World Journal of Surgical Oncology

REVIEW

Open Access

Systematic review and meta-analysis: diagnostic accuracy of exosomes in pancreatic cancer

Xinyi Xu[1†], Chunyue Long[2†], Meng Li[1†], Chen Shen[1], Qiuwen Ye[3], Yong Li[3], Hongyang Li[3], Xia Cao[1*] and Jun Ma[3*]

Abstract

Background Early, non-invasive identification can generally enhance the survival rate for asymptomatic pancreatic cancer (PC). This systematic review and meta-analysis is conducted to evaluate the precision of diagnosing PC using serum and duodenal fluid exosomes.

Methods Following the guidelines of PRISMA (Preferred Reporting Items for Systematic Review and Meta-Analyses), searches were conducted in the PubMed, Embase, Cochrane Library, and Web of Science databases in April 2024. A study was considered appropriate if it provided diagnostic precision and accuracy for patients with pancreatic cancer. The combined diagnostic impact was assessed by calculating the area beneath the aggregated SROC curve, and the quality of the studies included was evaluated using the QUADAS-2 checklist. All statistical evaluations and graphical representations utilized STATA 14.0.

Results Employing the terms "exosomes" and "pancreatic cancer" along with the search methodology, research was conducted across PubMed, Web of Science, Cochrane, and Embase databases. A total of 1202 studies were extracted from the databases, out of which nine were ultimately selected based on specific inclusion and exclusion standards. Across eight studies, exosomes were isolated from serum, while in a different one, they were taken from duodenal fluid. This document conducts subgroup analyses focusing on various types of exosome biomarkers, their origins, isolation techniques, and methods for analyzing biomarkers. Within the subset of exosome biomarker types, the group with exosomal cell surface proteoglycan exhibited the greatest combined sensitivity (0.96 (95% CI = 0.81–0.99) and specificity (0.90 (95% CI = 0.83–0.95)). Additionally, the set of exosomal cell surface proteoglycans showed the highest aggregated diagnostic ratio (215.92), combined positive likelihood ratio (9.96), area under the curve (0.93), and kombiniertes negative Likelihood-Ratio (0.05). The combined sensitivity of serum-derived exosomes stood at (0.86 (95% CI = 0.77–0.92)), the collective specificity at (0.83 (95% CI = 0.77–0.89)), the aggregate positive likelihood ratio at (5.22), the combined diagnostic ratio at (31.48), the overall area beneath the curve at (0.91), and the combined negative likelihood ratio at (0.17). Within the subgroup examination of exosome isolation techniques, ultracentrifugation emerged as the most sensitive method (0.90 (95% CI = 0.74–0.97)), the most

†Xinyi Xu, Chunyue Long and Meng Li contributed equally to this work.

*Correspondence:
Xia Cao
caoxia@kmmu.edu.cn
Jun Ma
mddiou@163.com
Full list of author information is available at the end of the article

specific method (0.89 (95% CI = 0.83–0.93)), the top positive likelihood ratio (8.35), the highest diagnostic ratio (76.48), the largest combined curve area (0.92), and the smallest negative likelihood ratio (0.11) in the aggregated data. Within the subset of biomarker analysis methods, the aggregate sensitivity via qRT-PCR was (0.84 (95% CI = 0.74–0.90)), the collective specificity (0.78 (95% CI = 0.64–0.87)), the aggregate diagnostic ratio (18.11), the aggregate area under the curve (0.88), the aggregate positive likelihood ratio (3.77), and the combined negative likelihood ratio (0.21).

Conclusion Overall, exosomes are still valuable in the diagnosis of pancreatic cancer. In subgroup analyses, the proteoglycan found on exosomal cell surfaces is highly valuable for diagnosing pancreatic cancer. The more frequent separation method used in the nine included studies was ultracentrifugation, and it did demonstrate good data. Nonetheless, to verify their practicality and usefulness in clinical environments, a significant amount of clinical trials are still necessary.

Keywords Pancreatic cancer, Exosomes, Diagnostic significance, Biomarkers, Meta-analysis

Introduction

PC, a deadly type of cancer, frequently presents with symptoms such as jaundice, abdominal discomfort, weight reduction, steatorrhea, and the aggravation of existing or new diabetes. Around 90% of pancreatic cancers fall under the category of pancreatic ductal adenocarcinomas (PDAC) [1], placing PDAC as the world's seventh most common cancer cause and fourth in the West, following lung, colorectal, and breast cancers. Worldwide cancer data for 2020 revealed close to 496,000 fresh instances of PDAC and over 466,000 related fatalities, with the death rate for PDAC almost coinciding with its occurrence [2]. Factors contributing to the risk of pancreatic cancer include smoking, diabetes mellitus, body mass index, alcohol dependence, pancreatitis, microbial, environmental, and occupational origins, familial cancer history [3], and stature [4].

Preliminary assessments for suspected PC patients involve serological examinations and abdominal scans [5], and the urgent requirement for early pancreatic cancer detection is clear, yet hindered by the absence of distinct symptoms and efficient screening techniques. The sole serum biomarker sanctioned by the US Food and Drug Administration (FDA), Carbohydrate antigen 19 − 9 (CA19-9) [6], due to its low sensitivity (0.80, 95% CI = 0.72–0.86) and specificity (0.75, 95% CI = 0.68–0.80), it is unable to meet the requirements of clinical diagnosis, which highlights the urgency of adopting new methods.

Lately, the focus on liquid biopsies has intensified due to their minimally invasive nature and their capacity for ongoing tracking of cancer development. Presently, liquid biopsies may target circulating tumor cells (CTC), circulating tumor DNA (ctDNA), noncoding RNA (ncRNA), messenger RNA (mRNA), and extracellular vesicles (EV), offering insights into tumor genomics, transcriptomics, and proteomics [7]. All cells generate exosomes, which are extracellular vesicles that transport nucleic acids, proteins, lipids, and metabolites. Under typical physiological or pathological conditions, they serve as intermediaries in cell-to-cell communication and influence different facets of cellular biology [8]. Growing research indicates the vital involvement of tumor-derived exosomes (TEX) in the development of cancer. Exosomes and their contents may serve as indicators of cancer outlook, targets for treatment, or even transporters of anti-cancer medications [9]. Consequently, employing exosomes as an indicator for diagnosing pancreatic cancer shows potential.

As far as we are aware, there hasn't been a publication of a systematic review on exosomal biomarkers used in diagnosing pancreatic cancer in the past three years. To assess the effectiveness of exosomal biomarkers (such as surface proteins, miRNAs, and circRNAs) in diagnosing pancreatic cancer, this study embarked on an in-depth review and analysis of crucial research in relevant diagnostic areas.

Methods

Our team formulated a methodical assessment protocol, grounded in the PRISMA standards and the Diagnostic Test Accuracy Assessment Program [10]. Before being published, the systematic assessment was recorded on PROSPERO under the identifier CRD42024552283 and the link is https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42024552283.

Strategy for search

By April 30, 2024, an extensive search plan was formulated to guarantee the discovery of all pertinent literature pertinent to the research's goals. Employing these keywords, our extensive search encompassed all databases: Utilizing text terms and subject headings (Mesh or equivalent terms), we executed detailed, methodical searches, subsequently incorporating them into these

Table 1 Detailed reporting of inclusion/exclusion criteria

Criteria for Inclusion	Study group (P): individuals identified with pancreatic cancer
	Intervention (I): assessment of exosome biomarkers' diagnostic precision for pancreatic cancer
	Control (C): individuals in good health without pancreatic cancer
	Outcome indicators (O): total count of true positives (TP), false positives (FP), false negatives (FN), and true negatives (TN) as shown in a 2×2 league table or deducible through study computations
	Study category (S): observational research focusing on the precision of exosome biomarkers in diagnosing pancreatic cancer(More credibility; avoidance of confounding factors; applicability and generalisability)
Criteria for Exclusion	Studies involving animals(Clinical applicability issues)
	Insufficient data for creating a 2×2 league table(Insufficient data for analysis)
	Case studies, meta-analyses, reviews, or commentaries(Case studies: sample sizes too small; meta-analyses, reviews, commentaries: duplication of inclusion, lack of independence, can affect accuracy, potential for bias)
	Review articles lacking peer review, book chapters, and frequent citations(Review articles lacking peer review: cannot be quality assured, lacks credibility; book chapters: uncertainty in study design and methodology, data may be incomplete, lack of peer review; frequently citations: duplication of inclusion, lack of independence, affects accuracy, possible bias)

online databases with Boolean operators: PubMed, Web of Science, Cochrane, and Embase. Utilizing data from the Emtree and Mesh databases, we conducted a search in these databases for "exosomes" and "pancreatic cancer," identifying all pertinent keywords. A pair of investigators, Xu and Li, independently explored various literature sources. Through an extensive review of existing literature on the topic, we guaranteed that all relevant articles were considered. Furthermore, to ensure the inclusion of all pertinent literature on the diagnostic precision of exosomes for early pancreatic cancer detection, we manually reviewed the references in each paper and systematically examined the online database contents. Table S1 offers an extensive methodology for searching the PubMed, Web of Science, Cochrane, and Embase databases.

Criteria for inclusion/exclusion

A summary of the past three years' research on the diagnostic significance of exosomes in pancreatic cancer patients is provided, encompassing studies on exosomes extracted from bodily fluids (such as serum and duodenal fluid) and those exploring various exosome types.

Criteria for Inclusion: Study group (P): individuals identified with pancreatic cancer; Intervention (I): assessment of exosome biomarkers' diagnostic precision for pancreatic cancer; Control (C): individuals in good health without pancreatic cancer OR patients with chronic pancreatitis; Outcome indicators (O): total count of true positives (TP), false positives (FP), false negatives (FN), and true negatives (TN) as shown in a 2×2 league table or deducible through study computations; Study category (S): observational research focusing on the precision of exosome biomarkers in diagnosing pancreatic cancer. For detailed information on inclusion and exclusion criteria, see Table 1.

Criteria for Exclusion: Studies involving animals; Insufficient data for creating a 2×2 league table; Case studies, meta-analyses, reviews, or commentaries; Review articles lacking peer review, book chapters, and frequent citations.

Extracting data

Our meta-analysis will incorporate these details from each research: lead author, publication year, country of origin, count of researchers involved, origins of exosomes, techniques for isolating and purifying exosomes, identified relevant biomarkers, procedures for extracting exosome biomarkers, methods for analyzing biomarkers, and various types of false positives (FP), false negatives (FN), true positives (TP), and true negatives (TN). The FP, FN, TP, and TN data, derived from 2×2 tables, were calculated based on sensitivity and specificity, except in cases where these were excluded from the study. Should additional details be needed, the authors of the study were reached out to.

Evaluation of quality

A pair of investigators (Xu and Li) separately assessed the methodological soundness of the studies in question, focusing on bias risk and relevance, utilizing the revised QUADAS-2 tool, recommended by the Cochrane Collaboration [11]. To assess bias risk, questions across four areas were evaluated: (1) choosing cases (considering potential bias in patient selection), (2) assessing trials (the bias in the execution or interpretation of the trials), (3) establishing a gold standard (the bias in the implementation or interpretation of the trials), and (4)

Table 2 Bibliographic information included primary studies

	Title	First author	Year	County	Ref.
1	CD63-positive extracellular vesicles are potential diagnostic biomarkers of pancreatic ductal adenocarcinoma.	Haruki Odaka	2022	Japan	[12]
2	Glypican1 identifies cancer exosomes and facilitates early detection of cancer.	Sonia A. Melo	2022	USA	[13]
3	Highly Sensitive Exosome Detection for Early Diagnosis of Pancreatic Cancer Using Immunoassay Based on Hierarchical Surface-Enhanced Raman Scattering Substrate	Juan Li	2022	China	[14]
4	Exosomal circular RNA hsa_circ_0006220, and hsa_circ_0001666 as biomarkers in the diagnosis of pancreatic cancer	Lu Hong	2022	China	[15]
5	Exosomal glypican-1 discriminates pancreatic ductal adenocarcinoma from chronic pancreatitis.	P.Moutinho-Ribeiro	2022	Portugal	[16]
6	Serum exosomal miR-451a acts as a candidate marker for pancreatic cancer	Jia Chen	2022	China	[17]
7	Circulating cancer-associated extracellular vesicles as early detection and recurrence biomarkers for pancreatic cancer	Yusuke Yoshioka	2022	Japan	[18]
8	Dual Tumor Exosome Biomarker Corecognitions Based Nanoliquid Biopsy for the Accurate Early Diagnosis of Pancreatic Cancer	Zhiguo Yu	2023	China	[19]
9	MicroRNA-20a in extracellular vesicles derived from duodenal fluid is a possible biomarker for pancreatic ductal adenocarcinoma.	Takashi Taniguchi	2024	Japan	[20]

Table 3 The workflow data from the included studies

No.	First author	Exosomes Source	Type	Exosome Isolation	Biomarker Extraction	Biomarker Analysis
1	Haruki Odaka	Serum	Platelet-derived EVs	MagCaptureTM Exosome Isolation Kit	/	ELISA
2	Sonia A. Melo	Serum	Cell surface proteoglycan	Ultracentrifugation	/	Western Blots
3	Juan Li	Serum	Cell surface proteoglycan	Ultracentrifugation	/	H-SERS substrate, magnetic beads @ exosomes @ SERS detection probes (MEDP)
4	Lu Hong	Serum	Exosomal circular RNA	Total Exosome Isolation Kit	TRIzol LS	qRT-PCR
5	P. Moutinho-Ribeiro	Serum	Cell surface proteoglycan	Ultracentrifugation	ELISA	flow cytometry
6	Jia Chen	Serum	exomiR	exoEasy Maxi Kit	TRIzol	qRT-PCR
7	Yusuke Yoshioka	Serum	G protein-coupled receptor, epidermal growth factor	Ultracentrifugation	M-PER Mammalian Protein Extraction Reagent, Thermo Scientific	Western Blots
8	Zhiguo Yu	Serum	Cell surface proteoglycan	Ultracentrifugation	/	flow cytometry, ELISA
9	Takashi Taniguchi	DF	exomiR	Ultracentrifugation	miRNeasy Mini Kit	qRT-PCR

examining case progression and timing (the bias in processes like patient inclusion and exclusion, the time gap between trials, and other procedures). Furthermore, in evaluating issues of clinical appropriateness, the initial three domains will be mentioned. The risk levels for each region were categorized as low, high, or indeterminate, with the two researchers collaborating to clarify these classifications.

Analysis of statistics

For every analysis conducted, the statistical software STATA 14.0, developed by Stata Corporation in University City, TX, USA, was employed. The computation of sensitivity, specificity, and both positive and negative likelihood ratios, along with diagnostic superiority ratios, was performed using TP, FP, FN, and TN data, derived from meta-analysis outcomes of diagnostic accuracy data

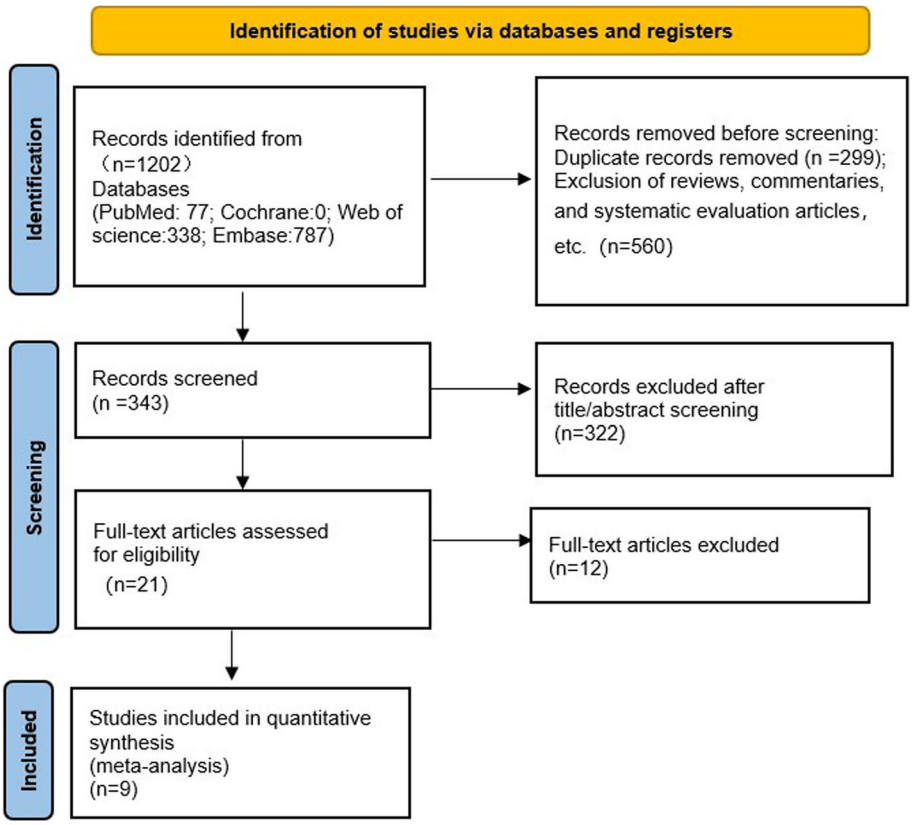

Fig. 1 Conducting a literature review and choosing studies systematically as per PRISMA standards

via the 'midas' command. Utilizing the "midas" feature of the random-effects model, estimations were made for combined sensitivity, specificity, SROC curves, combined positive and negative likelihood ratios, and diagnostic advantage ratios, varying with biomarker types (like platelet-derived EVs, cell surface proteoglycan, Exocet), surface proteoglycan, exosomal circular RNA, exomiR, exosome source, exosome isolation method, and biomarker analysis approach. Results are presented using a forest plot that illustrates the respective 95% confidence intervals. Spearman's rho was employed in our assessment of the threshold impacts. To assess the diversity among studies, Cochran's Q-test and Zhou & Dendukuri's I^2 statistic were employed.

Analysis of subgroups

Analyses of subgroups for Sen, Spe, PLR, NLR, SROC, and DOR were conducted based on the type of exosome

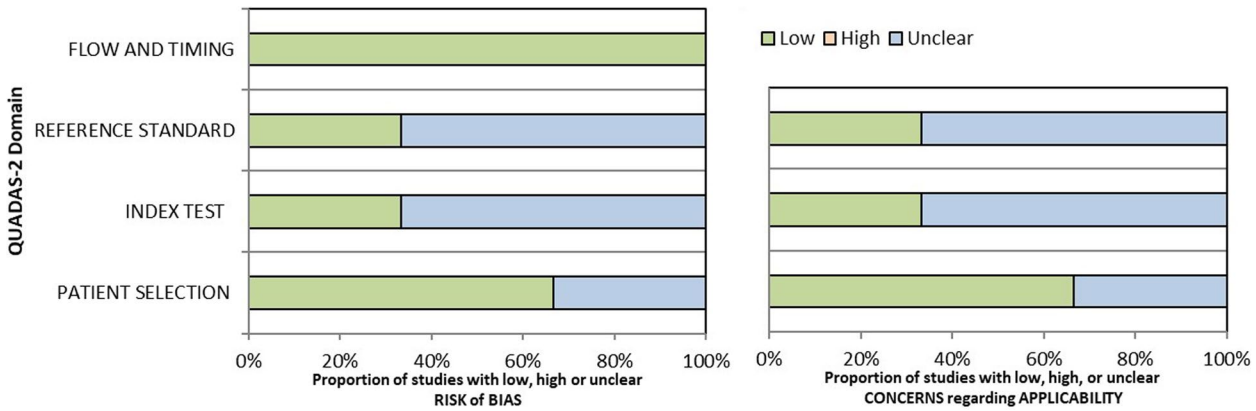

Fig. 2 Evaluation of the studies' quality in the meta-analysis was conducted through the QUADAS-2 checklist

Table 4 Diagnostic accuracy data from nine studies of exosomal biomarkers

First author	Biomarker name	Type	Sen (%)	Spe (%)	PLR	NLR	DOR
Haruki Odaka	$CD63^+$-EVs	Platelet-derived EVs	0.821	0.846	5.33	0.21	25.20
	$CD41^+$-EVs	Platelet-derived EVs	0.718	0.744	2.80	0.38	7.40
	$CD61^+$-EVs	Platelet-derived EVs	0.718	0.641	2.00	0.44	4.55
	$CD63^+$-EVs & CA19-9	Platelet-derived EVs	0.897	0.872	7.01	0.12	59.33
Sonia A. Melo	$GPC1^+$ crExos	Cell surface proteoglycan	1.000	1.000	/	0.00	/
Juan Li	LRG1-Exos	Cell surface proteoglycan	0.743	0.933	11.09	0.28	40.48
	GPC1-Exos	Cell surface proteoglycan	0.800	0.933	11.94	0.21	26.14
	LRG1-Exos & GPC1-Exos	Cell surface proteoglycan	0.914	0.867	6.87	0.10	69.46
Lu Hong	hsa_circ_0006220	Exosomal circular RNA	0.774	0.726	2.82	0.31	9.08
	hsa_circ_0001666	Exosomal circular RNA	0.968	0.516	2.00	0.06	31.95
	hsa_circ_0006220 & hsa_circ_0001666	Exosomal circular RNA	0.742	0.871	5.75	0.30	19.42
P. Moutinho-Ribeiro	$GPC1^+$crExos	Cell surface proteoglycan	0.983	0.862	7.12	0.02	361.19
Jia Chen	miR-451a	exomiR	0.801	0.867	6.01	0.23	26.17
Yusuke Yoshioka	$GPRC5C^+$EV	G protein-coupled receptor	0.519	0.889	4.68	0.54	8.64
	$EPS8^+$EV	Epidermal growth factor	0.667	0.833	3.99	0.40	9.99
Zhiguo Yu	$GPC1^+$EV	Cell surface proteoglycan	1.000	0.909	10.99	0.00	/
Takashi Taniguchi	MicroRNA-20a	exomiR	0.820	0.860	5.86	0.21	27.98

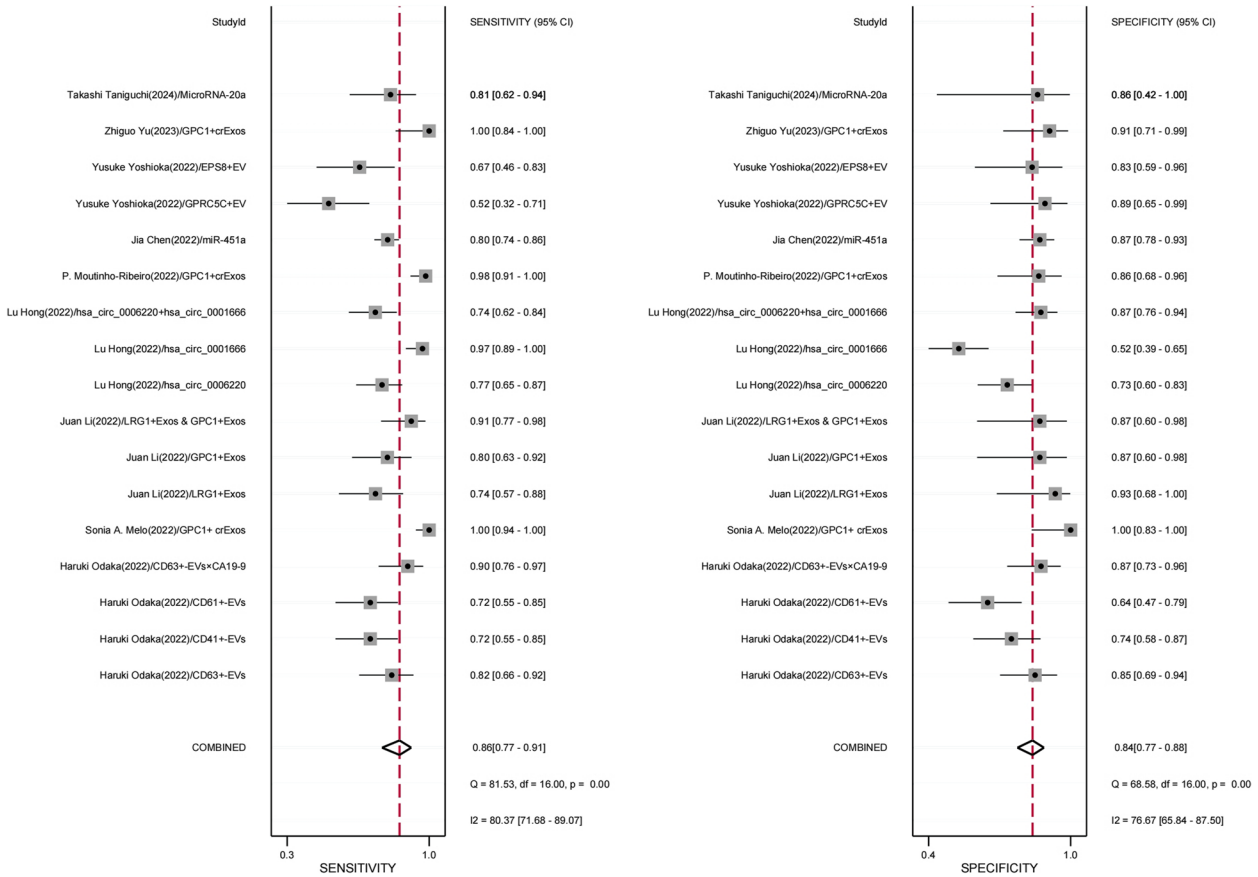

Fig. 3 Forest plot showing the pooled sensitivity and specificity of exosomes for distinguishing pancreatic cancer patients from controls

(serum), method of isolation (either commercial kit or ultra-rapid), type of biomarker (such as cell surface proteoglycan or RNA), and the method of biomarker analysis (qRT-PCR).

Bias in publication

The Deeks funnel plot and the asymmetry test were employed to examine publication bias. P values below 0.1 were deemed indicative of potential bias in publication.

Results

Results of the search

Utilizing an online database search method, 1202 articles were obtained, encompassing PubMed 77, Web of Science 338, Cochrane 0, and Embase 787. After pinpointing and eliminating 299 repeated publications, we kept 21 records, omitting 560 articles due to their document type and 322 based on their title and abstracts. After reviewing the complete texts of all 21 records, we discarded 12 articles, adhering to our set criteria for inclusion and exclusion. Ultimately, this comprehensive assessment and meta-analysis encompassed nine pertinent studies [12–20] from four countries—China, the US, Portugal, and Japan (see Tables 2 and 3). Figure 1 illustrates the methodology for the literature review and selection studies following PRISMA standards.

Evaluation of quality

The outcomes of our quality evaluation employing the QUADAS-2 checklist are depicted in Fig. 2 and Table S2. In the area of the reference standard, most studies were rated as unclear, which may be because the original studies were not detailed enough in describing the reference standard used and failed to fully explain the reasonableness and accuracy of the reference standard, which made it difficult for us to judge their risk of bias in this area. There was a little lack of clarity in this study regarding reference standards and indexing tests, which is one of the limitations of this study. Future studies should describe the selection of reference standards and the implementation process of indexing tests in more detail to reduce the risk of bias and improve applicability. Most of the studies in key areas such as flow and timing were rated as low risk, which indicates that our study was more reliable in

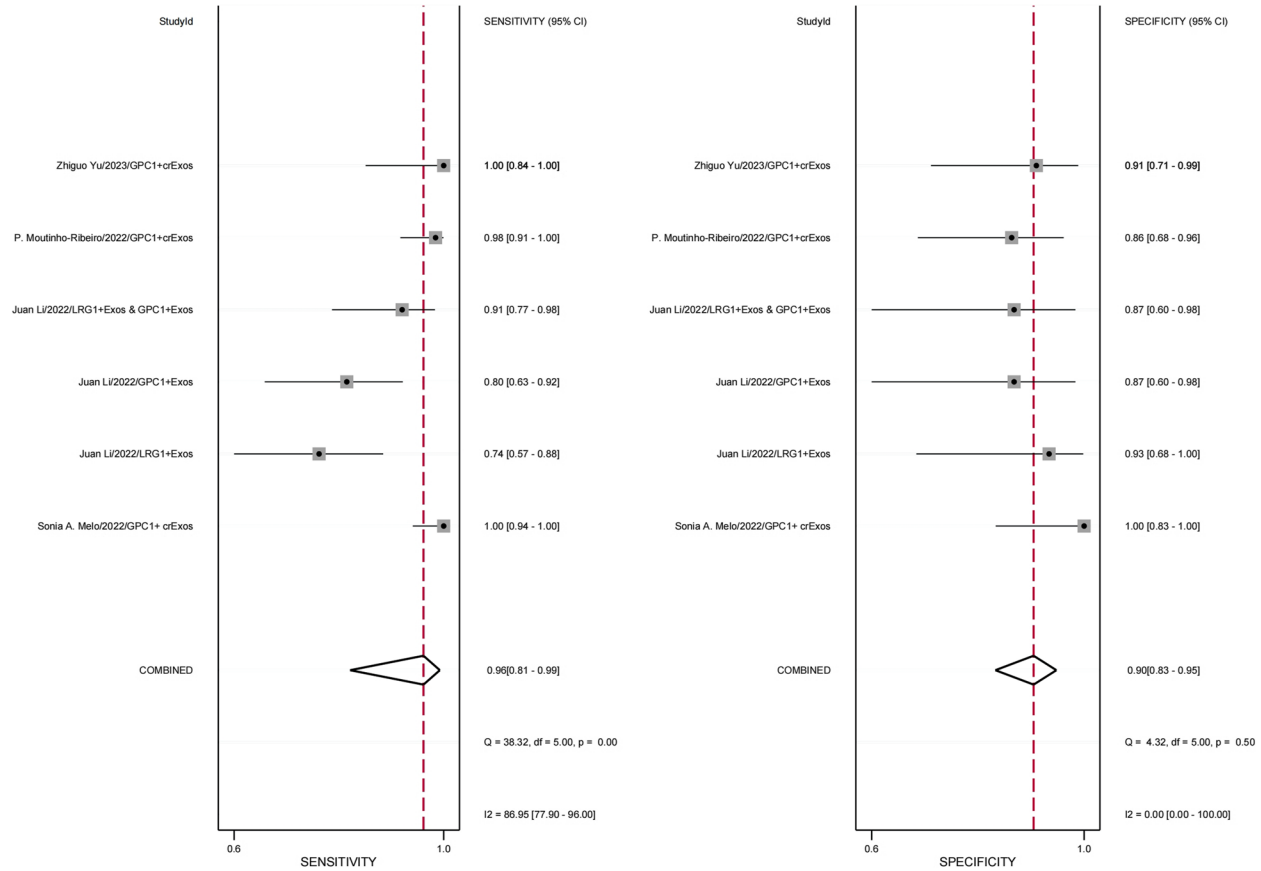

Fig. 4 Forest plot showing the pooled sensitivity and specificity of exosomal cell surface proteoglycan for distinguishing pancreatic cancer patients from controls

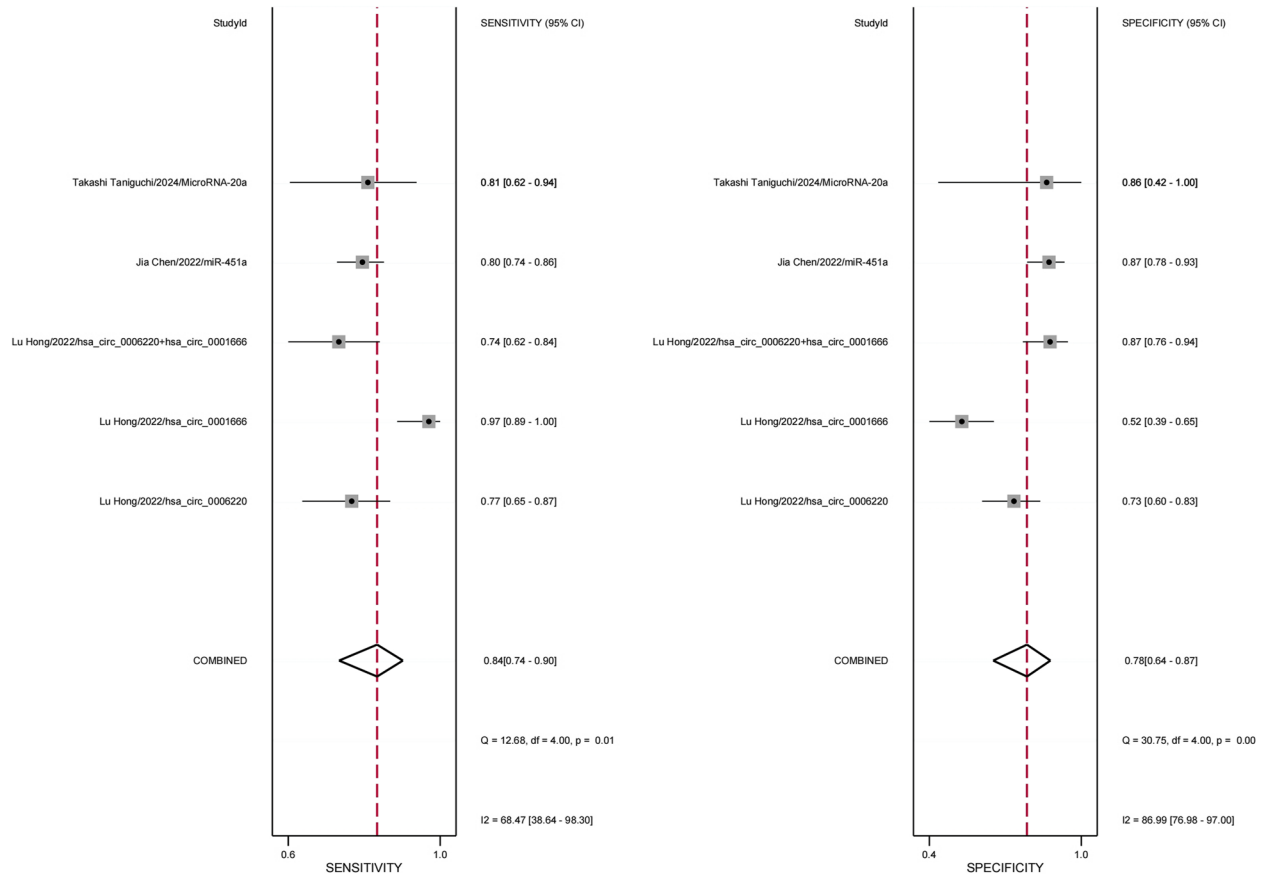

Fig. 5 Forest plot showing the pooled sensitivity and specificity of exosomal RNA for distinguishing pancreatic cancer patients from controls

terms of process and time control, which guarantees the accuracy of the study results. Generally, Sonia, Yu, and Takashi published three articles [13, 19, 20] that satisfied the criteria and evaluation criteria for each segment of the risk of bias and applicability assessment section.

Comprehensive review of diagnostic precision

Various biomarkers such as Sen, Spe, PLR, NLR, and DOR, and various indicators related to their diagnostic significance, are presented in Table 4 and S3. And we summarised the key results in Table S4.

Enhancing diagnostic precision, a comprehensive meta-analysis was conducted on 17 pieces of data from nine different studies, encompassing 820 individuals (518 with pancreatic cancer and 302 without), focusing on five types of biomarkers. This included six biomarkers for exosomal cell surface proteoglycan, five for exosomal RNA, three for platelet-derived EVs, G protein-coupled receptors, and biomarkers for epidermal growth factors.

Figure 3 illustrates the aggregate sensitivity and specificity, along with the respective 95% confidence intervals for all exosomes differentiating pancreatic cancer patients from healthy individuals, as well as the combined sensitivity and specificity and 95% confidence intervals for the exosomes' cell surface proteoglycans (Fig. 4), the combined sensitivity and specificity, and the respective 95% confidence intervals for the RNA of the exosomes and the exosomes. Graphical representation of the forest (circular exosome RNA, exomiR, Fig. 5). Combined sensitivity stood at 0.86 (95% CI = 0.77–0.91), while the overall specificity was 0.84 (95% CI = 0.77–0.88). The cell surface proteoglycan exosome group exhibited the greatest collective sensitivity (0.96 (95% CI = 0.81–0.99)), coinciding with their peak collective specificity (0.90 (95% CI = 0.83–0.95)). Figure 6 depicts the combined sensitivity and specificity of serum-derived exosomes. The combined sensitivity and specificity of various exosome isolation techniques (kit, ultracentrifugation) are depicted in Figs. 7 and 8, where ultracentrifugation leads in combined sensitivity (0.90 (95% CI = 0.74–0.97) and highest overall specificity (0.89 (95% CI = 0.83–0.93)). Figure 9 displays the aggregate sensitivity and specificity of biomarkers analyzed through qRT-PCR, revealing a combined sensitivity of 0.84 (95% CI = 0.74–0.90) and a specificity of 0.78 (95% CI = 0.64–0.87).

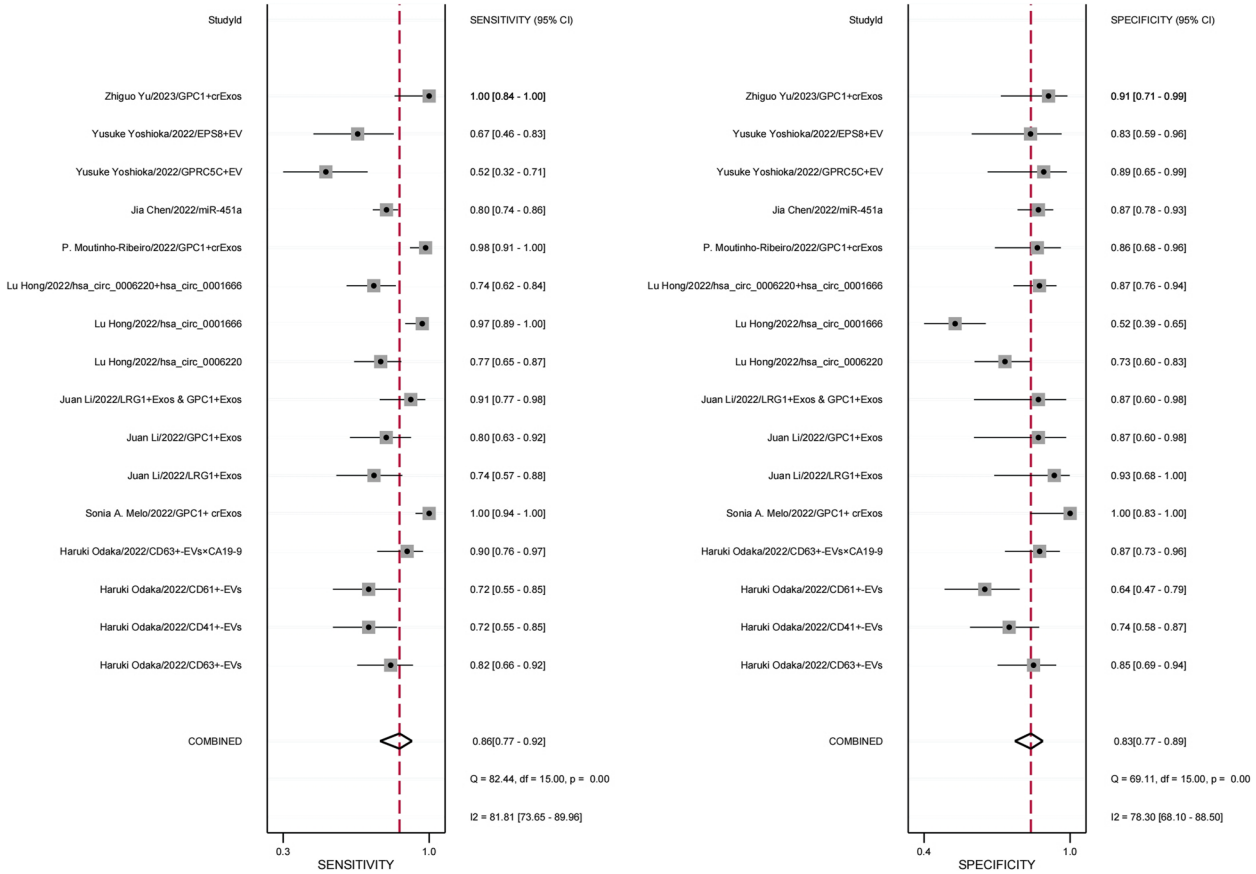

Fig. 6 Forest plot showing the pooled sensitivity and specificity of exosomes isolated from serum for distinguishing pancreatic cancer patients from controls

Figure 10 illustrates the aggregated Summary Receiver Operating Characteristic Curves (SROC) for assessing the precision of biomarker diagnoses. The SROC curves, showing an area under the curve (AUC) of 0.91 (95% CI = 0.88–0.93) for biomarker pooling, also concurred with the earlier stated results. Upon methodically omitting each research, the absence of comparative variances indicated a consistency in our results (Fig. 11).

The summarized SROC curves for various subgroups (exosome biomarker type, source, isolation method, and biomarker analysis method) are depicted in Figures S1-S4. The forest diagrams in Figures S5-S9 illustrate the combined positive likelihood ratios (PLR) and negative likelihood ratios (NLR) for the aggregate PLR and NLR, including various subgroups and their respective 95% confidence intervals. The pooled PLR stood at 5.20 (95% CI = 3.61–7.49) and NLR (0.17 (95% CI = 0.10–0.28)). Within these, Figures S10-S14 depict the aggregated Diagnostic Odds Ratio(DOR) for various subgroups, including exosomal biomarker type, source, isolation method, and biomarker analysis method. The combined DOR stood at (30.58 (95% CI = 14.62–63.97)).

Bias in publication

The Deeks funnel plot was employed to investigate potential biases in publication. Figure S15 in the meta-analysis reveals an absence of noticeable bias in the publication of biomarkers ($P > 0.1$).

Discussion

Consequently, there's a need for innovative, faster, and more accurate diagnostic techniques due to the ongoing difficulty in detecting cancer early. The stagnation in conventional cancer detection methods results in numerous cases being identified too late for effective therapy. Prompt and delicate detection is crucial for successful cancer therapy [21]. Pancreatic cancer, a malignant digestive system tumor, often leads to a bleak outlook, placing it third globally in cancer-related fatalities, with a mere 12% five-year survival rate [22], and causing around 227,000 deaths annually worldwide. Nearly every individual suffering from pancreatic cancer succumbs to metastasis, with a significant number exhibiting mutations in the K-ras oncogene and the deactivation of several tumor suppressor genes [23]. Despite the intricate and

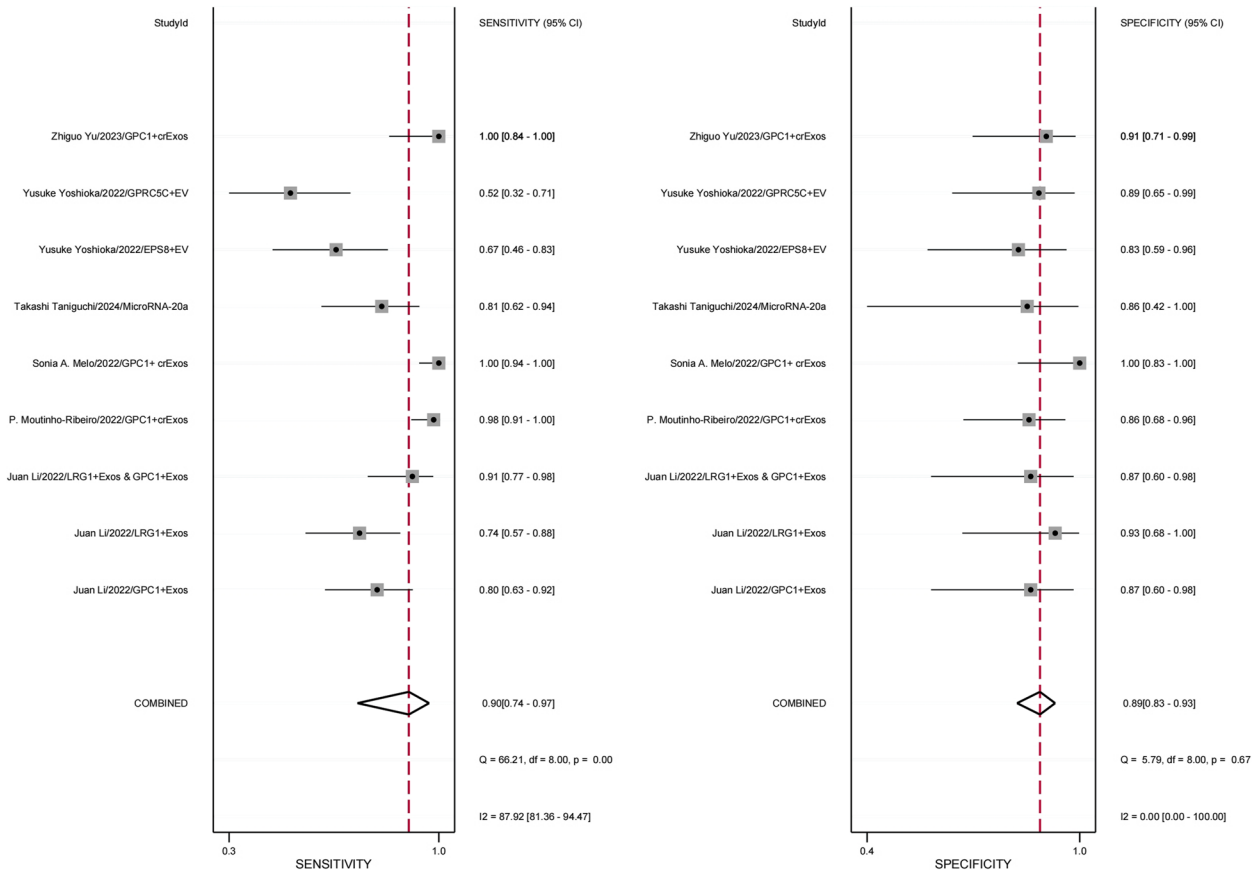

Fig. 7 Forest plot showing the pooled sensitivity and specificity of exosome isolation using ultracentrifugation for distinguishing pancreatic cancer patients from controls

varied causes of pancreatic cancer, factors like smoking and familial history play a significant role. Around 20% of pancreatic tumors result from smoking, with smokers having a higher mutation rate than nonsmokers [24]. The carbohydrate antigen 19 – 9 (CA19-9), also called sialyl Lewis a (sLe^a), stands as the most frequently utilized and presently employed benchmark biomarker for pancreatic cancer [25]. Additional biomarkers include circulating tumor cells (CTC), circulating tumor DNA (ctDNA), non-coding RNA (ncRNA), and extracellular vesicles (EVs) or exosomes [7].

An extensive review and meta-analysis of relevant studies from January 1, 2022, to April 30, 2024, were conducted to assess exosomes' effectiveness in diagnosing pancreatic cancer (PC). The main objective of this research was to explore the capabilities of exosomes as a non-intrusive diagnostic technique for PC. A thorough exploration of the latest databases over the past three years was undertaken to identify all exosome biomarkers associated with PC. Our review encompassed research evaluating the diagnostic value of exosomes, such as platelet-derived EVs, cell surface proteoglycans, exosomal cyclic RNA, and exomiR.

Initially, our evaluation encompassed nine studies' diagnostic tests that employed exosomal biomarkers. The main purpose of this meta-analysis is to determine the diagnostic value of exosomes in pancreatic cancer; therefore, we believe that we should choose an observational study without external interventions to ensure the objectivity of the data analysis and make the results more credible. Previous studies [26–28] indicate that the technique used for isolating and analyzing exosomes influences the outcomes of diagnostic assessments. Consequently, the research explored the progression from isolating exosomes to isolating and analyzing biomarkers. Lately, ultracentrifugation has become the favored technique for isolating exosomes [29]. The studies constituting this analysis utilized two distinct methods for isolating exosomes: ultracentrifugation and the use of commercial kits (Figs. 7 and 8). Indeed, each measurement derived from the ultracentrifugation technique in the data we examined exceeded those from standard commercial kits.

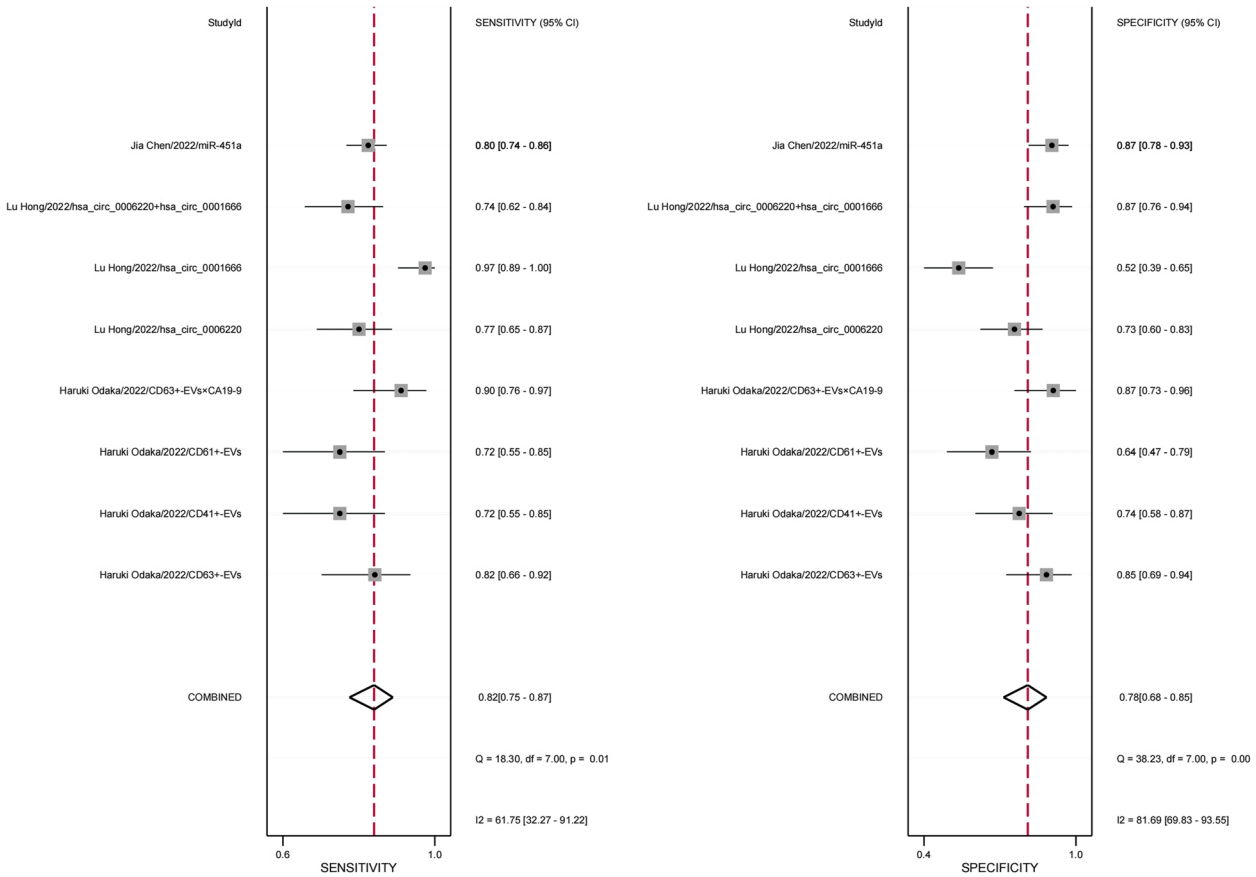

Fig. 8 Forest plot showing the pooled sensitivity and specificity of using the kit to isolate exosomes for differentiating pancreatic cancer patients from controls

Our team conducted a comprehensive assessment and meta-analysis to determine the diagnostic significance of exosomal biomarkers in PC. The findings indicated that the overall exosomes' sensitivity and specificity stood at 0.86 (95% CI = 0.77–0.91) and 0.84 (95% CI = 0.77–0.88), in that order. The AUC is broadly acknowledged as a robust measure for evaluating the total precision of diagnostic examinations, A meta-analysis of 2022 [30] which data from studies from 2009 to 2020 were pooled and analyzed, and the AUC values of CA19-9 were compared with the exosomal biomarkers evaluated in the corresponding studies, and the AUCs of the exosomal biomarkers in five of the studies were better than those of CA19-9, suggesting that exosomal biomarkers have a high diagnostic value for pancreatic cancer. A diagnostic efficiency is deemed high when the AUC exceeds 0.9 [31]. In the process of using exosomes to diagnose pancreatic cancer in this paper, the integrated area under the curve (AUC) of the detected PC reached 0.91, indicating a significant diagnostic effect.

It has been documented that Glypican-1 (GPC1) identifies cancer exosomes and holds diagnostic significance in the initial stages of pancreatic cancer. It has been demonstrated that the membrane-anchored protein GPC1 is excessively expressed in various cancers, notably in the breast and pancreas. In certain breast and pancreatic cancer cell lines, levels of GPC1 transcripts and proteins are higher than in non-cancerous cells [32]. The protein known as Leucine-rich α−2-glycoprotein-1 (LRG1) [33], belonging to the eight-repeat leucine-rich repeat sequence (LRR) protein family, has been shown in past research to play a role in tumor development by encouraging angiogenesis across multiple cancers such as pancreatic, lung, bladder, and colon cancer. The protein markers in these exosomes demonstrate notable sensitivity and specificity and have been employed in numerous research projects for the early detection of pancreatic cancer. Indeed, this research demonstrated unparalleled sensitivity and specificity in diagnosing pancreatic cancer compared to other studies in this paper. CD63 [34], initially discovered as a surface antigen prevalent in early-stage melanoma cells, was found in various cancers (such as malignant melanoma, ovarian, lung adenocarcinomas, breast, and colon cancers) to have an inverse relationship

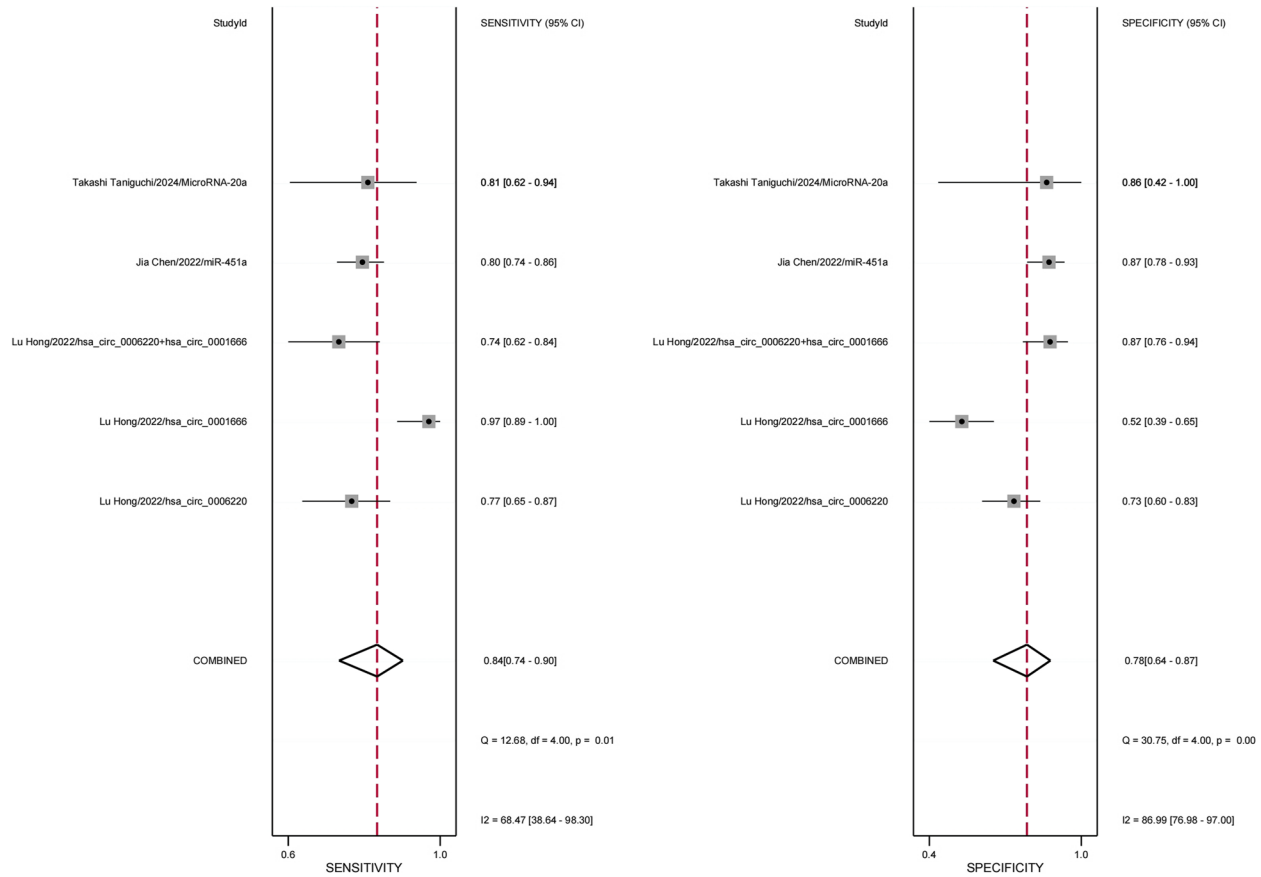

Fig. 9 Forest plot showing the pooled sensitivity and specificity of exosomal biomarkers analyzed using qRT-PCR for distinguishing pancreatic cancer patients from controls

between CD63 expression and tumor malignancy, potentially aiding in cancer progression diagnosis. The extent of cancer cell invasion and metastasis can be assessed by observing $CD63^{+}$ exosome levels.2023 Research [35] revealed that EVs in patients with severe COVID-19 infection exhibited elevated CD41 platelet marker levels, and this study indicated an increase in CD41 in PC patients, potentially hinting at the patient's disease condition and its severity. Growing research indicates that the imbalance in circular RNAs (circRNAs) is vital in the evolution and advancement of cancer [36]. This paper's included article examined the diagnostic significance of hsa_circ_0006220 and hsa_circ_0001666 in pancreatic cancer, revealing a high expression of hsa_circ_0006220 and hsa_circ_0001666 in the plasma exosomes of pancreatic cancer patients versus healthy individuals [15].

Despite conducting an extensive systematic review and meta-analysis adhering to the most recent diagnostic standards, this research faces multiple constraints. Initially, the existing body of work on exosomal diagnosis of pancreatic cancer (PC) remains limited, even after thorough research. The limited quantity of studies and participants implies a need for additional research to verify the function of exosomes in diagnosing PC. In addition, most of the studies included in this analysis were conducted in Asia, and the differences in genetic background, living environment, and lifestyle between Asian populations and other populations lead to the fact that the applicability of our findings to different populations may be limited, and further validation may be needed in other populations, and it is hoped that in the future there will be more studies conducted on other populations to better expand the applicability of the findings. The third significant constraint lies in the diversity of biomarkers. Our findings were unavoidably influenced by the statistical diversity in the type of exosome, ethnic background, the size of the sample, and the methods of extraction. The findings of our study suggest that despite certain limitations, exosome surface protein assays are highly predictive of PC. Increasing evidence suggests that exosomal biomarkers could be an effective method for diagnosing cancer. We aspire for additional studies by fellow scholars to validate these results.

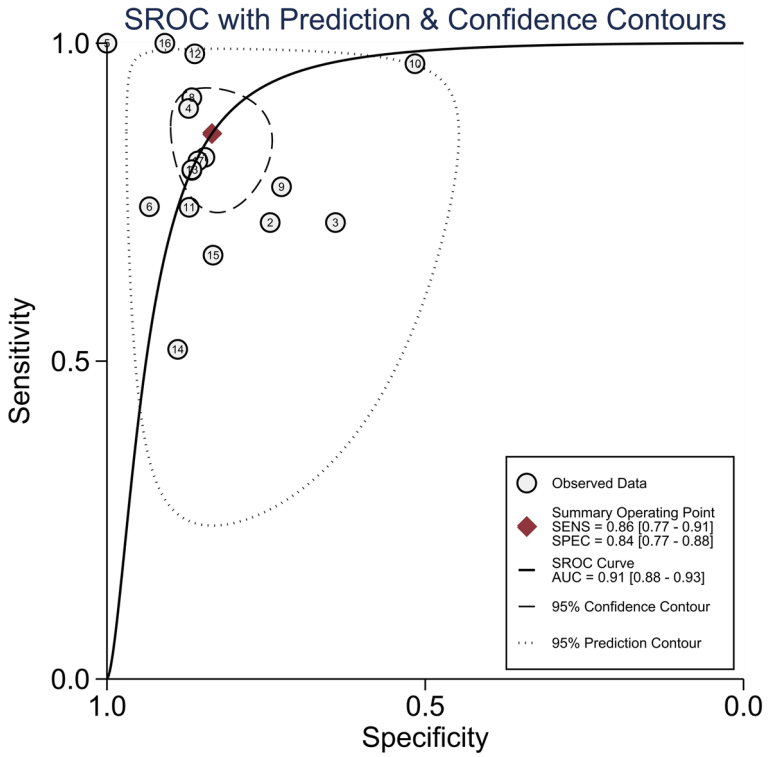

Fig. 10 Summary of the SROC for the biomarkers studied

Currently, the prognosis for pancreatic cancer is poor, but scientists are actively searching for ways to treat it, and research has found that [37] Oncolytic viruses can be effectively targeted in tumor cells by systemic administration and have improved patient survival. Pancreatic cancer patients should also remain hopeful and actively cooperate with diagnosis and treatment.

Conclusion

This meta-analysis resulted in the inclusion of nine studies on exosomes and PC after reviewing 1,202 initial research papers based on inclusion and exclusion criteria and after removing duplicates and excluding reviews, case studies, meta-analyses, unrelated articles, and studies with insufficient data. Among them, the exosomal

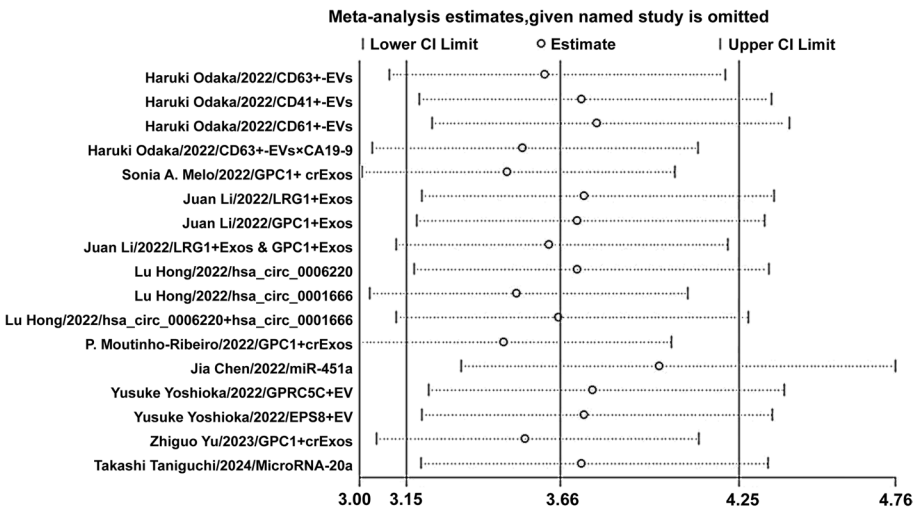

Fig. 11 Sensitivity analysis of exosomal biomarkers

surface proteoglycan marker had the highest diagnostic value for pancreatic cancer. Enhanced diagnostic precision was noted in biomarkers of proteoglycan on the surface of exosomal cells. The results also revealed that the exosomal cell surface proteoglycan biomarker demonstrated the greatest combined DOR, PLR, AUC, specificity, and sensitivity. Consequently, we deduced that employing exosomal cell surface proteoglycan biomarkers is the optimal approach for pancreatic cancer diagnosis. Of course, there are some limitations in this study (limited number of studies, limited study population, etc.), according to the consensus of diagnostic accuracy studies in the field, ideally, studies should be as low-risk as possible in each QUADAS-2 domain. The present study comes close to this standard in some respects, but further improvements are needed in reference standards and indexing tests. However the results of this paper indicate that the value of exosomes in the diagnosis of pancreatic cancer is still considerable, and we eagerly look forward to more relevant studies in the future to validate this result to advance the clinical work as well as to make it more broadly applicable to a wider population.

Abbreviations

PC	Pancreatic Cancer
PDAC	Pancreatic ductal adenocarcinomas
MRI	Magnetic Resonance Imaging
CT	Computed tomography
Sen	Sensitivity
Spe	Specificity
PLR	Positive likelihood ratio
NLR	Negative likelihood ratio
SROC	Summary receiver operating characteristic
DOR	Diagnostic Odds Ratio
DF	Duodenal fluid

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12957-025-03666-9.

Supplementary Material 1.

Authors' contributions

Xu and Meng Li wrote the main manuscript text , Xu and Long prepared figures. Shen , Ye, Yong Li and Hongyang Li extract the data.Cao and Ma revised the article.All authors reviewed the manuscript.

Funding

Thanks for the financial support from the Basic Research Program of Yunnan Province (grant No. 202401AY070001-267), Kunming Medical University Graduate Education Innovation Fund(grant No. 2024S268).

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Central Laboratory, The Second Affiliated Hospital of Kunming Medical University, 374, Dianmian Road, Kunming 650101, China. [2]Department of Clinical Laboratory, The Second Affiliated Hospital of Kunming Medical University, Kunming, China. [3]Department of Hepatobiliary and Pancreatic Surgery, The Third Affiliated Hospital of Kunming Medical University, Yunnan Cancer Hospital, 519, Kunzhou Road, Kunming 650105, China.

Received: 15 October 2024 Accepted: 19 January 2025
Published online: 14 February 2025

References

1. Stoffel EM, Brand RE, Goggins M. Pancreatic Cancer: changing Epidemiology and New approaches to Risk Assessment, early detection, and Prevention. Gastroenterology. 2023;164(5):752–65.
2. Tonini V, Zanni M. Early diagnosis of pancreatic cancer: what strategies to avoid a foretold catastrophe. World J Gastroenterol. 2022;28(31):4235–48.
3. Klein AP. Pancreatic cancer epidemiology: understanding the role of lifestyle and inherited risk factors. Nat Rev Gastroenterol Hepatol. 2021;18(7):493–502.
4. Yuan C, et al. The age-dependent association of risk factors with pancreatic cancer. Ann Oncol. 2022;33(7):693–701.
5. Faur AC, Lazar DC, Ghenciu LA. Artificial intelligence as a noninvasive tool for pancreatic cancer prediction and diagnosis. World J Gastroenterol. 2023;29(12):1811–23.
6. Xing H, et al. Diagnostic value of CA 19 – 9 and Carcinoembryonic Antigen for Pancreatic Cancer: a Meta-analysis. Gastroenterol Res Pract. 2018;2018:p8704751.
7. Wang K, et al. Liquid biopsy techniques and pancreatic cancer: diagnosis, monitoring, and evaluation. Mol Cancer. 2023;22(1):167.
8. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. Science. 2020;367(6478):eaau6977.
9. Dai J, et al. Exosomes: key players in cancer and potential therapeutic strategy. Signal Transduct Target Ther. 2020;5(1):145.
10. McInnes MDF, et al. Preferred reporting items for a systematic review and Meta-analysis of Diagnostic Test Accuracy studies: the PRISMA-DTA Statement. JAMA. 2018;319(4):388–96.
11. Whiting PF, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med. 2011;155(8):529–36.
12. Odaka H, et al. CD63-positive extracellular vesicles are potential diagnostic biomarkers of pancreatic ductal adenocarcinoma. BMC Gastroenterol. 2022;22(1):153.
13. Melo SA, et al. Glypican-1 identifies cancer exosomes and detects early pancreatic cancer (523, pg 177, 2015). Nature. 2022;610(7932):E15–7.
14. Li J, et al. Highly sensitive exosome detection for early diagnosis of pancreatic Cancer using Immunoassay based on hierarchical surface-enhanced Raman scattering substrate. Small Methods. 2022;6(6):e2200154.
15. Hong L, Xu L, Jin L, Xu K, Tang W, Zhu Y, Qiu X, Wang J. Exosomal circular RNA hsa_circ_0006220, and hsa_circ_0001666 as biomarkers in the diagnosis of pancreatic cancer. J Clin Lab Anal. 2022;36(6):e24447.
16. Moutinho-Ribeiro P, et al. Exosomal glypican-1 discriminates pancreatic ductal adenocarcinoma from chronic pancreatitis. Dig Liver Disease. 2022;54(7):871–7.
17. Chen J, et al. Serum exosomal miR-451a acts as a candidate marker for pancreatic cancer. Int J Biol Markers. 2022;37(1):74–80.
18. Yoshioka Y, et al. Circulating cancer-associated extracellular vesicles as early detection and recurrence biomarkers for pancreatic cancer. Cancer Sci. 2022;113(10):3498–509.
19. Yu Z, et al. Dual Tumor Exosome Biomarker co-recognitions based Nanoliquid Biopsy for the Accurate Early diagnosis of pancreatic Cancer. ACS Nano. 2023;17(12):11384–95.

20. Taniguchi T, et al. MicroRNA-20a in extracellular vesicles derived from duodenal fluid is a possible biomarker for pancreatic ductal adenocarcinoma. DEN Open. 2024;4(1):e333.
21. Tiyuri A, et al. Diagnostic accuracy of serum derived exosomes for hepatocellular carcinoma: a systematic review and meta-analysis. Expert Rev Mol Diagn. 2023;23(11):971–83.
22. Halbrook CJ, et al. Pancreatic cancer: advances and challenges. Cell. 2023;186(8):1729–54.
23. Li D, et al. Pancreatic cancer. Lancet. 2004;363(9414):1049–57.
24. Vincent A, et al. Pancreatic cancer. Lancet. 2011;378(9791):607–20.
25. Luo G, et al. Roles of CA19-9 in pancreatic cancer: Biomarker, predictor and promoter. Biochim Biophys Acta Rev Cancer. 2021;1875(2):188409.
26. Kanchanapally R, et al. Drug-loaded exosomal preparations from different cell types exhibit distinctive loading capability, yield, and antitumor efficacies: a comparative analysis. Int J Nanomed. 2019;14:531–41.
27. Tang YT, et al. Comparison of isolation methods of exosomes and exosomal RNA from cell culture medium and serum. Int J Mol Med. 2017;40(3):834–44.
28. Van Deun J, et al. The impact of disparate isolation methods for extracellular vesicles on downstream RNA profiling. J Extracell Vesicles. 2014;3.
29. Lobb RJ, et al. Optimized exosome isolation protocol for cell culture supernatant and human plasma. J Extracell Vesicles. 2015;4:27031.
30. Sha M, et al. Role of circulating exosomal biomarkers and their diagnostic accuracy in pancreatic cancer. JGH Open. 2023;7(1):30–9.
31. Zhu Y, et al. Diagnostic value of various liquid biopsy methods for pancreatic cancer: a systematic review and meta-analysis. Med (Baltim). 2020;99(3):e18581.
32. Melo SA, et al. Glypican-1 identifies cancer exosomes and detects early pancreatic cancer. Nature. 2015;523(7559):177–82.
33. Liu P, et al. Alterations of plasma exosomal proteins and motabolies are associated with the progression of castration-resistant prostate cancer. J Transl Med. 2023;21(1):40.
34. Pols MS, Klumperman J. Trafficking and function of the tetraspanin CD63. Exp Cell Res. 2009;315(9):1584–92.
35. Aharon A, et al. Extracellular Vesicles of COVID-19 Patients Reflect Inflammation, Thrombogenicity, and Disease Severity. Int J Mol Sci. 2023;24(6):5918.
36. Wang X, et al. Hsa_circ_0001666 promotes non-small cell lung cancer migration and invasion through miR-1184/miR-548l/AGO1 axis. Mol Ther Oncolytics. 2022;24:597–611.
37. Hamidi-Sofiani V, et al. Oncolytic viruses and pancreatic cancer. Cancer Treat Res Commun. 2022;31:100563.

Publisher's Note