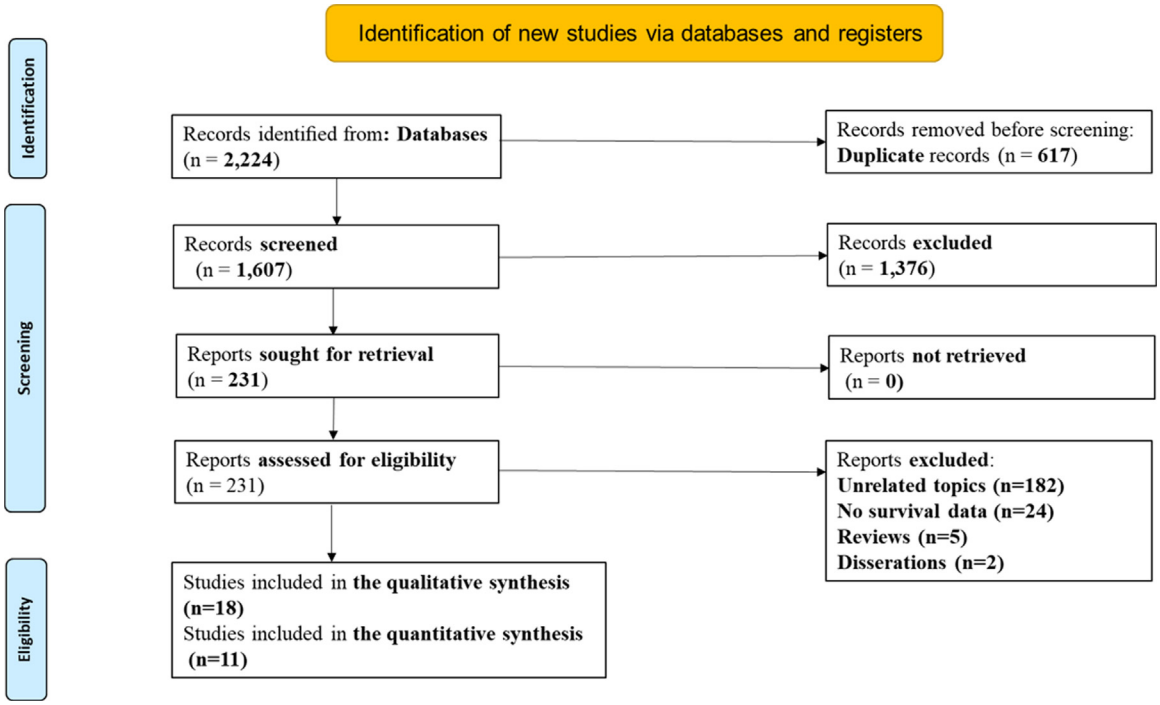



**Fig 1.** PRISMA 2020 flow-chart. (Color version of figure is available online.)

$I^2$ = 0, *P*= 0.93, Fig s2(B); AHR = 2.58, CI:1.63−4.07, $I^2$ = 0, *P*= 0.88, Fig s2(A)) were revealed in PDAC patients with positive exosomal biomarkers. Samples were taken at baseline, before initiation of treatment, in the studies reporting on PFS. Some of the studies reporting on OS did not specify the sampling time. Analyses included all disease stages.

**The association between detectable exosomal micro RNAs and survival in PDAC.** The univariate and multivariate subgroup analyses of cases with positive ExmiRs showed an increased risk for mortality (UHR = 4.08, CI:2.16−7.69, $I^2$ = 46.9, *P*= 0.15, Fig 3, AHR = 2.39, CI:1.64−3.50, $I^2$ = 0, *P*= 0.6, Fig s3). These reference biomarkers were: ExmiR−451−a, ExmiR−200−b within exosomes positive for epithelial cells adhesion molecule (EpCAM), ExmiR−222, and ExmiR−21.[11,18,21,23,25] Information on sampling time was not fully available. Analyses included all disease stages.

**The association between detectable exosomal biomarkers with survival according to resectability status.** Positive exosomal biomarkers were not associated with a significantly increased risk for mortality according to the subgroup analysis for unresectable PDAC (UHR:2.51, CI:0.55−11.43, $I^2$ = 90.3, *P*< 0.001, Fig 2(C)). The concentration of exosomes in the plasma, EpCAM within serum exosomes, and KRAS mutations of the exosomal DNA were the reference biomarkers.[3,17,22] On the other hand, the risk for mortality was significantly higher in the resectable cases with positive exosomal biomarkers (UHR:5.55, CI:3.24−9.49, $I^2$ = 0, *P*= 0.89 Fig 2 (B)). Samplig was performed before surgery in this subgroup and the reference biomarkers were ExmiR45−a and exosomal phosphatidylethanolamine (PE34:1).[11,18,26]

Data extracted from the studies ineligible for quantitative synthesis are summarized in Table 2. They revealed a poorer prognosis for patients with a resectable disease stage and increased level of exosomal epithelial adhesion molecule (EpCAM) or glypican-1 (GPC-1) positive exosomes.[27,28] Also, detection of exosomal long non-coding RNA SOX2 overlapping transcript (lnc−Sox2ot), c-Met positive exosomes, increased levels of exosomal circular RNA phosphodiesterase (Exo cric−PDE8A), exosomal long non-coding RNA urothelial carcinoma-associated1 (ExlncRNA-UCA1), and ExmiR-301a-3p were associated with increased mortality risk across various PDAC stages.[29−33]

**Reporting biases.** The results for risk of bias assessment are detailed in Supplementary Figs 4 and 5. The overall risk of bias for both OS and PFS was low for statistical analysis reporting, study confounding, study participation, prognostic factor measurement, and moderate for study attrition. The risk of bias for outcome

**Table 1.** Characteristics of the included studies

| Study | Country | Sample size (% female) | Disease stage | Marker | Detection method[b] | Sample origin | Time of sample collection | Outcomes |
|---|---|---|---|---|---|---|---|---|
| Allenson, et al. (2017)[17] | Multicentric | 39 (46.2) | All stages | exoDNA KRASmut exo concentration | NTA ddPCR | plasma | Pre−treatment | OS, DFS |
| Bernard, et al. (2019)[22] | USA | 104 (42.3) | Metastatic | exoDNA KRASmut | UC, ddPCR | plasma | Pre−treatment | OS, PFS |
| Chang, et al. (2020)[20] | Taiwan | 165 (57.6) | All stages | sEV−EZR | SEC, NTA, immunoblot | plasma | — | OS |
| Giampieri, et al. (2019)[3] | Italy | 18 (5.2) | Unresectable[c] | exoEpCAM;exoCXCR4; exoCD9;exoCD81 | UC,ELISA | serum | Pre/Post−treatment | OS, PFS |
| Goto, et al. (2018)[23] | Japan | 32(46.9) | All stages | ExmiR−21;ExmiR−451a; ExmiR−191 | Exo isolation &quantification kits qRT−PCR | serum | Pre−treatment | OS |
| Kawamura, et al. (2019)[11] | Japan | 55 (40) | I−II UICC | ExmirR−451a; ExmiR−4525; ExmiR−21 | UC, TEM, qRT−PCR | PVB PB | Pre−treatment | OS, PFS |
| Li, Yanfang, et al. (2018)[32] | China | 56 (23.1) | All stages | Exo cric−PDE8A | qRT−PCR | plasma | — | OS |
| Li, Jiang, et al. (2018)[33] | China | 56 (76.8) | All stages | Exo lnc−Sox2ot;Exo lnc−AK296146 | UC, exo extraction kit, qRT−PCR | plasma | — | OS |
| Li, Tao, et al. (2018)[21] | China | 73 (80.8) | All stages | ExmiR−222 | Exo isolation &quantification kits, TEM, qRT−PCR | plasma | — | OS |
| Melo, et al. (2015)[24] | USA | 20 (50) | All stages | GPC1+crExo | IG−TEM FACS ELISA | serum | Pre/Post−treatment | OS |
| Reese, et al. (2020)[25] | Germany | 56 (36) | II−IV UICC | ExmiR−200b; ExmirR−200c;EpCAM +ExmiR200b;EpCAM +ExmiR200c | UC, Western blot, RT−qRT−PCR | serum | Pre−treatment | OS |
| Takahasi, et al. (2018)[18] | Japan | 50 (44) | I−II UICC | ExmiR−451a | UC, TEM, qRT−PCR | plasma | Pre−treatment | OS, DFS |
| Tao, et al (2019)[26] | China | 22 (50) | I−II AJCC 7th edition | Targeted lipidomics | SEM, Western blot, exo precipitation kit, MRM−MS | plasma | Pre−treatment | OS |
| Amrollahi, et al, (2019)[27] | USA | 21 (38.1) | Resectable[a] | exoEpCAM | FF-nPES | plasma | — | OS |
| Buscail, E. et al, (2019)[28] | France | 22 (9) | Resectable[a] | exoGPC1 | Exo isolation kit, Western blot | serum PVB | Pre-treatment | OS, PFS |
| Guo, Z., et al. (2020)[29] | China | 46 (41.3) | All stages | ExlncRNA-UCA1 | Exo isolation kit, NTA,Western blot, qRT-PCR | serum | — | OS |
| Lux, A., et al. (2019)[30] | Germany | 29 (—) | All stages | Exo c-Met, exo PD-L1 | Exo isolation kit, flow cytometry | serum | Pre-treatment | OS |
| Wang, X., et al. (2018)[31] | China | 50 (42) | All stages | ExmiR-301a-3p | Exo isolation kit, NTA, qRT−PCR | serum | — | OS |

Abbreviations: UICC−Union fir International Cancer Control, exo−exosomes, exoDNA KRASmut−KRAS mutations of exosomal DNA, sEV−EZR−small extracellular vesical Ezrin, exoEpCAM−exosomal epithelial cell adhesion molecule, exoCXCR4−exosomal chemokine receptor 4, exoCD exosomal cluster of differentiation, ExmiR−exosomal micro ribonucleic acid, Exo cric−PDE8− exosomal circular ribonucleic acid phosphodiesterase 8A, Exo lnc−Sox2ot−exosomal long non-coding RNA SRY−box transcription factor 2 (SOX2) overlapping transcript, ExlncRNA-UCA1−exosomal long non-coding RNA urothelial carcinoma associated-1, GPC1+crExo−concentration of glypican−1 positive circulating exosome; EpCAM+ExmiR−EpCAM positive exosomal micro RNA, NTA−nanoparticle tracking analysis, ddPCR−droplet digital polymerase chain reaction, ELISA−enzyme−linked immuno−sorbent assay, qRT−PCR−quantitative real−time polymerase chain reaction, IG−TEM−Immunogold Transmission Electron Microscopy, FACS− flow cytometry analysis, RT−qRT−PCR − real−time quantitative reverse−transcription polymerase chain reaction, MRM−MS−multiple reaction monitoring mass spectrometry, PVB−portal venous blood, PB−peripheral blood, Pre−treatment−before treatment, Post−treatment−after treatment; OS−overall survival, PFS−progression−free survival; DFS−disease−free survival; UC−ultracentrifugation; SEM−scanning electron microscopy; FF−nPES−far-field nanoplasmon-enhanced scattering (FF-nPES); NTA−nanoparticle tracking analysis; PD−L1−Programmed death-ligand 1;SEC−size exclusion chromatography.

[a]as defined in each article.

[b]detailed in Annex3.

[c]locally advanced, metastatic; — not available.

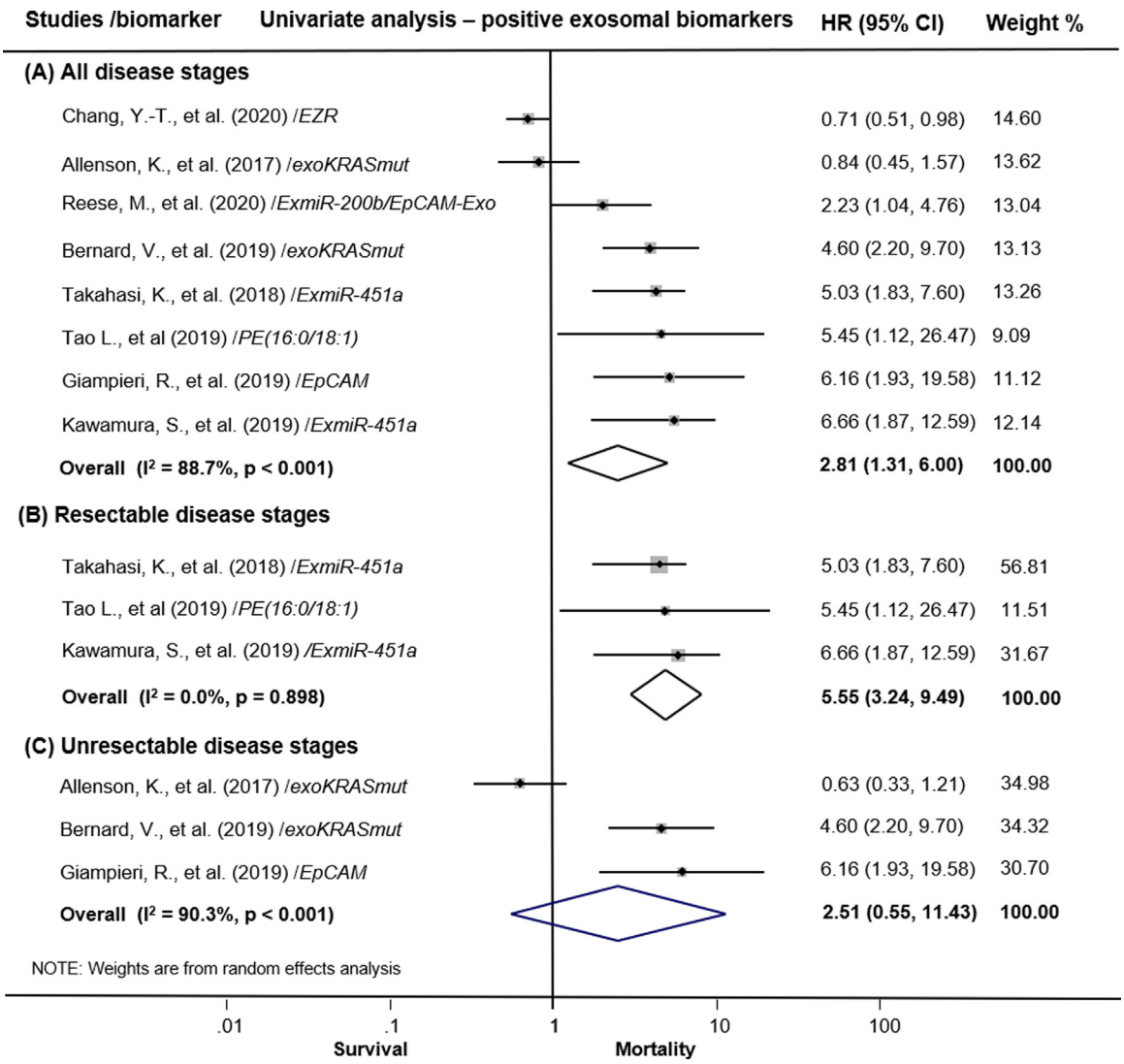



**Fig 2.** Pooled univariate analysis of association between positive exosomal biomarkers and overall survival in pancreatic adenocarcinoma: (A) all disease stages; (B) resectable disease stages; (C) unresectable disease stages.

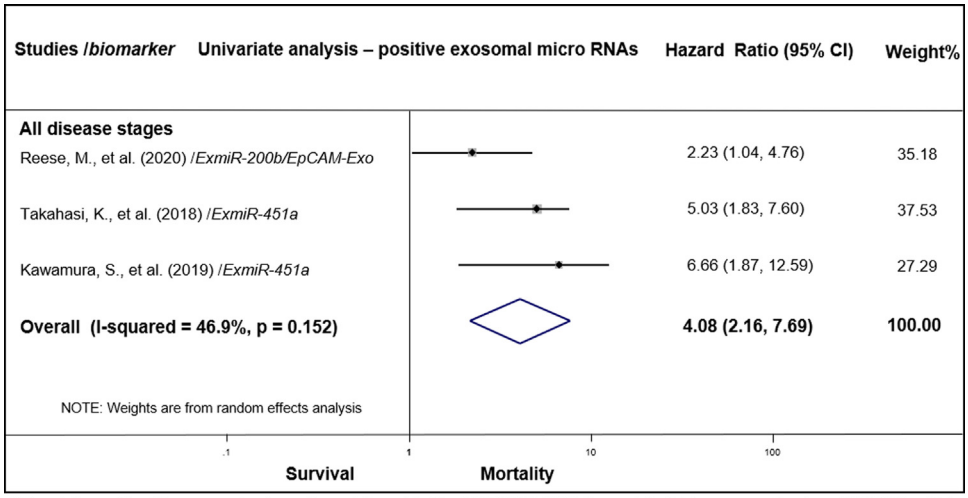
measurement was moderate for OS and low for PFS. The overall risk of bias was low-moderate for OS and low for PFS.

The low number of available publications precluded publication bias assessment.

## DISCUSSION

We performed a systematic review and meta-analysis on the prognostic role of exosomal biomarkers in PDAC. Patients with positive exosomal biomarkers had decreased OS and PFS. Detection of positive exosomal biomarkers before surgery in resectable cases revealed an increased risk for progression. Nevertheless, we did not detect a significant association between exosomal biomarkers and mortality in unresectable disease stages. All researched biomarkers were involved in tumor development and invasion processes.

Production of exosomes is increased in the malignant cells since the initial phases of tumorigenesis.[23,36,37] Amongst circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA), exosomes performed best as diagnostic biomarkers in PDAC according to a meta-analysis published in 2020 with a sensitivity of 93% and a specificity of 92%.[38] Besides the tumor itself, they also carry information about the tumor microenvironment, which determines its behavior and, therefore, its prognosis.[11]




Abbreviations: ExmiR–exosomal micro ribonucleic acid, ExmiR-200b/EpCAM-Exo –detectable exosomal micro RNA200b within exosomes positive for epithelial cell adhesion molecule, HR –hazard ratio, CI –confidence interval;

**Fig 3.** Pooled univariate analysis of association between detectable exosomal micro RNAs and overall survival in pancreatic adenocarcinoma−all stages.

**Table 2.** Summary results of the studies ineligible for quantitative synthesis

| Author | Sample size (% + exo bmk) | Disease stage | OS (months) | | p-value | PFS (months) | | p-value |
|---|---|---|---|---|---|---|---|---|
| | | | *+ exo bmk* | *-exo bmk* | | *+ exo bmk* | *-exo bmk* | |
| Amrollahi, P et al. 2019[27] | 21 (52.38) | Resectable | 12.17 | 17.3 | 0.03 | — | — | — |
| Buscail, E. et al. 2019[28] | 22 (50.00) | Resectable | 5.8 | 16.43 | 0.04 | 3.4 | 8 | 0.01 |
| Guo, Z. et al. 2020[29] | 46 (50.00) | All stages | — | — | 0.008 | — | — | — |
| Lux, A et al. 2019[30] | 29 (34.48) | All stages | 9.47 | 21.67 | <0.001 | — | — | — |
| Wang, X. et al. 2018[31] | 50 (60.00) | All stages | — | — | 0.01 | — | — | — |
| Li, Z., Yanfang W., et al (2018)[32] | 56 (50.00) | All stages | — | — | 0.01 | — | — | — |
| Li, Z., Peng, J., et al (2018)[33] | 56 (49.02) | All stages | — | — | 0.02 | — | — | — |

Abbreviations: %+exo bmk –percentage of patients with positive exosomal biomarkers; - exo bmk –negative exosomal biomarkers, OS – overall survival, PFS –progression-free survival, — not available; as defined in each article.

Positive exosomal biomarkers indicated an increased risk for progression across all disease stages in our analysis. Some of the studies revealed an association with the T (tumor) and N (node) stages suggesting a correlation with tumor burden.[11,18] Still, in the cohort of Giampieri et al., there was no association with the presence of metastases.[3]

According to our results, exosomal biomarkers detected before surgery in resectable cases are associated with decreased overall survival. In their research, Kawamura et al. compared the exosomal biomarkers isolated from the peripheral blood with those isolated from the PVB sampled right before resection.[11] They hypothesize that PVB exosomes characterize more accurately the pancreatic microenvironment as they had higher sensitivity and specificity than the peripheral blood exosomes to indicate disease recurrence.[11] Although scarce, the available evidence shows a trend towards a more rapid tumor spread in PDAC patients with detectable exosomal biomarkers in the blood. If positive, they might indicate a need for treatment adjustment, therefore they should prompt a closer disease follow-up.

Our analysis did not demonstrate an increased risk for mortality in the unresectable cases with positive exosomal biomarkers. However, only 3 studies were available to test this hypothesis, and the associated heterogeneity was above 90%. One of the studies revealed baseline exosomal KRAS mutant allele frequency (MAF) $\geq$5% as the only predictor of PFS in a multivariate analysis of 104 metastatic cases.[22] Moreover, in the same cohort, the detection of an exosomal KRAS

MAF ≥ 1% on serial sampling during chemotherapy anticipated progression and was proposed as an indicator of treatment resistance.[22] It preceded the increase of serum CA19−9 level and concurrent radiological progression with a median of 50 days.[22] In a cohort of 22 locally advanced and metastatic PDAC cases, positive exosomal biomarkers correlated with a lower treatment response rate, poorer performance status, and decreased overall survival.[3] Similarly, in another cohort of 41 patients with advanced progressive solid malignancies, time to treatment failure was significantly shorter if plasma exosomal biomarkers were detected.[22,39] Even if the evidence is yet limited, exosomal biomarkers might better stratify the unresectable PDAC cases in which systemic therapy will be beneficial. Prospective trials on exosomal biomarkers-based therapeutic decisions are necessary to confirm this premise.

Micro RNAs (miRNAs) were identified as essential modulators of multiple pathways of carcinogenesis and as indicators of chemotherapy resistance in PDAC.[25,40,41] Our analysis indicated a decreased OS for patients with various PDAC stages and positive ExmiRs. Regarding PFS, one of the studies reported unchanged risk for recurrence in cases resected with curative intent and positive ExmiRs detected preoperatively.[25] Still, in the cohort of Goto et al. comprising 22 patients with all PDAC stages, detection of ExmiRs at baseline was associated with a lack of chemotherapy response.[23] Micro RNAs were proved to be involved in therapy resistance in other malignancies like colorectal or non-small lung cancer.[9,25] Nevertheless, the efficacy of ExmiRs as predictive biomarkers can only be confirmed by prospective controlled trials.

The highly desmoplastic nature of PDAC might raise concerns regarding the performance of exosomes isolated from blood as tools for guiding disease management, still their concentration is higher in PC patients than in healthy controls.[17]

**Implications for research and clinical practice.** Available data suggest that intensification of disease monitoring in PDAC patients with positive exosomal biomarkers is appropriate. Nevertheless, the complex nature of exosomes is what precludes them from yet entering clinical routine. In 2018, the International Society of Extracellular Vesicles (ISEV) published a position paper on the "minimal information for the study of extracellular vesicles (EVs)" providing recommendations for their isolation and characterization according to their purpose.[12] The heterogeneity of our results most likely reflects the methodological and populational differences across the included studies. Since heterogeneity can bring to question the relevance of our results, we should discuss it in detail. Most frequently, the methods for separation and concentration of exosomes used in the included studies were −ultracentrifugation, size exclusion chromatography, precipitation kits − alone or in combination. There are differences between them regarding recovery, specificity, runtime or costs. The ISEV recommends the methods should be described to the extent the experiment is reproducible and to use highly purified EVs when they are attributed biomarkers.[12] Also, combined methods may be more efficient.[12] For the characterization of exosomes −nanoparticle tracking analysis, scanning electron microscopy, transmission electron microscopy and flow cytometry analysis were among the used techniques. Exosomes isolation and characterization is time-consuming and requires sophisticated and expensive devices. The development of isolation kits to surmount this shortcoming will increase their accessibility. Immune purification to concentrate tumor-specific exosomes could increase sensitivity and specificity of the analysis.[42] The common ground across the eligible studies was, besides analysis of biomarkers associated with tumor development and aggressiveness, as previously mentioned, a low risk of bias regarding prognostic factor measurement -with clearly described methods for sampling and isolation of biomarkers. This allowed us to summarize in meta-analysis some of the available data on the prognostic role of exosomal biomarkers in PDAC and emphasize their potential as a liquid biopsy tool for clinical practice. Concerning study population −there were dissimilarities in disease stages, treatment types, and follow-up periods; therefore, studies on more homogenous populations will generate more clinically relevant data.

Isolation of exosomes from pancreatic juice (PJ) was proved feasible and the exosomal biomarkers could distinguish between PDAC and premalignant lesions or benign pancreatic diseases with an accuracy of up to 91%.[43−45] Although the alterations harbored by the PJ exosomes are more specific for the PDAC and tumor microenvironment, pancreatic fluid seems more suitable in diagnosis settings rather than for diseases follow-up, since sampling is invasive and more costly.[43]

**Strengths and limitations.** The strengths of our meta-analysis are: (1) to our knowledge, being the first one on the topic, (2) the rigorous methodology, and (3) performing subgroup analyses for clinically relevant scenarios like resectable vs unresectable disease stages. Still, several limitations must be pointed out: (1) a limited number of studies available for meta-analytical calculations; (2) the low number of available articles, insufficient to perform publication bias assessment; (3) the statistical heterogeneity present in some of the analyses and (4) the moderate-high risk of bias for some of the selected studies.

## CONCLUSION

As our data suggest, the detection of exosomal biomarkers in the blood of PDAC patients is associated with an increased risk for mortality, disease recurrence, or chemotherapy resistance. Although vigilant monitoring of such cases seems justified, standardization across circulating exosome-based studies and prospective trials on exosome-based decisions are still necessary before developing clear recommendations on their use for guiding PDAC management.

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Conflicts of interest: All authors have read the journal's policy on disclosure of potential conflicts of interest. There are no conflicts of interest to declare.

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### PRIOR PRESENTATIONS

The results in this manuscript have not been previously presented in any form before submission.

### DATA AVAILABILITY STATEMENT

The data underlying this article will be shared on reasonable request to the corresponding author.

### ETHICS APPROVAL

Not applicable.

### CONSENT TO PARTICIPATE

Not applicable.

## SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.trsl.2022.01.001.

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