

ORIGINAL ARTICLE

# Role of circulating exosomal biomarkers and their diagnostic accuracy in pancreatic cancer

Menazir Sha,<sup>\*,†</sup> Basir Kunduzi,<sup>‡</sup> Saied Froghi,<sup>†,§</sup> Alberto Quaglia,<sup>¶</sup> Brian Davidson<sup>†,§</sup> and Giuseppe K Fusai<sup>†,§</sup>

<sup>\*</sup>University College London, Medical School, <sup>†</sup>Division of Surgery and Interventional Sciences/UCL, <sup>§</sup>Department of HPB and Liver Transplantation,

<sup>‡</sup>Department of Cellular Pathology, Royal Free Hospital and <sup>¶</sup>Renal Transplant Unit, Guy's Hospital, London, UK

## Key words

cancer biomarker, exosomal biomarker, pancreatic cancer.

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## Correspondence

Saied Froghi, University College London; HPB & Liver Transplant, Wellington Hospital, St Johns Wood, London, UK.

Email: [sidfrg@doctors.org.uk](mailto:sidfrg@doctors.org.uk); [saied.froghi.13@ucl.ac.uk](mailto:saied.froghi.13@ucl.ac.uk)

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## Abstract

**Background and Aim:** New biomarkers have the potential to facilitate early diagnosis of pancreatic cancer (PC). Circulating exosomes are cell-derived protein complexes containing RNA that can be used as indicators of cancer development. The aim of this review is to evaluate the current literature involving PC patient groups for highly accurate exosomal biomarkers.

**Methods:** The literature search followed Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. Eight-hundred and seventy-five studies were identified across various databases (Ovid MEDLINE, Embase, and Cochrane) published between 2009 and 2020. Nine studies fulfilled the inclusion criteria: human PC patients, diagnosis as outcome of interest, serum biomarker of exosomal content, reporting of diagnostic values, and disease progress. Area under the curve (AUC) of the exosomal biomarker was compared against that of CA19-9.

**Results:** Nine papers were reviewed for relevant outcomes based on the inclusion criteria. These studies involved 565 participants (331 PC, 234 controls; male/female ratio 1.21; mean age 64.1). Tumor staging was reported in all studies, with 45.6% of PC patients diagnosed with early-stage PC (T1–2). The mRNA panel (ARG1, CD63, CK18, Erbb3, GAPDH, H3F3A, KRAS, ODC1) and GPC 1 reported the highest performing sensitivity and specificity at 100% each. The microRNA panel (miR-10b, miR-21, miR-30c, miR-181a, and miR-let7a), mRNA panel (ARG1, CD63, CK18, Erbb3, GAPDH, H3F3A, KRAS, ODC1), and GPC 1 showed a perfect AUC of 1.0. Five studies compared the AUC of the exosomal biomarker against CA19-9, each being superior to that of CA19-9.

**Conclusion:** The potential of exosomal biomarkers remains promising in PC diagnosis. Standardization of future studies will allow for larger comparative analyses and overcoming contrasting findings.

## Introduction

Pancreatic cancer (PC) has the fourth highest mortality in relation to cancer in Europe.<sup>1</sup> Although new treatment for many cancers has improved survival, novel interventions have had a minimal impact on PC survival. The median survival for PC patients is 18 months, with less than 10% surviving to 5 years.<sup>1,2</sup> Only a minority of patients at presentation can undergo a potentially curative resection,<sup>3</sup> and the majority of resected patients die of recurrence. Prognostic outcomes may greatly improve if there is a shift from patients being diagnosed from late stage to an early or precancerous stage.<sup>4,5</sup> Unfortunately, there has been little progress in the research on potential diagnostic tools that enable earlier diagnosis.<sup>6</sup> Current strategies for diagnosis include various radiological imaging techniques along with endoscopic retrograde cholangiopancreatography (ERCP) and biopsies among other common practices for tumor

confirmation. Unfortunately, radiological imaging alone is often inadequate because it has only moderate sensitivity and specificity for detecting PC. In addition, early pancreatic lesions are of particular importance, and these are often missed by these modalities completely.<sup>7</sup> Newer studies have looked into the role of artificial intelligence and deep learning methods in diagnosing PC mainly hinging on detecting early CT changes.<sup>8,9</sup> Screening of “at-risk” individuals has been proposed as the optimal solution. Although at-risk patients can be identified, there is currently no clinically established strategy for screening these patients.<sup>6,10</sup>

Carbohydrate antigen 19-9 (CA19-9) is presently the only biomarker for PC in clinical use. CA19-9 is principally used to measure therapeutic response and to determine prognosis, although it has also frequently been used in the diagnosis of PC.<sup>11,12</sup> However, there are some challenges to the use of

CA19-9 as a screening tool. First, it has been reported to have sub-optimal sensitivity (41–86%) and specificity (33–100%).<sup>13,14</sup> Within an asymptomatic population, mass screening is rather ineffective because of its poor positive predictive value.<sup>15,16</sup> Also, only 65% of early-stage pancreatic ductal adenocarcinoma (PDAC) patients present with elevated CA19-9 in sera.<sup>17</sup> CA19-9 has frequently been demonstrated to be elevated in various other gastrointestinal malignancies as well as benign conditions such as pancreatitis.<sup>18,19</sup> Furthermore, CA19-9 is not expressed by around 5–10% of the Caucasian population who possess a Lewis-negative genotype.<sup>20</sup> Thus, it is vital to explore novel biomarkers that may be more effective in the diagnosis of PC.

Circulating biological markers have been proposed as a potential screening solution for PC patients. Serum biomarkers, in particular, hold great clinical potential, as they can be obtained noninvasively in comparison to tissue markers. These biomarkers can be difficult to detect, as they are diluted by the various other components of blood.<sup>21,22</sup> However, recent technological advances in gene-expression microarrays, proteomics, and immunology have facilitated biomarker research.<sup>23</sup>

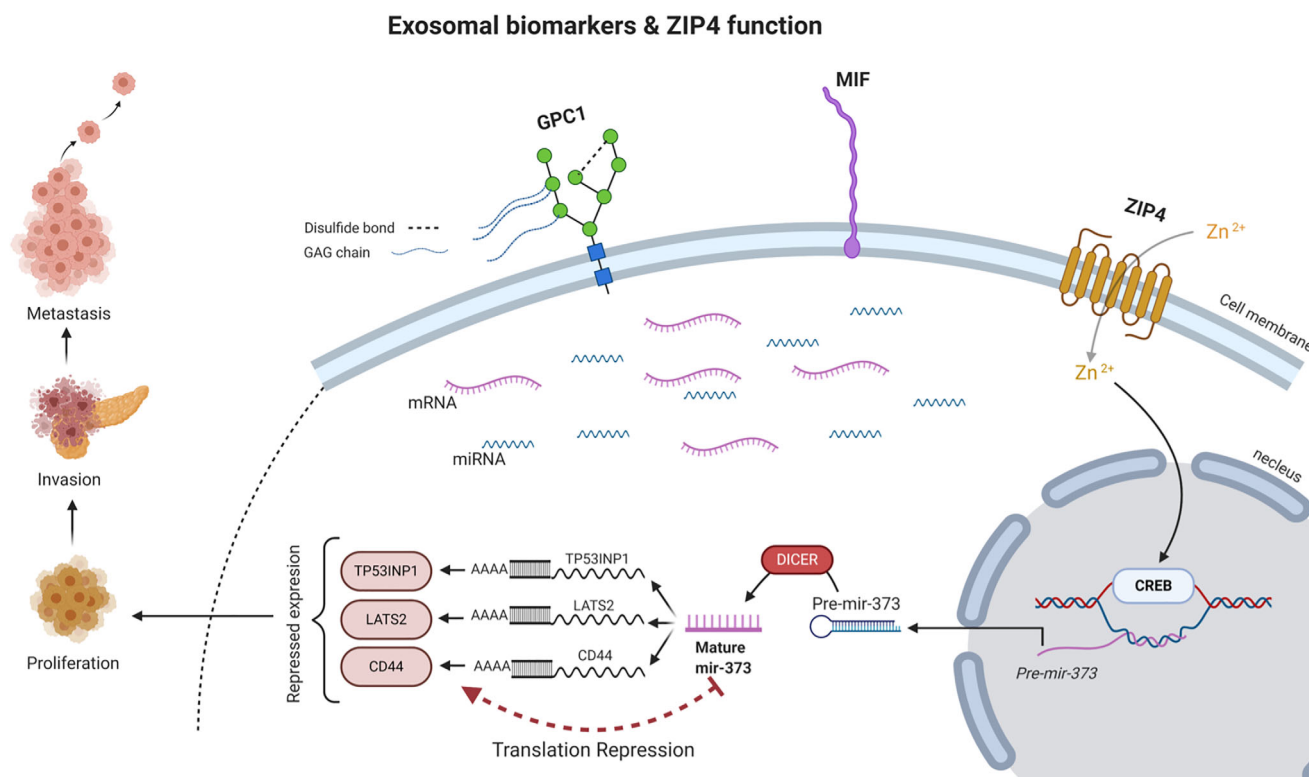
Numerous biomarkers exist, ranging from inflammatory markers and metabolites to gene sequences and circulating tumor cells.<sup>24</sup> Of all the potential biomarkers, exosomes (extracellular vesicles) in particular present a promising candidate for diagnostics in relation to cancer.

Exosomes are extracellular vesicles that are released by almost every type of cell, including cancer cells (Fig. 1).<sup>25,26</sup> These vesicles contain a variety of intracellular contents such as DNA, mRNA, miRNA, proteins, and metabolites.<sup>27</sup> Studies support the theory that they are mediators of intercellular signaling that dynamically respond to the different stressors of the secreting cell microenvironment (implying further specificity).<sup>28</sup>

They are important in normal physiologic processes, such as immune response, lactation, and neuronal function, but emerging evidence has also found that exosomes are involved in the development of numerous pathologies such as neurodegenerative diseases and cancer.<sup>29</sup>

Every cell in the body releases exosomes including cancer cells.<sup>30</sup> Exosomes from cancer cells facilitate communication with other cells. Cancer cells have also been shown to secrete exosomes at a higher rate than normal cells.<sup>31</sup> Exosomes show high levels of heterogeneity that are derivative of the cell that produces them.<sup>32</sup> Specifically within PC, genomic pleomorphism is abundant with known mutations (KRAS, BRCA) and other more novel ones associated with chromatin modification, DNA damage repair, etc.<sup>33</sup> Thus, exosomes potentially contain numerous specific markers that can be identified as diagnostic of PC.<sup>29,33</sup>

Exosomes are particularly novel as biomarkers because just a decade ago their detection was considered too difficult and ineffective. Recent technological advances in immunoassay procedures, for example, have helped develop new, effective, timely,



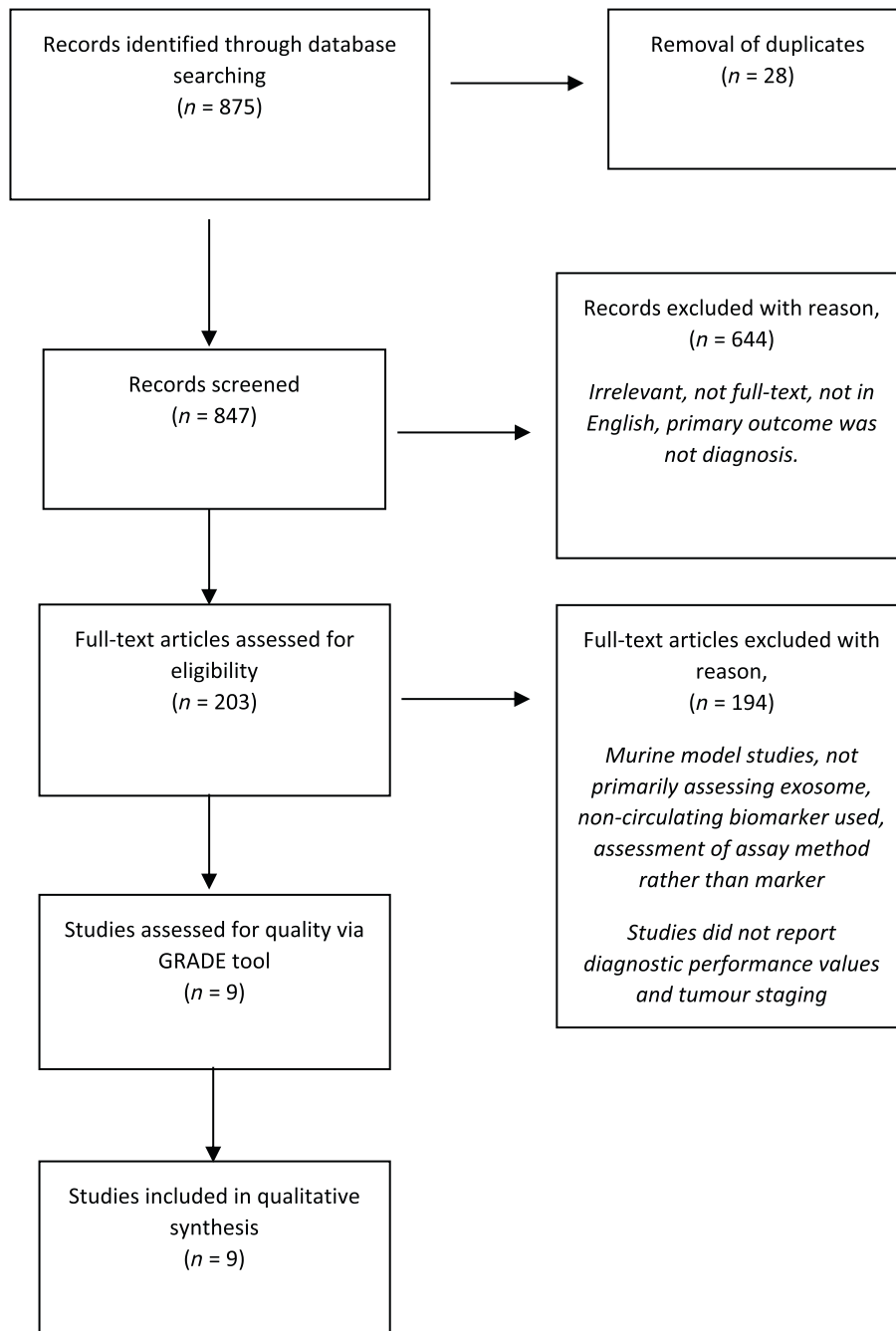
**Figure 1** Membrane-bound surface proteins (GPC 1, MIF, and ZIP 4) have been found to be upregulated in pancreatic cancer-derived exosomes. ZIP 4, an ion transporter, regulates intracellular zinc homeostasis. The differential expression of ZIP 4 has been reported in pancreatic cancer.

and precise mechanisms to identify exosomes and analyze their molecular contents.<sup>34</sup> This, coupled with an increase in our etiological understanding of cancer and the function of exosomes, has led to an increased focus on the potential diagnostic capability of circulating exosomes in cancer.<sup>35–37</sup>

The aim of this review is to evaluate the current literature involving PC patient groups for highly accurate exosomal biomarkers, highlight comparisons with CA19-9 where reported, and identify performance discrepancies between studies.

## Methods

**Search strategy.** A literature search was performed by M.S., S.F. on November 2020 using the MeSH/EMTREE terms “pancreas,” “cancer,” “diagnosis,” “detection,” “exosome,” “biomarker,” and “marker” as keywords in a Boolean search on the electronic databases of Ovid MEDLINE(R), Embase, and Cochrane Central Register of Controlled Trials. The search was limited to full-text articles available in English. Our review



**Figure 2** Preferred Reporting Items for Systematic Reviews and Meta-Analyses flowchart detailing study inclusion.

focused on identifying observational studies. Also, in concordance with the “novel” aspect of the review, only publications from the last 10 years were included. Duplicates were then removed with the use of citation software. An additional search using Google Scholar was performed to ensure discovery of all suitable studies.

**Study selection criteria.** Titles and abstracts of all studies retrieved from the literature search were reviewed by two authors (M.S. and S.F.) to determine relevance.

**Inclusion criteria.** Studies were included according to the following criteria: (1) Population of interest were human PC patients; (2) Outcome of interest involved diagnosis of the cancer; (3) Biomarker assessed was that of an exosome or exosomal content; (4) Biomarkers were accessible in serum (noninvasively); (5) Diagnostic performance values (the ability to distinguish between PC and controls) of biomarkers were included: specifically, sensitivity, specificity, and area under the curve (AUC); (6) Disease progress, such as tumor staging, was reported to establish diagnostic performance in different stages of disease.

**Exclusion criteria.** Studies were excluded according to the following criteria: (1) Publications in the form of letters, case reports, editorials, or reviews; (2) Studies assessing only animal models; (3) Studies only evaluating prognostic and/or predictive element(s) of biomarkers; (4) Studies with no control group; (5) Studies which primarily assessed biomarker detection method rather than their diagnostic capability.

**Quality assessment.** Two reviewers (M.S., S.F.) assessed quality of eligible studies via the Grading of Recommendations Assessment, Development and Evaluation (GRADE) quality of evidence tool, recommended by the Cochrane Collaborative Group.<sup>38</sup> Studies were scored as high, moderate, low, or very low. Only studies determined to be of “moderate” to “high” quality via the GRADE tool were preserved for inclusion in the review. Consensus using the author coefficient of agreement was achieved to resolve differences between reviewers.

**Data collection and handling.** This review was prospectively registered on PROSPERO.<sup>39</sup> We report our findings according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statements (PRISMA) guidelines.<sup>40</sup> Data were extracted from all included studies and collated for the parameters of interests. Study characteristics that were extracted included biomarker(s) examined, detection method, number of patients, mean age, gender ratio, diagnostic performance of biomarker(s), predictive values, and any comments regarding the study. Sensitivity, specificity, and AUC values of biomarkers were compared. Where individual biomarkers were repeated between studies, their diagnostic accuracies were compared for any differences. Where reported, the AUC values of CA19-9 were compared to the exosomal biomarker assessed in the respective study. Statistical analyses were conducted using Graph-Pad Prism 8 (GraphPad Software, CA, USA).

**Table 1** Quality assessment of eligible studies using the GRADE tool

Author, year, reference	Study type	GRADE score
Lewis <i>et al.</i> , 2018 <sup>46</sup>	Case-control	Moderate
Li <i>et al.</i> , 2018 <sup>47</sup>	Case-control	Moderate
Jin <i>et al.</i> , 2018 <sup>45</sup>	Case-control	Moderate
Kitagawa <i>et al.</i> , 2018 <sup>41</sup>	Case-control	Moderate
Ko <i>et al.</i> , 2017 <sup>42</sup>	Case-control	Moderate
Lai <i>et al.</i> , 2017 <sup>43</sup>	Case-control	Moderate
Melo <i>et al.</i> , 2015 <sup>48</sup>	Case-control	Moderate
Yang Z <i>et al.</i> , 2020 <sup>49</sup>	Case-control	Moderate
Wu <i>et al.</i> , 2020 <sup>44</sup>	Case-control	Moderate

## Results

The complete study inclusion process compliant with PRISMA guidelines is illustrated in Figure 2. The initial search yielded 875 articles, which included duplicates, reviews, and studies that failed to meet the inclusion criteria. Twenty-eight duplicates were removed, and 847 records were further screened.

A total of 672 articles were excluded during screening. Reasons included articles were irrelevant, were not in English, lacked full-text availability, or the primary focus of the study was not specifically on the diagnosis of PC.

Upon full-text article screening, a further 194 studies were excluded. These were deemed in line with our exclusion criteria, such as assessment of murine models rather than humans, not utilizing a serum biomarker, or the study assessing an assay or spectrometry method rather than the actual biomarker in question. Some studies did not report the diagnostic performance values (sensitivity, specificity, AUC) and did not mention the tumor staging of the PC group. A total of nine articles deemed eligible for inclusion were assessed by the GRADE quality of evidence tool (Table 1) and scored “moderate.” These nine articles were used for the qualitative review. These studies were case-control studies.

Of the included studies, four assessed genetic exosomal contents<sup>41–44</sup> as biomarkers and four assessed exosomal proteins.<sup>45–48</sup> The gene arrays and proteins assessed differed between most studies (Table 2). One study subsequently used a multianalyte panel including both genetic exosomal contents and exosomal proteins.<sup>49</sup> In total, the 9 studies included 331 patients and 234 controls.

The median size of the study was 40 and ranged from 31 to 136 participants (Table 2). The median size of the PC population was 29.5 (range: 17–71 patients) and the median size of the control population was 18.5 (range: 6–79 controls). The male to female ratio among the patients was 1.21, and the mean age was 64.1 years. All studies included information of tumor staging to better appreciate the diagnostic utility at various stages of cancer presentation. One study included quantitative assessment of biomarker performance between early- and late-stage PC.<sup>47</sup> Li *et al.* showed that the discriminatory sensitivity for macrophage migration inhibitory factor (MIF) was 95.7% for early-stage pancreatic cancer (stage 1 and 2) *versus* Stage 3 cancer.

Pathological validation of PC and its subtypes were performed in some of the included studies (Table 2). Three studies did not histologically validate the PDAC patients included.<sup>42,46,49</sup>

**Table 2** Summary of studies reviewed

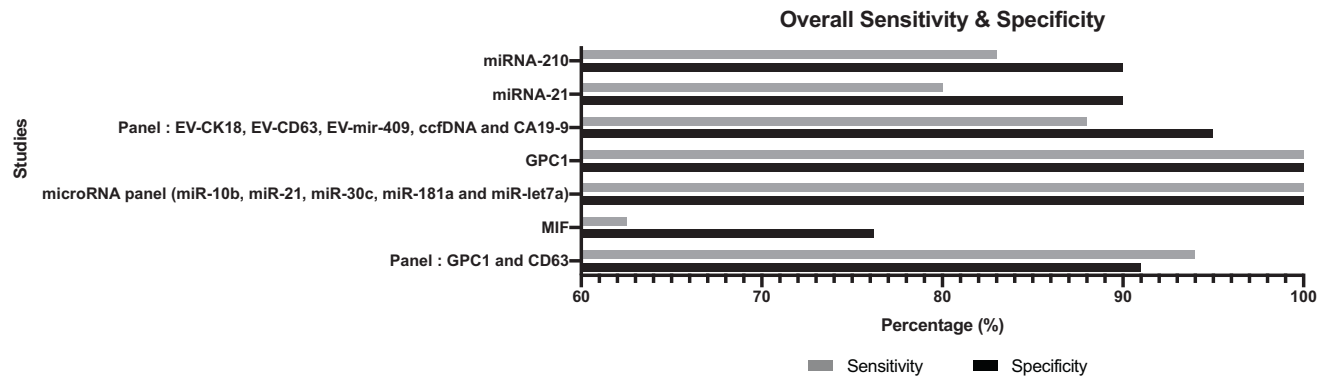
Author, year, reference	Exosomal Biomarker(s)	Detection method	n (patients/controls)	Mean age (MF)	Cancer sub-type and pathology information	Tumor staging	Biomarker performance (Sen: %; Spe: %; AUC)	CA19-9 performance in patient group where reported (AUC)	Comments
Lewis et al., 2018 <sup>46</sup>	Panel of GPC1 and CD63	ACE immunoassay	20/11	63.7	PDAC. No histologic confirmation	T3N0: 40%, T3N1: 60%	94: 91; 0.99	—	Also distinguished PC patients from those with benign pancreatic conditions
Li et al., 2018 <sup>47</sup>	MIF	SERS immunoassay	71/32	60.08	PDAC histologically diagnosed	T1-2: 10% T3: 90%	62.5; 76.2; 0.886	—	MIF distinguished P1-2 from P3-4 with a sensitivity of 95.7%
Jin et al., 2018 <sup>45</sup>	ZIP4	ELISA	24/46	61.3	Pancreatic adenosquamous carcinoma, 1 squamous cell carcinoma of body and tail. Pathologically diagnosed	T1-2: 46% T3-4: 54%	AUC: 0.8931	—	
Kitagawa et al., 2018 <sup>41</sup>	mRNAs (WASF2, ARF6) and snoRNAs (SNORA74A, SNORA25)	RT-qPCR	27/13	60-69	PDAC pathologically diagnosed	T0-2B: 89% T3-4: 11%	AUC: >0.9 WASf2: 0.943 ARF6 0.940 SNORA74A: 0.909 SNORA25: 0.903	0.897	WASF2 mRNA may be particularly useful, as it was the most highly correlated with pancreatic cancer risk
Ko et al., 2017 <sup>42</sup>	mRNA panel (ARG1, CD63, CK18, ErbB3, GAPDH, H3F3A, KRAS, ODC1)	RT-qPCR	17/17	66	Metastatic pancreatic cancer. No histologic confirmation.	T1-2: 82% T3-4: 18%	Overall AUC: 1.0	—	
Lai et al., 2017 <sup>43</sup>	microRNA panel (miR-10b, miR-21, miR-30c, miR-181a, and miR-let7a)	RT-qPCR	29/6	67.34	PDAC confirmed by cytopathology	T1-2: 93% T3-T4: 7%	Panel and individual biomarkers: 100; 100; 1.0	0.92 (sensitivity 86%)	Study showed decrease in exosome biomarker detection after resection of tumor

(Continues)

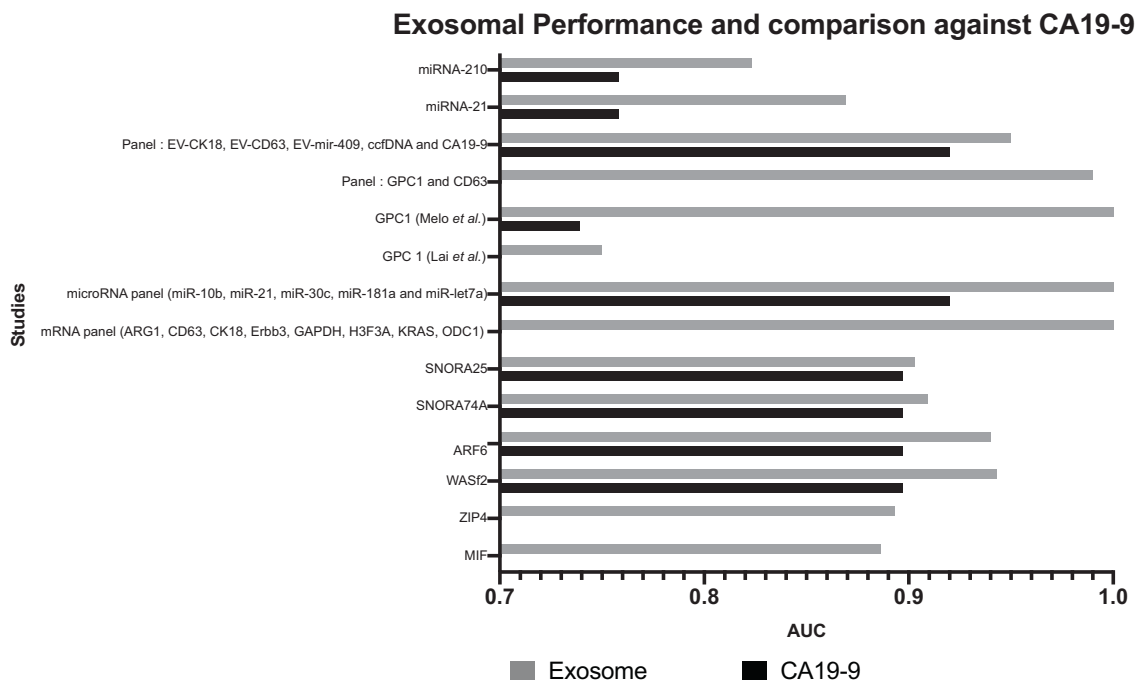
**Table 2** (Continued)

Author, year, reference	Exosomal Biomarker(s)	Detection method	n (patients/controls)	Mean age (MF)	Sex	Cancer sub-type and pathology information	Tumor staging	Biomarker performance (Sen: %; Spe: %; AUC)	CA19-9 performance in patient group where reported (AUC)	Comments
Melo <i>et al.</i> , 2015 <sup>48</sup>	GPC1	Immunogold labeling, TEM, RT-qPCR	56/20	70	28/28	PDAC—histologically validated	T1–2: 66% T3–T4: 34%	100; 100; 1.0	0.739	Study showed decrease in biomarker post resection. Higher overall survival rate was associated in patients with lower GPC levels post resection. Best overall biomarker was CA19-9. The best performing individual EV mRNA marker was CK18. The best performing EV miRNA marker was miR-409
Yang <i>et al.</i> , 2020 <sup>49</sup>	Panel including EV-CK18, EV-CD63, EV-mir-409, ccfDNA, and CA19-9	RT-qPCR, ECLIA immunoassay	57/79	65.3	66/70	PDAC. No histological confirmation. Control included three IPMN	T0N0m1: 1.75% T1–2: 43.9% T3–T4: 54.4%	88.95; AUC: 0.95;	0.92	
Wu <i>et al.</i> , 2020 <sup>44</sup>	miRNA-21, miRNA-210	qPCR	30/10	59.1	26/14	PC confirmed by either pathology or cytology	T1–2: 20%, T3–4: 80%, ENET staging method was used. European Neuroendocrine Tumor Society	miRNA 21 80, 90, 0.869 miRNA-210 83, 90, 0.823 Mir21/210: 93, 80 miR21/CA19-9: 90, 90 miR-210/CA 19-9: 90/90	0.758	The biomarkers showed higher sensitivity and specificity when combined with CA 19-9. The sensitivity and specificity of both miRNA-21 and miRNA-210 improved to 90% each

ACE, angiotensin convertin enzyme; AUC, area under the curve; CA19-9, carbohydrate antigen 19-9; PC, pancreatic cancer; PDAC, pancreatic ductal adenocarcinoma; IPMN, intraductal papillary mucinous neoplasm; MIF, macrophage migration inhibitory factor.



**Figure 3** Overall reported sensitivity and specificity of exosomal biomarkers reported from studies.



**Figure 4** Overall area under the curve (AUC) comparison between exosomal biomarkers and CA19-9 values (where reported) from each study. The exosomal biomarker performed better with higher AUC values in each study.

Lai *et al.* used cytopathology to confirm PDAC.<sup>43</sup> Wu *et al.* used either cytology or pathology for validation.<sup>44</sup>

Some studies also included assessment of biomarker utility in benign pancreatic conditions.<sup>46</sup> Sensitivity and specificity values ranged from 62.5 to 100 and from 76.2 to 100, respectively (Fig. 3). Ko *et al.* (mRNA panel ARG1, CD63, CK18, Erbb3, GAPDH, H3F3A, KRAS, ODC1) and Melo *et al.* (GPC 1) reported the highest performing sensitivity and specificity at 100% each. Li *et al.* (MIF) reported the lowest sensitivity and specificity at 62.5% and 76.2%, respectively.

The nine studies reported the AUC values of 13 exosomal biomarkers (Table 2). The overall AUC values ranged from 0.823 to 1.0 (Fig. 4). The highest AUC performance, all showing

1.0, was reported by Lai *et al.* (microRNA panel miR-10b, miR-21, miR-30c, miR-181a, and miR-let7a), Ko *et al.* (mRNA panel ARG1, CD63, CK18, Erbb3, GAPDH, H3F3A, KRAS, ODC1), and Melo *et al.* (GPC1). Wu *et al.* (miRNA 210) reported the lowest AUC of 0.823. Five studies<sup>41,43,44,48,49</sup> compared the AUC of the exosomal biomarker against CA19-9 (Table 2). Where reported, the AUCs of the exosomal biomarkers were superior to that of CA19-9 (Fig. 4).

Individual biomarker discrepancy was identified between reported AUC of GPC 1 (Melo *et al.* and Lai *et al.*). Melo *et al.*<sup>48</sup> found that it had a perfect sensitivity and specificity of 100% and that it was superior to CA19-9 in distinguishing PC patients. Contrastingly, Lai *et al.*,<sup>43</sup> found that GPC1 did not

have perfect sensitivity and specificity: they determined the AUC to be 0.75 when comparing GPC1 in PC patients to normal controls. Additionally, they found that GPC1-positive exosomes failed to adequately distinguish between PC and pancreatitis. This was further supported by Lewis *et al.*<sup>46</sup> who conducted a bivariate analysis of both GPC1-positive exosomes and CD63 exosomal proteins and reported that GPC1 did not exhibit perfect diagnostic capability.

Lai *et al.* and Wu *et al.* both assessed the performance of miRNA-21. Sensitivity, specificity, and AUC differed (100%, 100%, and 1.0 vs 80%, 90%, and 0.869) between their studies.

## Discussion

A clinically reliable biomarker must demonstrate high sensitivity and specificity. In the context of PC diagnostics, it should be able to accurately discern PC from healthy control samples and it should also have a high AUC to enable detection. Establishing the accuracy of exosomal biomarkers is vital for further exploring their advantages in the earlier diagnosis of PC patients compared to existing methods.

In technical aspects, it should be able to be stored easily and not present a problem for clinicians or technicians to obtain. Also, its detection should be fairly rapid and cheap to help facilitate clinical application. It has been shown that earlier detection, in stages I/II of the classification of PC stages, improves prognosis<sup>50,51</sup> because often it is feasible for the patient to receive tumor resection. Furthermore, research has demonstrated that certain preneoplastic conditions precede tumor formation by nearly 8 years.<sup>52,53</sup> This offers a window of opportunity to pre-emptively detect susceptibility to pancreatic tumor formation, paving the way for curative treatment and, consequently, better prognosis.

The potential of multiple exosomal biomarkers has been assessed in these studies. A key limitation identified was the differing diagnostic performances of the same biomarker between studies (GPC1 and miRNA-21). Melo *et al.* had a larger group of PC and healthy patients, and included murine model investigations to support their findings, which therefore should not be disregarded easily. Some studies did not report the individual AUC values of the biomarkers used within the panel,<sup>42,46</sup> which did not allow for discrepancies to be identified for repeated biomarkers (CD63). Differences between the studies suggest that further investigation is required. Standardizing evaluations can help improve the reproducibility of results. In addition, the difference in plasma processing and immunoassay techniques can lead to differences in biomarker detection.

Various detection methods may yield different outcomes in biomarker performance. In the studies reviewed, RT-qPCR or q-PCR was conventionally used for miRNA and mRNA panels, and varying immunoassay methods were used for exosomal proteins (Table 2). The variation in detection methods of studies evaluating GPC1-positive exosomes might have contributed to the differing results.<sup>43,46,48</sup> With technological advancements, newer and more capable methods are coming to fruition. Studies should aim to assess the effectiveness for each detection method and establish which is most suitable to the detection and examination of circulating exosomes as well as other circulating biomarkers.

CA19-9 is the most commonly used validated serum biomarker that is currently used in practice. A comparison of

exosomal biomarkers that show superior performance against CA19-9 will encourage further large-scale studies. Not all the studies compared their exosomal biomarker's performance directly against that of CA19-9. Nonetheless, where reported, the AUC of the exosomal biomarkers was superior to that of CA19-9 in all the five studies. Standardization of exosomal diagnostic evaluations to include CA19-9 comparisons will be useful in identifying the most promising biomarkers.

Where studies include different stages of PC in the patient population, biomarker performance between early- and late-stage PC should be analyzed for any discrepancies. Of the reviewed study, only that of Li *et al.* reported the sensitivity, specificity, and AUC of MIF between Stage I–II versus Stage III PC.<sup>47</sup> Such reporting would facilitate a better understanding of the accuracy of the biomarker being investigated.

It is also important to highlight the sub-types of PC in diagnostic studies. From a histologic point of view, only a few studies clearly stated the specific sub-types included (PDAC, adenosquamous, squamous cell, etc.) and the modality used to verify the diagnosis. Histologic verification should be performed and standardized in future studies involving PC populations.

The performance of individual exosomal biomarkers should not just be evaluated in isolation. Studies in this review that used a panel of biomarkers<sup>42,43,46</sup> reported better outcomes compared to the performance of the biomarker in isolation. Ko *et al.*<sup>42</sup> reported that only a panel of more than four genes could achieve this diagnostic accuracy, and no one single gene could solely provide such a result. They suggest that this is due to the great heterogeneity that exists among people such that several genes were differentially expressed among individuals in the group. This offers an interesting perspective of genetic biomarkers, implying that only a panel of multiple genes can be sufficient, as heterogeneity does not allow for one single gene to be significant enough to provide robust diagnosis for the wider population. The heterogenous nature of these exosomes can be of further benefit by evaluating a combination of high-performing biomarkers.

All reviewed studies were case-control studies and had the limitation of a small sample size. Future studies should look to validate findings of these studies in larger, multicenter trials. This can extend to prospective cohort studies and examine whether earlier disease detection with the marker is translated into improved survival. This would facilitate assessing the ability of markers to predict prognosis and response to treatment.

## Conclusion

In conclusion, the potential of exosomal biomarkers remain promising in PC diagnosis, but more work needs to be done in the standardization of future studies for larger comparative analyses to overcome contrasting findings and reliably establish key pragmatic markers for clinical practice.

*Ethics statement:* This article does not contain any studies with human participants or animals performed by any of the authors.

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