

REVIEW

Open Access



Multibiomarker panels in liquid biopsy for early detection of pancreatic cancer – a comprehensive review

Kim-Lea Reese¹, Klaus Pantel^{1*} and Daniel J. Smit^{1*}

Abstract

Pancreatic ductal adenocarcinoma (PDAC) is frequently detected in late stages, which leads to limited therapeutic options and a dismal overall survival rate. To date, no robust method for the detection of early-stage PDAC that can be used for targeted screening approaches is available. Liquid biopsy allows the minimally invasive collection of body fluids (typically peripheral blood) and the subsequent analysis of circulating tumor cells or tumor-associated molecules such as nucleic acids, proteins, or metabolites that may be useful for the early diagnosis of PDAC. Single biomarkers may lack sensitivity and/or specificity to reliably detect PDAC, while combinations of these circulating biomarkers in multimarker panels may improve the sensitivity and specificity of blood test-based diagnosis. In this narrative review, we present an overview of different liquid biopsy biomarkers for the early diagnosis of PDAC and discuss the validity of multimarker panels.

Keywords Blood-based biomarkers, Early-stage diagnosis, Liquid biopsy, Multimarker panel, Pancreatic cancer

Background

The most common type of pancreatic cancer is pancreatic ductal adenocarcinoma (PDAC), which accounts for more than 90% of all pancreatic cancers [1]. PDAC-related precancerous conditions include pancreatic intraepithelial neoplasms (PanINs), intraductal papillary mucinous neoplasms (IPMNs), and mucinous cystic neoplasms (MCNs). The etiology of pancreatic cancer is not fully understood, but several risk factors are associated with PDAC. In addition to common cancer risk factors, including age, obesity, genetic predispositions, smoking and alcohol consumption, the factors conferring the

highest risk are type 2 diabetes mellitus and chronic pancreatitis [2].

Pancreatic cancer is the seventh most commonly diagnosed cancer and the fourth most frequent cause of cancer-related deaths in Europe; it accounted for almost as many diagnoses (140,116 cases) as deaths (132,134 deaths) in 2020 [3]. Despite tremendous efforts in research and new therapies resulting in increased survival rates of patients with other cancer types, pancreatic cancer still has a low 5-year survival rate of approximately 10%, with a median overall survival (OS) of less than six months [4]. One of the main reasons is late diagnosis, as patients do not show specific early clinical symptoms [5]. At the time of PDAC detection, less than 20% of tumors are eligible for curative resection [6]. However, surgery followed by adjuvant systemic chemotherapy is the best therapeutic option, significantly increasing the 5-year survival rate [7]. At advanced tumor stages with metastases, the only remaining treatment is systemic chemotherapy, which has a low response rate and a high resistance

*Correspondence:

Klaus Pantel
pantel@uke.de
Daniel J. Smit
d.smit@uke.de

¹ Institute of Tumor Biology, University Medical Center Hamburg-Eppendorf, Martinistraße 52, Hamburg 20246, Germany



© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

rate [6]. Consequently, it is highly important to develop diagnostic tests that enable the detection of early-stage PDAC (AJCC/UICC stages I and II) to improve the OS and progression-free survival (PFS) of patients.

PDAC is mainly diagnosed through medical imaging methods, including computer tomography, magnetic resonance imaging, magnetic or endoscopic retrograde cholangiopancreatography, and endoscopic ultrasound-guided fine needle aspiration. However, a clear diagnosis is not always possible because of the retroperitoneal location of the pancreas and the small size of early-stage PDAC lesions [6, 8]. In addition, screening for the early diagnosis of PDAC by imaging is not practical, as it is neither cost nor time efficient and involves exposure to radiation [8].

Liquid biopsy (LB) is a minimally invasive procedure that allows the sampling and analysis of body fluids, thus enabling cancer diagnosis, treatment monitoring, surveillance, and prognostication [9]. The samples can be obtained from various body fluids, including urine, saliva, cerebrospinal fluid, bone marrow, or blood [10]. In recent years, blood has been one of the most popular analytes, as blood sampling is easy, cost-effective, and repeatable [11]. Biomarkers that can be detected in the blood include circulating tumor cells (CTCs), circulating host cells, including cancer-associated fibroblasts (CAFs) or circulating endothelial cells (CECs), circulating cell-free RNA and DNA (cfRNA, cfDNA), extracellular vesicles (EVs) and proteins [12] (Fig. 1). However, to date, no single biomarker or multimarker panel can reliably diagnose PDAC, especially in the early stages.

In this review, we summarize potential biomarkers and detection methods for blood-based liquid biopsy and discuss their implications for the early-stage detection of PDAC. Moreover, we review publications on multimarker panels for PDAC diagnosis and highlight their importance in the early diagnosis of pancreatic cancer.

Methods

The literature search for this narrative review regarding multimarker panels was carried out in PubMed on 4th July 2024 via the following terms:

("biomarker panel*" OR "marker panel*" OR "multi biomarker*" OR "multi marker*" OR "marker combin*" OR "biomarker combin*") AND (pancrea*) AND (cancer OR carcinoma OR tumor* OR adenocarcinoma*) AND (sera OR serol* OR plasma OR blood OR "liquid biops*" OR "fluid biops*").

The literature search was restricted to articles in English and yielded 126 papers. Reviews and case reports were excluded, as were studies that did not involve research on humans, PDAC, diagnosis, or blood-based liquid biopsy. According to these criteria, a total of 57 publications were further analyzed for this review. A flow chart can be found in Fig. 2. The data extracted from the publications included study details (author, year of publication, country), biomarker details (biomarkers used, detection method, fluid type), patient cohort details (patient numbers, stage, controls), and statistical details (sensitivity, specificity, area under the curve (AUC)). If separate data for early-stage PDAC (pathological AJCC/UICC stages I and II according to the 8th edition of the staging manual) and late-stage or all-stage PDAC diagnosis were provided, only the values for early-stage PDAC were included.

Liquid biopsy biomarkers for PDAC

Currently used tumor markers for PDAC

A commonly used serological biomarker for PDAC is carbohydrate antigen 19–9 (CA19-9), also referred to as sialyl Lewis-A, which currently represents the only FDA-approved marker. Increased levels of CA19-9 have been reported in PDAC patients compared with healthy individuals [13, 14]. The concentration of CA19-9 and

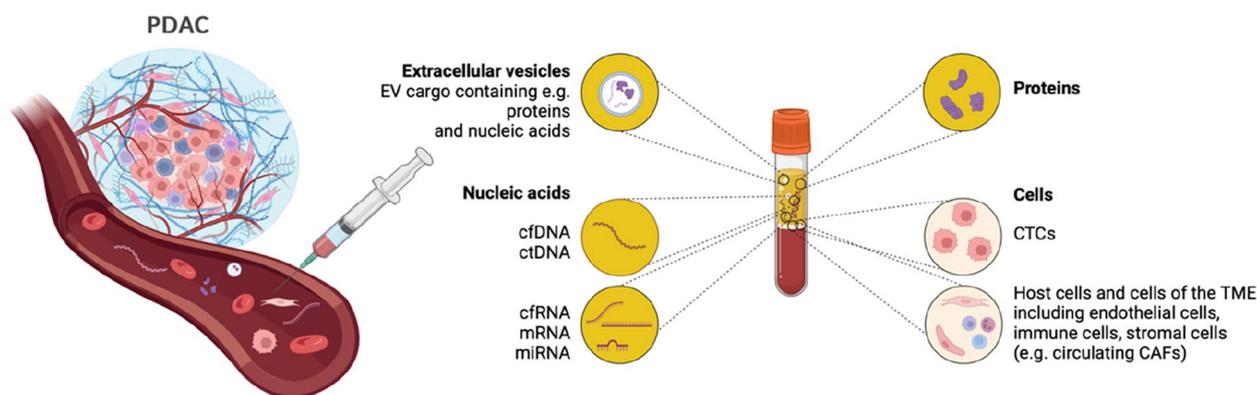


Fig. 1 Overview of (blood-based) liquid biopsy analytes for the early detection of pancreatic cancer. The figure was created with BioRender.com under academic license

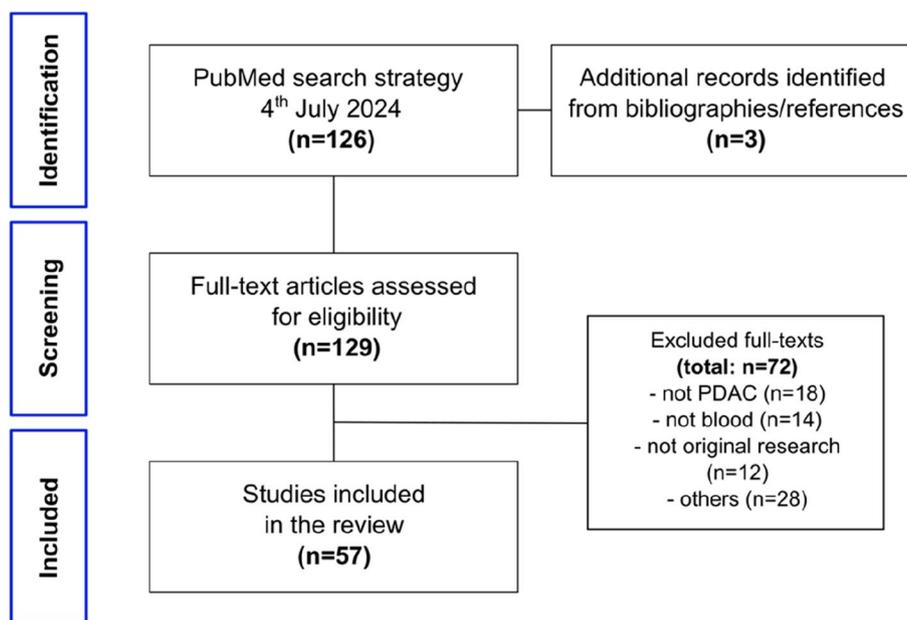


Fig. 2 Overview of the results from the literature search and selection of included studies

its sensitivity as a diagnostic marker increase with increasing PDAC stage [15, 16], with the most pronounced increase detected between AJCC/UICC stage II and stage III. However, especially in early stages (e.g., stage I [17]), the level of CA19-9 is similar to that in various benign conditions, precancerous lesions, and other malignancies (e.g., colorectal cancer, gastric cancer, hepatocellular carcinoma), resulting in low specificity [14, 15]. With respect to its use for diagnostic purposes, it is important to consider that 6% of Caucasians and 22% of non-Caucasians who lack Lewis antigen A cannot produce CA19-9, subsequently leading to false-negative results [18]. Consequently, international guidelines do not recommend its use as a diagnostic method but rather as a longitudinal marker in patients with detectable CA19-9 at baseline [16]. Owing to the lack of robust biomarkers, various markers have been investigated as possible candidates with increased sensitivity and specificity for PDAC diagnosis. The following sections present an overview of cellular and acellular liquid biopsy-based biomarkers for PDAC diagnosis.

Cellular biomarkers

One group of biomarkers analyzed in liquid biopsy is cells that have detached from their site of origin and entered the bloodstream. These can be derived from tumor or noncancerous host cells that are part of the tumor micro-environment (TME) (e.g., immune cells, fibroblasts, and endothelial cells) [19].

Tumor cells detected in the blood are referred to as circulating tumor cells (CTCs) [9]. CTCs are highly heterogeneous even if derived from the same patient [20]. Compared with classical diagnostic biopsies (e.g., fine needle biopsy), CTCs are able to provide a more representative image of tumor heterogeneity [21–23]. However, detecting CTCs is still challenging, as approximately one CTC is detectable among more than a million other blood cells (e.g., erythrocytes, leukocytes, and platelets), and CTCs have a short half-life of only 1–2.4 h [24, 25]. In addition to the number of CTCs, their genome, transcriptome, proteome and functional properties can be analyzed [26].

With respect to PDAC, a meta-analysis of 19 studies revealed that more than half of patients (707 out of 1320 patients analyzed) had detectable CTCs in their blood [27]. These patients had lower OS and PFS rates than CTC-negative patients did, highlighting the adverse prognostic effect of CTCs in PDAC patients. However, most patients in the studies included in the meta-analysis were in advanced tumor stages (stage III and IV: 61%), with only 31% in stage II and only 8% in stage I. The low number of CTCs, particularly in early PDAC stages, may lead to false negative results and low sensitivity [12, 28]. A potential explanation for the low CTC number in PDAC could be the filtration of CTCs in the liver before they reach the peripheral blood vessels and the reduced blood flow within the cancerous pancreas [29, 30]. This limits the analysis of CTCs as possible biomarkers for early diagnosis, but

with the emergence of novel, more sensitive analysis techniques (e.g., in vivo CTC capture devices [31]) and techniques that allow processing of larger volumes [32, 33], this limitation may be overcome [34]. Despite the impaired sensitivity that could arise from the different methods used or the heterogeneity of CTCs, the specificity of CTCs for PDAC diagnosis has been reported in several studies to reach > 90% [35–37].

In PDAC, cells make up only a small part of the tumor, while the largest part is the dense stroma that forms the tumor microenvironment [38]. Compared with other solid tumors, PDAC has the most pronounced desmoplastic stroma reaction, which generates a physical barrier around the tumor, thereby impairing radical resection and increasing therapy resistance [39, 40]. Although the composition and structure of the stroma varies between patients, it consists of several main components [41]. Noncellular components, including glycoproteins, fibronectins, collagens, and enzymes, form the extracellular matrix (ECM). The cellular components include endothelial, immune, and stromal cells, including pericytes, and local cancer-associated fibroblasts (CAFs). These host cells can also detach from the TME, enter the bloodstream, and be analyzed as possible liquid biopsy biomarkers for PDAC (e.g., as circulating CAFs (cCAFs)).

CAFs are key components of the TME, and they are near or in direct contact with cancer cells [38]. The three different major types of CAFs, myofibroblast CAFs (myCAFs), inflammatory CAFs (iCAFs), and antigen-presenting CAFs (apCAFs), are associated with distinct functions and phenotypes [42]. These functions include the production of cytokines, chemokines, metabolites, enzymes, and ECM molecules to prevent or promote tumor growth [43]. cCAFs are found in the blood of patients with various tumors, including PDAC, where they are linked to a poorer prognosis in advanced stages [44–46]. Only one study examined cCAFs in six PDAC patients and reported an association between the presence of cCAFs and poorer clinical outcomes as well as lower OS rates at metastatic stages [47]. However, to our knowledge, there are currently no studies on the role of cCAFs in the early stages of PDAC, and such studies are crucial for identifying their suitability as biomarkers for early PDAC diagnosis.

Circulating nucleic acids

Circulating tumor DNA (ctDNA) is a type of cell-free DNA (cfDNA) derived from tumor cells that can be found in the bloodstream. ctDNA can be released by cells undergoing apoptosis and necrosis or can be actively

transported through the cell membrane [48]. Since only up to 1% of the cfDNA in the blood of early-stage patients originates from tumors, most detectable circulating nucleic acids are cfDNA from noncancer cells, which limits the ability to detect ctDNA [49, 50]. The amount of ctDNA in the blood varies between different tumors and increases up to 40% in advanced tumor stages [50, 51]. The half-life of ctDNA is estimated to be between 16 and 114 min, which makes isolation more challenging [52, 53]. ctDNA can be detected due to specific alterations in the tumor and can be examined for mutations, DNA integrity, gene fusion, copy number variation, or methylation status [54, 55]. The high concordance between mutations in ctDNA and those in tumor tissue makes it suitable as a biomarker that provides information about the primary tumor even if it is inaccessible [56].

Analysis of genomic aberrations in all-stages PDAC tissue revealed a panel of four genes, namely, *KRAS*, *CDKN2A*, *TP53*, and *SMAD4*, with mutation frequencies of 90%, 90%, 70%, and 55%, respectively, as the main genomic drivers of PDAC [57–61]. Interestingly, mutations in these genes can be detected in preneoplastic PanIN lesions; notably, *KRAS* is the first event, and subsequent alterations in *CDKN2A*, *TP53*, and *SMAD4* can be detected in higher-grade PanINs [62, 63]. These mutations lead to increased proliferation, dysregulation of the cell cycle and an impaired DNA damage response [59]. As *KRAS* mutations are among the initiating mutations during the development of PDAC, *KRAS* mutations are interesting biomarkers for the early diagnosis of PDAC [64]. On the basis of the molecular profile of PDAC, several studies have used these mutations for ctDNA detection [64]. However, germline mutations in cfDNA or clonal hematopoiesis of indeterminate potential (CHIP) in noncancerous cells, especially related to *KRAS* (approximately 30%) [65], may lead to false-positive results and should be considered in related evaluations [55, 66, 67].

A meta-analysis of seven retrospective studies on the utility of ctDNA as a liquid biopsy biomarker revealed a sensitivity of 64% (95% CI 0.58–0.70), a specificity of 92% (95% CI 0.88–0.95), and an AUC of 0.9478 across all PDAC stages [28]. With approximately only one molecule of ctDNA in every 5 mL of plasma, the moderate sensitivity is presumably the result of minute amounts of released ctDNA, especially in the early tumor stages, when the rates of apoptosis and necrosis are lower [68, 69].

In addition to somatic cancer alterations, epigenetic traits (e.g., methylation, fragmentation) can also be examined in ctDNA. Epigenetic alterations have recently received much attention, as they may also provide

tissue-specific information that helps to determine the organ in which the tumor originates. Nicholson et al. analyzed the cfDNA methylation pattern in a prospective study of 5,461 participants with suspected cancer and were able to detect different tumors with a sensitivity of 66.3% in all stages and 24.4% in stage I patients, with a specificity of 98.4% [70]. A recent publication by García-Ortiz et al. reviewed studies analyzing the ctDNA methylation status in PDAC and concluded that the use of a single epigenetic biomarker does not allow for the diagnosis of early-stage PDAC and suggested that a multimarker panel would be more efficient [71]. Moreover, fragmentomic approaches focusing on fragment size, fragment ends, and end motifs can reveal differences between ctDNA and cfDNA [72]. ctDNA from cancer patients is shorter than nontumor cfDNA, and its features differ between different tumor entities, which enables the identification of the tissue of origin [73, 74]. Cristiano et al. were able to detect pancreatic tumors with a sensitivity of 71% at a specificity of 95% on the basis of the cfDNA fragment size [54]. These studies underscore the promising value of cfDNA-based approaches that are independent of the presence of genomic signatures.

In addition to cfDNA, cancer cells also release cell-free RNA (cfRNA) into the circulation [75, 76]. In addition to intracellular coding messenger RNAs (mRNAs), which are required for protein synthesis, noncoding RNAs, including microRNAs (miRNAs), are potential biomarker candidates [77]. cfRNAs are highly stable, as they are typically packed in extracellular vesicles or attached to lipid or protein complexes rather than circulating freely in the bloodstream [78–82].

miRNAs are noncoding, single-strand RNAs with an average length of 22 nucleotides that are highly evolutionarily conserved among various species [83]. miRNAs can regulate their target mRNAs at the posttranscriptional level by affecting their translation and stability [84–86]. In cancer patients, altered expression of miRNAs has been reported [87]. Numerous studies examining the role of various miRNAs as LB biomarkers in PDAC have been conducted and reviewed elsewhere [57]. In a meta-analysis, Peng et al. examined miRNAs from 46 studies involving 4,326 patients with pancreatic cancer [88]. The diagnostic performance of miRNA panels, which included 4.5 miRNAs on average (range: 2–12 miRNAs), was compared to that of single miRNAs and interestingly exhibited no significant diagnostic benefit. The combined results yielded a sensitivity of 79% (0.77–0.81), a specificity of 77% (0.75–0.79), and an AUC of 0.85 (0.81–0.87). Considering only early-stage PDAC (up to stage IIA), the diagnostic value decreased slightly to a sensitivity of 79% (0.76–0.82), a specificity of 74% (0.68–0.79), and an AUC of 0.81 (0.77–0.84) [88].

Proteins

Proteins are important for communication between cancer cells and host cells in the TME [89]. Proteins can be located on the membrane surface of cells but can also be secreted in vesicles or released into the circulation [90]. A wide range of different circulating proteins, including cytokines, chemokines, carbohydrate antigens, growth factors, inflammatory factors, glycoproteins, and apolipoproteins, orchestrate numerous biological processes [91–93]. Proteins released by cancer cells can regulate the development and progression of cancer by promoting invasion and metastasis [89]. Several proteins are up- or downregulated in the blood of PDAC patients compared to that of healthy donors or benign tumor patients [94]. Hence, many circulating proteins have been analyzed as potential biomarkers for PDAC and are reviewed in more detail elsewhere [95]. However, interestingly, the sensitivity and specificity of most single proteins do not exceed those of CA19-9 [5]. A meta-analysis by Kane et al. compared 250 prospective and retrospective studies published before July 2020 on all stages of PDAC; the results revealed an AUC of 0.85 for CA19-9 alone and 0.783 for novel single biomarkers [96].

Extracellular vesicles

Extracellular vesicles (EVs) are lipid-bound and secreted particles that comprise three classes of vesicles: exosomes (30–150 nm), microvesicles (50–1000 nm), and apoptotic bodies (500–5000 nm). EVs can be released by several cell types, including neurons, epithelial cells, and fibroblasts, as well as cancer cells [97–99]. Exosomes are particularly interesting for liquid biopsy approaches because they contain many molecules, including lipids, metabolites, nucleic acids (e.g., miRNAs and mRNAs), and proteins, that are protected from degradation by the EV membrane [100]. As exosomes transfer these molecular cargoes to recipient cells through cell–cell interactions or even over large distances, e.g., between different organs, they are important for cellular communication [101].

Exosomes influence tumor malignancy by regulating the tumor microenvironment, angiogenesis, tumor growth, invasion, and metastasis, including epithelial–mesenchymal transition, immunomodulation, and chemoresistance [98, 102–106]. Moreover, cancer cells, including PDAC cells, secrete more exosomes than non-cancerous cells [107, 108].

In a meta-analysis on the potential utility of extracellular vesicle cargo as biomarkers for PDAC, Jia et al. examined 39 studies including 2,037 PC patients [109]. Seventeen studies on EV RNAs, 16 on EV proteins, and 16 on EV biomarker panels were evaluated across all tumor stages. The most reported molecules were the EV RNAs miR-21 and miR-10b and the EV proteins GPC1

and EphQ2. A sensitivity of 84% (95% CI: 81–86%) and a specificity of 89% (95% CI: 86–91%) were obtained from the pooled values of EV RNAs and EV proteins. In contrast to analysis of the previously described biomarker types, the analysis of EVs in early PDAC stages I and II led to an increased sensitivity of 90% (95% CI: 87–93%) and specificity of 94% (95% CI: 92–95%). Interestingly, EVs as markers seem to perform at least as well and possibly even better in earlier stages (although only modestly with almost overlapping CIs) than in advanced stages [109]. One potential explanation could be that patients with advanced PDAC suffer from dysregulated EV secretion due to cancer-related effects, including cachexia and dysregulated metabolic processes. These findings indicate that exosomes and their cargo are potential biomarkers for the early diagnosis of PDAC.

Multimarker analysis

Numerous single biomarkers for the diagnosis of PDAC have been investigated, as a single marker can facilitate diagnostic assay development and implementation in routine clinical practice. However, the investigated markers have low sensitivity and specificity for diagnosis. Considering the high degree of patient diversity and tumor heterogeneity, a multimarker panel can provide complementary value and seems to perform better than single biomarkers do.

The literature search yielded 57 papers that analyzed multibiomarker panels in blood to diagnose PDAC. As some publications included two different panels, the total number of multibiomarker panels assessed was 63. Among these panels, 57 included proteins, 10 included RNA, 6 included EVs, 4 included cfDNA, 2 included metabolites, and 1 included CTCs. An overview of these studies [16, 110–165] can be found in Table 1.

Many publications started by analyzing single biomarkers and later combined them with one or more other biomarkers. The addition of biomarkers led to increased sensitivity and specificity and improved AUC values in these studies. For example, Capello et al. calculated an AUC of 0.730 for TIMP1, 0.832 for LRG2, and 0.821 for CA19-9 to distinguish early-stage PDAC patients from healthy controls [133]. The combination of all three protein markers increased the AUC to 0.887, and adding 5 metabolites to the protein panel further increased the AUC to 0.924 [161]. This observation was quantified in the above-mentioned meta-analysis by Kane et al. [96]. The pooled AUC for studies with single biomarkers was 0.803, which was significantly lower than the multibiomarker panel AUC of 0.898. However, this analysis was performed on all stages of PDAC and did not focus particularly on the early stages.

The investigated biomarkers were combined with CA19-9 analysis in 55 of the 63 studies, and only 8 studies did not include CA19-9 in their panel [129, 150–155, 165]. Adding CA19-9 to other biomarkers improved the diagnostic power. For example, Dong et al. examined the proteins POSTN and CA242 in early-stage PDAC patients versus healthy controls and reported an AUC of 0.92 for their combination [139]. The addition of CA19-9 to the panel increased the AUC to 0.98.

Most biomarker panels included only protein markers (46 out of 63 studies) that were analyzed directly from the blood or isolated from EVs. In the studies on early-stage PDAC, the AUCs ranged from 0.76–0.98 (Fig. 3). The protein panels consisted of two proteins in 7 studies, three proteins in 11 studies, and four or more proteins in 5 studies, although the number of proteins did not appear to directly correlate with the reported AUC. The biomarker panels included numerous different proteins, whereas only some proteins, including CA19-9, CEA, and MUC5AC, were found in several panels. Hinestroza et al. isolated EVs from the blood of early PDAC patients and healthy controls and analyzed a panel of 13 proteins within EVs, resulting in a sensitivity of 95.7% and specificity of 99.5% [147].

Only five studies focused on other types of biomarkers, namely, cfDNA [150, 151] or RNA [152–154]. Eissa et al. analyzed the cfDNA methylation pattern of the *BNC1* and *ADAMTS1* genes and reported the ability to distinguish early-stage PDAC patients from mixed controls, with an AUC of 0.95 [150, 151]. Ganepola et al. compared the miRNAs miR-642b, miR-885-5p, and miR-22 between stage II PDAC patients and healthy controls as well as high-risk patients, resulting in an AUC of 0.97 [152]. A prospective study analyzing the 2'-O-methylated miRNAs miR-28-3p, miR-143-3p, and miR-151a-3p in 135 individuals was performed by Yang et al. The panel identified 20 out of 28 early-stage PDAC patients, resulting in an AUC of 0.81 [154].

Furthermore, several studies have investigated multiomic panels by combining analyses of CTCs, cfDNA, metabolites, or miRNAs with proteins [155–165]. The only reviewed study that included CTCs for PDAC diagnosis was performed by Chen et al. [159]. The authors isolated and quantified CTCs from whole blood and added CA19-9 analysis to distinguish all-stage PDAC patients from mixed controls with an AUC of 0.95. Two studies analyzed cfDNA in combination with different proteins: Cohen et al. focused on four proteins (CA19-9, CEA, HGF, OPN) and *KRAS* mutations in cfDNA, distinguishing early-stage PDAC patients from healthy controls with a sensitivity of 64% and a specificity of 99.5% [160]. In a different cfDNA analysis approach, Berger et al. quantified ctDNA and

Table 1 Overview of multimarker studies for the (early) diagnosis of PDAC using liquid biopsy

Biomarker	SN [%]	SP [%]	AUC	Total patients [N] (female)	Stage	Control	Detection method	Fluid	Country	Reference
Protein panels—all PC stages										
CA19-9+OPN+CHI3L1	93	81	N/A	52 PDAC (N/A)	II, III	43 HC	PLA	plasma	USA	Chang, 2009 [110]
CA19-9+TFPI	84	90	0.94	37 PDAC (15)	I:0,II:1,III:2,IV:9,N/A:25	15 HC	ELISA	plasma	USA	Balaseshthil, 2011 [111]
CA19-9+ICAM-1+OPG	78	94	0.91	173 PDAC (95)	I:4,II:37,III:20,IV:41,N/A:71	120 HC	MIA	serum	USA	Brand, 2011 [112]
CA19-9+CEA+TIMP-1	71	89	0.83	173 PDAC (95)	I:4,II:37,III:20,IV:41,N/A:71	70 BD	MIA	serum	USA	Brand, 2011 [112]
CA19-9+Cathepsin D+MMP-7	89	77	0.91	139 PDAC (50)	N/A	74 HC, 72 CP	MIA	serum	South Korea	Park, 2012 [113]
CA19-9+AACT+THBS1+HPT	77	90	0.92	37 PC (21)	I:A:3,IB:1,IIA:6,IIIB:8,IIIC:2,IV:17	30 HC, 30 DM, 30 PC, 30 CP, 22 OJ	ELISA	serum	USA	Nie, 2014 [114]
CA19-9+CEA+Cyfra 21-1	30	95	0.68	135 PC (56)	N/A	540 HC	MIA	serum	USA	Nolen, 2014 [115]
CA19-9+IL-6+IP-10+PDGF	81	92	0.88	43 PDAC (19)	resectable:31, advanced:12	7 BD	ELISA+cytokine assay	serum	UK	Shaw, 2014 [116]
CA19-9+IL-6+IP-10+IL-8	N/A	N/A	0.91	43 PDAC (19)	resectable:31, advanced:12	17 CP	ELISA+cytokine assay	serum	UK	Shaw, 2014 [116]
CA19-9+AACT+THBS1+SAAV peptide	100	96	0.99	26 PDAC (15)	IA:2 ⁺ , IB:1 ⁺ , IIA:3 ⁺ , IIB:7 ⁺ , IV:24 ⁺	27 HC	ELISA+MS	serum	USA	Nie, 2014 [117]
CA19-9+sLeX	76	78	N/A	109 PC (N/A)	N/A	30 BD	ELISA	plasma	USA	Tang, 2015 [118]
CA19-9+APOE+IIH3+APOA1+APOL1	95	94	0.99	40 PC (N/A)	N/A	34 HC	ELISA+MS	serum	China	Liu, 2017 [119]
CA19-9+FWII+fibrinogen+albumin+conjugated bilirubin+ALP	N/A	N/A	0.95	67 PDAC (36)	I-III	18 IPMN	coagulation assay	plasma	Finland	Mattila, 2018 [120]
CA19-9+TSP-2	91	99	0.95	263 PDAC (140)	I:II:57, III:IV:206	230 IAR	ELISA	serum	Taiwan	Peng, 2019 [121]
CA19-9+sTRA	54	95	0.86	71 PDAC (31)	I:17, II:40, III:5, IV:9	20 HC, 9 PC, 15 CP, 8 BD, 24 DM	ELISA	plasma	USA	Staal, 2019 [122]
CA19-9+AGP1	89	97	0.96	52 PC (23)	N/A	34 HC	immunoturbidimetry, ELISA	serum	Sweden	Zhou, 2019 [123]
CA19-9+CA125+CEA+APOA1+APOA2+TTR	93	96	0.99	60 PDAC (18)	I:18, II:24, III:10, IV:8	191 HC	immunoturbidimetry, ELISA	plasma	South Korea	Kim, 2020 [124]
CA19-9+CLU+C5+KLKB1+PPBP+IFRD1+IGFBP2+ICAM1+C4BPA+PTPRJ+ECM1+VIM+C4BPB+SERPINA5+TTR	86	97	0.97	65 PDAC (27)	IA:1, IB:0, IIA:11, IIB:15, IIC:10, IV:93	59 HC, 13 BD	MS	plasma	South Korea	Kim, 2021 [125]
CA19-9+S100A2+S100A4+CA125	N/A	N/A	0.91	120 PDAC (59)	I+II:9, III+IV:111	80 HC	ELISA	serum	Australia	Mehta, 2021 [126]
CEACAM5+TLR3+MUC16+THBS2+FASLG+CTSV+VEGEFA+FCRLB+ERBB4+ANXA1+ERBB2+OCP+NT5E+CCN1+TGFA	92	90	0.91	218 PDAC (N/A)	N/A	249 NC	ELISA, OLINK	serum	UK	Nené, 2023 [127]
CA19-9+CEA+CA125	82	85	0.90	65 PDAC (N/A)	N/A	700 HC	ECLIA	serum	China	Cao, 2023 [128]
IL-7R+PLD4+ID3	73	79	0.76	33 PDAC (N/A)	N/A	80 BD	RT-PCR	plasma	USA	Jang, 2023 [129]
Protein panels—early PC stages (stage I-II)										
CA19-9+SYCN+REG1B	39	95	0.87	20 PDAC (14)	I, II	92 HC	ELISA	plasma	Canada	Makawita, 2013 [130]
CA19-9+SYCN+REG1B	68	95	0.92	40 PDAC (18)	I, II	47 HC	ELISA	serum	Canada	Makawita, 2013 [130]

Table 1 (continued)

Biomarker	SN [%]	SP [%]	AUC	Total patients [N] (female)	Stage	Control	Detection method	Fluid	Country	Reference
CA19-9+CA125+LAMC2	78	63	0.76	27 PDAC (N/A)	IA:5, IB:5, IIA:17	17 BD	ELISA	serum	Canada	Chan, 2014 [16]
CA19-9+sLeX+type 1 N-acetyl-lactosamine	80	84	N/A	50 PDAC (27)	I:3, II:47	10 BD	ELISA	plasma	USA	Tang, 2016 [131]
CA19-9+IGFBP2+IGFBP3	N/A	N/A	0.90	38 PDAC (14)	I:4, II:34	65 HC	MS	plasma	Japan	Yoneyama, 2016 [132]
CA19-9+LRG1+TIMP1	67	95	0.89	39 PDAC (18)	IA:6, IB:10, resectable (no TNM data):23	82 HC	ELISA, MS	plasma	USA	Capello, 2017 [133]
CA19-9+TFPI+TNC-FN III-C	75	82	0.83	98 PDAC (55)	IA:7, IB:8, II:1, IIA:40, IIB:42	61 HC	ELISA	plasma	USA	Balaseshthil, 2017 [134]
CA19-9+MUC5AC	75	83	0.84	63 PC (N/A)	IA:2, IB:4, IIA:18, IIB:46	35 HC, 30 BD, 43 CP	ELISA, RIA	serum	USA	Kaur, 2017 [135]
CA19-9+THBS2	N/A	N/A	0.96	88 PDAC (43)	I:4, IA:2, IB:4, IIB:37, IIA:15, IIB:26	140 HC	ELISA	plasma	USA	Kim, 2017 [136]
CA19-9+LRG1+TTR	78	94	0.91	50 PDAC (N/A)	I:4, II:46	68 HC, 21 BD	ELISA	plasma	South Korea	Park, 2017 [137]
CA19-9+MMF7	N/A	N/A	0.98	25 PDAC (N/A)	IA:2, IB:1, IIA:22	131 HC	ELISA, MIA	plasma	Italy	Resovi, 2018 [138]
CA19-9+CCN2+Col4+FN+PLG	N/A	N/A	0.92	25 PDAC (N/A)	IA:2, IB:1, IIA:22	30 CP	ELISA, MIA	plasma	Italy	Resovi, 2018 [138]
CA19-9+POSTN+CA242	92	97	0.98	38 PDAC (N/A)	IA:3, IB:5, IIA:30	37 HC	ELISA	serum	China	Dong, 2018 [139]
CA19-9+MIA	N/A	N/A	0.86	96 PDAC (N/A)	IA:13, IB:18, IIA:17, IIB:48	68 CP	MIA	serum	USA	Song, 2019 [140]
CA19-9+MIC-1	N/A	N/A	0.81	96 PDAC (N/A)	IA:13, IB:18, IIA:17, IIB:48	63 IPMN	MIA	serum	USA	Song, 2019 [140]
CA19-9+TFI1+TF2+TF3	33	100	0.85	18 PC (N/A)	I, II	8 BD	ELISA	serum	USA	Jahan, 2019 [141]
CA19-9+MUC5AC	77	89	0.89	30 PDAC (14)	N/A	34 HC, 29 BD, 35 Cholecholethiasis, 25 CP	ELISA, EIA	serum	China	Zhang, 2020 [142]
CA19-9+APOA4+CD14+CLEC3B+GSN+HRG+ITIH3+KLKB1+LRG1+SERPINF1+SERPING1+TIMP1	80	80	0.85	50 PDAC (N/A)	IA:3, IB:1, IIA:11, IIB:35	49 DM	ELISA, MS	plasma	USA	Peng, 2020 [143]
CA19-9+LRG1+TTR	92	88	N/A	248 PDAC (N/A)	I:20, II:228	347 HC/gallstones/cholecystitis	ELISA	plasma	South Korea	Choi, 2021 [144]
CA19-9+LRG1+TTR	94	91	N/A	657 PDAC (N/A)	I:39, II:618	609 HC	ELISA	plasma	South Korea	Lee, 2023 [145]
CA19-9+CA125	72	80	0.81	56 PDAC (N/A)	IA:5, IB:6, IIA:9, IIB:36	53 IPMN	ECLIA	serum	USA	Song, 2021 [146]
EV proteins: CA19-9+CA125+Cathepsin D+Ferritin+sE-selectin+IGFBP3+MIA+CA15-3+sFAS+TIMP1+sNeuropilin-1+MPO+bHCC	96	100	N/A	47 PDAC (33)	I:22, II:25	184 HC	IA	plasma	USA	Hinestrosa, 2022 [147]
CA19-9+CEA+ALCM+ANG+AXL+BAG3+BSG+CEACAM+COL18A1+EPCAM+HA+HP+ICAM+IGFBP2+IGFBP4+LCN2+LRG1+MMP2+MMP7+MMP9+MSLN+PARK7+PPBP+PRG4+SPRCL1+SPPI1+TGFB1+THBS1+TIMP1+TNFRSF1A+WEGFC	63	97	0.94	30 PDAC (12)	I:4, IIA:8, IIB:18	103 HC	ELISA	serum	USA	Firpo, 2023 [148]
DNA panels CA19-9+PIGR+YWF	N/A	N/A	0.98	28 PDAC (N/A)	IA:4, IB:5, IIB:2, IIB:17	28 HC	ELISA	serum	Australia	Byeon, 2024 [149]
BNC1+ADAMTS1 methylation	81	85	N/A	10 PC (N/A)	I:10	26 HC	PCR, MOB	serum	USA	Yi, 2013 [150]
BNC1+ADAMTS1 methylation	95	92	0.95	37 PDAC (13)	I:8, IIA:9, IIB:20	95 HC, 8 pan-creatitis	PCR	plasma	USA	Eissa, 2019 [151]

Table 1 (continued)

Biomarker	SN [%]	SP [%]	AUC	Total patients [N] (female)	Stage	Control	Detection method	Fluid	Country	Reference
RNA panels										
miR-642b + miR-885-5p + miR-22	91	91	0.97	11 PDAC (6)	II	11 HC, 11 IAR	qRT-PCR	plasma	USA	Ganepola, 2014 [152]
EV miRNA: miR-93-5p + miR-339-3p + miR-425-5p + miR-425-3p	80	95	0.89	15 PDAC (2)	I:3, II:2, III:3, IV:7	19 C	qRT-PCR	plasma	USA	Makler, 2023 [153]
miR-28-3p, miR-143-3p, and miR-151a-3p	N/A	N/A	0.81	14 PDAC (N/A)	I:8, II:6	107 C	qRT-PCR	plasma	China	Yang, 2024 [154]
multi-omics panels—all PC stages										
CD44v6 + Tspan8 + EpcAM + MET + CD104 + miR-1246 + miR-4644 + miR-3976 + miR-4306	100	80	N/A	131 PC (76)	N/A	30 HC, 20 BD, 25 C, 12 non-PC	qRT-PCR, flow cytometry	serum	Germany	Madhavan, 2015 [155]
CA19-9 + MIC-1 + miR-21	88	99	0.97	82 PC (35)	I:1, II:7, III:5, IV:23	88 HC	RT-PCR, ELISA	plasma	China	Yuan, 2016 [156]
CA19-9 + MIC-1 + miR-25	84	100	0.97	82 PC (35)	I:1, II:7, III:5, IV:23	88 HC	RT-PCR, ELISA	plasma	China	Yuan, 2016 [156]
CA19-9 + THBS2 + ctDNA quantification	87	92	0.94	52 PDAC (26)	I:14, II:17, III:21	15 IPMN, 32 pancreatitis	ELISA, Qubit	plasma	Germany	Berger, 2019 [157]
CA19-9 + EV: miR200b + miR200c	92	100	0.97	56 PDAC (20)	IIA:4, IIB:14, III:22, IV:24	22 HC + 11 CP	PCR, western blot	serum	Germany	Reese, 2020 [158]
CA19-9 + CTC quantification	91	91	0.95	80 PDAC (29)	I + II:40, III + IV:40	34 HC, 32 AP, 22 BD	NE-ImFISH, ECLIA	whole blood, plasma	China	Chen, 2022 [159]
Multi-omics panel—early PC stages (stage I-II)										
CA19-9 + CEA + HGF + OPN + ctDNA: KRAS mutation	64	100	N/A	221 PDAC (100)	I:29, II:192	182 HC	PCR, MIA	plasma	multi-center	Cohen, 2017 [160]
CA19-9 + LRG1 + TIMP1 + acetylspermidine + diacetylspermine + indole-derivative + two lysophosphatidylcholines	90	88	0.92	39 PDAC (18)	IA:6, IB:10, resectable (no staging data)23	82 HC	ELISA, MS	plasma	USA	Fahrman, 2019 [161]
CA19-9 + cf miRNA: miR30c-5p, miR340-5p, miR335-5p, miR23b-3p, miR142-3p + EV miRNA: miR145-5p, miR200b-3p, miR429, miR1260b, miR145-3p, miR216b-5p, miR200a-3p, miR217-5p	84	99	0.99	91 PDAC (N/A)	I:36, II:55	67 HC	qRT-PCR, ELISA	plasma + serum	USA, Japan, South Korea	Nakamura, 2022 [162]
CA19-9 + proline + creatine + palmitic acid	86	82	0.95	22 PDAC (11)	IA:2, IB:6, IIA:2, IIB:12	27 HC	MS	serum	China	Zhao, 2023 [163]
CA19-9 + hsa_circ_0060733 + hsa_circ_0006117 + hsa_circ_0064288 + hsa_circ_0007895 + hsa_circ_0007367	77	95	0.94	63 PDAC (N/A)	I, II	46 HC	qRT-PCR	plasma	USA	Xu, 2023 [164]
EV: CD63 + GPC-1 + HER2 + snRNA U6 + GPC-1 mRNA + miR-10b	N/A	N/A	0.93	15 PDAC (8)	IA:1, IB:9, IIA:3, IIB:2	15 HC	Co-PAR	plasma	China	He, 2024 [165]

Abbreviations: AC Acute pancreatitis, AUC Area under the curve, BD Benign disease, C Control (undefined), Co-PAR Codetection platform of Proteins and RNAs, CP Chronic pancreatitis, ECLIA electrochemiluminescence-based immunoassay, EIA Enzyme immunoassay, ELISA Enzyme-linked immunosorbent assay, DM Diabetes mellitus, HC Healthy control, IAR Individual at risk, IPMN Intraductal papillary mucinous neoplasm, MIA Magnetic immunoassay, MS Mass spectrometry, N/A Not available, PC Pancreatic cancer, PCR Polymerase chain reaction, PDAC Pancreatic adenocarcinoma, SN Sensitivity, SP Specificity, † As only TNM stage data were present in the original study, staging was assessed according to the 8th edition of the UICC staging manual

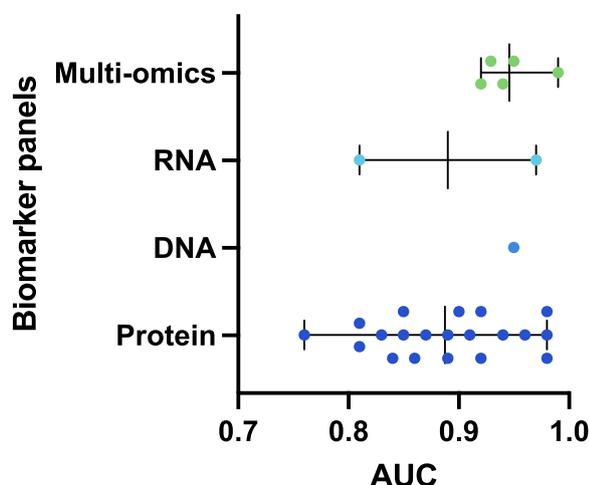


Fig. 3 Distribution of the AUC values of biomarker panels in identifying stage I and II PDAC patients. The AUC values derived from Table 1 for the biomarker panels for early-stage PDAC are plotted. The figure includes biomarker panels consisting of combinations of proteins (20 studies), DNA (1 study), RNA (2 studies) or multiomic markers (5 studies)

analyzed CA19-9 and THBS2 to differentiate between all-stage PDAC, IPMN and pancreatitis, with an AUC of 0.94 [157]. Metabolites were included in the studies of Fahrman et al. and Zhao et al. to distinguish early-stage PDAC patients from healthy controls. The biomarker panel of Fahrman et al. consisted of five metabolites (acetylspermidine, diacetylspermine, an indole-derivative, and two lysophosphatidylcholines) and three proteins (CA19-9, LRG1, and TIMP1), resulting in an AUC of 0.924 [161]. Zhao et al. combined three metabolites (proline, creatine, and palmitic acid) with CA19-9, and this panel had an AUC of 0.949 [163]. Seven studies involved combined analysis of proteins with cell-free RNAs or miRNAs isolated from EVs [155, 156, 158, 162, 164, 165]. Nakamura et al. combined CA19-9 with 5 cell-free miRNAs (miR30c-5p, miR340-5p, miR335-5p, miR23b-3p, and miR142-3p) and 8 EV-derived miRNAs (miR145-5p, miR200b-3p, miR429, miR1260b, miR145-3p, miR216b-5p, miR200a-3p, and miR217-5p) [162]. This biomarker panel had an AUC of 0.99 for distinguishing early-stage PDAC patients from healthy controls, indicating that it was the most precise diagnostic panel among all reviewed studies on early-stage PDAC. However, there were some limitations to this study, such as the modest sample size ($n=91$) and the lack of age-matched control groups, which need to be addressed before the biomarker panel can be applied in the clinic.

The analyzed biomarkers were tested on early-stage PDAC patient samples in 34 of the 63 studies and had AUCs in the range of 0.76–0.99 (Fig. 3). The protein panels had the lowest mean AUC of 0.89, and the range of AUC values was the widest. The multiomic panels had the highest mean AUC of 0.95, with a small range from 0.92–0.99, indicating that combining different omic biomarkers yields greater statistical power. However, compared with single markers or panels with only one type of marker (e.g., protein), multimarker panels, particularly multiomic panels, involve more elaborate integrative assays with potentially increased development time and greater complexity.

These studies indicate that several biomarkers perform well in detecting early-stage PDAC. However, for these markers to be used for the screening of risk groups, the sensitivity and specificity need to be increased to minimize the number of false positive and negative diagnoses. In particular, high sensitivity is difficult to reach, as PDAC shares numerous biomarkers and mutations (e.g., RAS mutations) with other diseases (e.g., colorectal cancer), benign diseases of the pancreas (e.g., pancreatitis) or its precancerous lesions (e.g., IPMN), and these other diseases have higher prevalence in the general population than PDAC [166, 167]. Diseased controls (e.g., those with precancerous conditions, pancreatitis, and pancreatic cysts) and individuals at risk for developing PDAC should be included in studies to minimize false-positive rates and gain further knowledge of the molecular tumorigenesis of PDAC. On the basis of these assumptions, screening for PDAC in the general population could be implemented in pancancer screening efforts rather than as a specific test for PDAC. A panel of multiple markers could be used to screen for several cancer types at the same time and therefore be used on a broader group of individuals. Two multimarker panel-based tests, CancerSEEK [168] and Galleri (GRAIL) [169–172], were developed to detect the early stages of multiple tumors, including PDAC. The CancerSEEK multimarker panel includes ctDNA and eight proteins (CA-125, CEA, CA19-9, PRL, HGF, OPN, MPO, and TIMP-1) to detect ovarian, liver, stomach, pancreatic, esophageal, colorectal, lung and breast tumors. Currently, the CancerSEEK test is only used in clinical trials (NCT04213326). The Galleri test by GRAIL, which is already commercially available, analyzes the whole-genome methylation of cfDNA to detect signals of more than 50 cancer types. In an independent validation set of 4,077 individuals, the test was able to identify 35 of 41 patients with early-stage pancreatic cancer, resulting in a sensitivity of approximately 60% at 99.5% specificity [171]. Moreover, in

another prospective study of 6,662 participants, the Galleri test detected a suspicious positive cancer signal in 92 cases [172]. After 12 months of follow-up, 35 of these 92 participants (38%) were confirmed to have a true positive cancer diagnosis, and 6,235 of the 6,549 (95.5%) participants without a cancer signal had true negative results, highlighting the feasibility of multicancer early detection (MCED) testing. Moreover, for the first time, this study assessed the subsequent diagnostic pathways and time to diagnostic resolution [172]. Notably, these studies by Schrag et al., are highly important for emphasizing the clinical utility of screening approaches (e.g., MCED tests), even in nonrisk groups.

Another study on the methylation of cfDNA, the THUNDER study, yielded high sensitivity for advanced stages but only approximately 35% sensitivity for stages I and II, with 98.9% specificity for detecting pancreatic tumors [173]. Although the specificity of the tests is sufficient, the sensitivity for early-stage diagnosis is lower than that of the PDAC-specific panels outlined in this review and therefore indicates that high-risk groups may benefit from a PDAC-specific screening approach. Moreover, the number of false-negative results leads to high costs for further diagnosis and insecurities for tested individuals. However, for some cancer types, the sensitivity reached relatively high values (e.g., 100% (CancerSEEK), 100% (Galleri), and 75% (THUNDER) for diagnosing stage I liver cancer). With respect to the diagnosis of advanced stages, the sensitivity was 80% to 100% for several cancer types in all tests. Further investigations on early-stage cancers are necessary to establish a reliable multicancer test, which would greatly improve the diagnosis of cancer.

In many of the reported PDAC studies in this review, several limitations affected the results of the analyses. Only 34 of the 63 studies assessed the analyzed biomarkers in early-stage PDAC patient samples. Notably, surgery is most efficient treatment in the early stages of PDAC and can significantly improve the OS of patients [5, 174, 175]. Thus, studies focusing on samples of early-stage patients are urgently needed to find suitable biomarkers for early diagnosis. Moreover, many studies analyzing single or multiple biomarkers had small sample sizes, resulting in low statistical power. Considering the high heterogeneity of patients and tumors, large cohorts (optimally from multiple centers) are needed to reliably assess biomarkers. Another limitation of most studies is the retrospective study design. Although many samples were collected prospectively, the analysis was performed retrospectively on chosen samples. Only three biomarker panels were tested in a prospective study [115, 129, 176], yielding a higher level of medical

evidence. Another important aspect for future studies is the standardization of preanalytical factors as well as methods used for detection. Multicenter evaluation of CTCs, DNAs, and miRNAs in standardized blood samples revealed significant differences between the technologies used at different centers [177–179], which prevents the comparison of results and thereby limits the development of novel diagnostic assays. Therefore, it is necessary to use standardized protocols for the handling of samples and the performance of assays to improve the quality of studies. Several international liquid biopsy consortia and societies, including the European Liquid Biopsy Society (ELBS) and the International Liquid Biopsy Standardization Alliance (ILSA), are currently collaborating on this task. The establishment of reference and uniform cutoff values for promising biomarkers is important for performing randomized prospective studies to obtain more robust medical evidence. These prospective studies must be hypothesis-driven and have defined enrollment criteria to avoid the risk of missing many specific features related to the complex clinical condition of pancreatic cancer to address unmet needs. Moreover, after these studies have been completed, it is highly important to systematically review or conduct a meta-analysis of the available studies to select the best features and guide further diagnostic assay development.

Conclusion and perspectives

This review of studies on using multimarker panels in blood samples to diagnose PDAC revealed that the use of panels of multiple biomarkers compared with single biomarkers improved the diagnostic power. Most studies have been performed on protein panels, whereas only a few have analyzed other biomarker types or even multiomic panels. Although many biomarkers had low diagnostic power alone, the combination of these biomarkers with CA19-9 increased the diagnostic power; thus, CA19-9 is part of many multibiomarker panels. For future studies, it is crucial to conduct prospective studies with standardized methods and to use samples of patients in the early stages to enable the development of a biomarker panel for early-stage PDAC diagnosis, allowing early therapeutic intervention. Owing to the low number of early-stage PDAC patients, collaborative efforts in a multicenter setting are needed. Currently, 361 ongoing studies on early-stage diagnosis of pancreatic cancer are listed at clinicaltrials.gov, illustrating the unmet need for reliable early detection methods. More than 40% of these ongoing studies (152 studies) include liquid biopsy-based diagnostics, and four of these liquid biopsy-based studies are MCED tests that utilize DNA methylation or multiomic panels.

One of these recent liquid biopsy-based studies was initiated by the EU-funded PANCAID consortium [180]. Researchers in the PANCAID consortium have committed their research toward finding novel minimally invasive multimarker panels for early PDAC detection. Findings related to the early-stage detection of primary disease may also apply to the early diagnosis of minimal residual disease in cancer patients who have undergone treatment with curative intent after diagnosis [181, 182]. The European Consortium GUIDE.MRD is currently tackling this ambitious task for the detection of ctDNA in patients with pancreatic, colorectal, and lung cancers [183].

Abbreviations

AC	Acute pancreatitis
apCAFs	Antigen-presenting CAFs
AUC	Area under the curve
BD	Benign disease
CA19-9	Carbohydrate antigen 19–9
CAF	Cancer-associated fibroblast
cCAF	Circulating CAF
cfDNA	Cell-free DNA
cfRNA	Cell-free RNA
CHIP	Clonal hematopoiesis of indeterminate potential
CP	Chronic pancreatitis
CTC	Circulating tumor cell
ctDNA	Circulating tumor DNA
DM	Diabetes mellitus
ECLIA	Electrochemiluminescent-based immunoassays
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
EV	Extracellular vesicle
HC	Healthy control
IA	Immunoassay
IAR	Individuals at risk
iCAF	Inflammatory CAF
IPMN	Intraductal papillary mucinous neoplasm
LB	Liquid biopsy
MCED	Multi-cancer early detection
MCN	Mucinous cystic neoplasm
MIA	Magnetic immunoassay
miRNA	MicroRNAs
MOB	Methylation on beads
mRNA	Messenger RNA
MS	Mass spectrometry
myCAF	Myofibroblast CAF
OS	Overall survival
PanIN	Pancreatic intraepithelial neoplasm
PC	Pancreatic cyst
PCR	Polymerase chain reaction
PDAC	Pancreatic ductal adenocarcinoma
PFS	Progression-free survival
PLA	Proximity ligation assay
RIA	Radioimmunoassay
SN	Sensitivity
SP	Specificity
TME	Tumor microenvironment

Acknowledgements

This work was funded by the European Union. Views and opinions expressed are those of the authors only and do not necessarily reflect those of the European Union or European Health and Digital Executive Agency (HADEA). Neither the European Union nor the granting authorities can be held responsible for them.

We would like to thank Sian Haynes-Ryterski for proofreading the manuscript.

Authors' contributions

K.-L.R. reviewed the literature and wrote the original draft of the manuscript. K.P. and D.J.S. reviewed the literature and critically revised the original draft. All authors have read and agreed to the published version of the manuscript.

Funding

Open Access funding enabled and organized by Projekt DEAL. K.P. received grant/research support from the EU/IHI GUIDE.MRD (No. 101112066), EU Horizon PANCAID (No. 101096309) and ERC Grant INJURMET (No. 834974). The position of K.-L.R. and in part the position of D.J.S. were funded through the PANCAID project within the EU Horizon Mission Cancer Program (No. 101096309).

K.P. and D.J.S. received funding from the Fleur Hiege Center for Skin Cancer Research (funded by the Hiege Stiftung) outside of this work.

Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

K.P. received honoraria outside of this work from NRich, BMS, Agena, Menarini, Novartis, Sanofi, Illumina, Abcam, MSD, Eppendorf, and Hummingbird. K.-L.R. and D.J.S. declare that they have no competing interests.

Received: 18 March 2024 Accepted: 16 August 2024

Published online: 02 September 2024

References

- Liou GY, Byrd CJ. Diagnostic bioliquid markers for pancreatic cancer: what we have vs. what we need. *Cancers (Basel)*. 2023;15(9):2446.
- Maisonneuve P, Lowenfels AB. Risk factors for pancreatic cancer: a summary review of meta-analytical studies. *Int J Epidemiol*. 2014;44(1):186–98.
- Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin*. 2021;71(3):209–49.
- Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. *CA Cancer J Clin*. 2021;71(1):7–33.
- Al-Shaheri FN, Alhamdani MSS, Bauer AS, Giese N, Büchler MW, Hackert T, et al. Blood biomarkers for differential diagnosis and early detection of pancreatic cancer. *Cancer Treat Rev*. 2021;96: 102193.
- Michl P, Löhr M, Neoptolemos JP, Capurso G, Rebours V, Malats N, et al. UEG position paper on pancreatic cancer. Bringing pancreatic cancer to the 21st century: Prevent, detect, and treat the disease earlier and better. *United European Gastroenterol J*. 2021;9(7):860–71.
- Neoptolemos JP, Stocken DD, Friess H, Bassi C, Dunn JA, Hickey H, et al. A randomized trial of chemoradiotherapy and chemotherapy after resection of pancreatic cancer. *N Engl J Med*. 2004;350(12):1200–10.
- Chhoda A, Lu L, Clerkin BM, Risch H, Farrell JJ. Current approaches to pancreatic cancer screening. *Am J Pathol*. 2019;189(1):22–35.
- Pantel K, Alix-Panabières C. Circulating tumour cells in cancer patients: challenges and perspectives. *Trends Mol Med*. 2010;16(9):398–406.
- Kamyabi N, Bernard V, Maitra A. Liquid biopsies in pancreatic cancer. *Expert Rev Anticancer Ther*. 2019;19(10):869–78.
- Lianidou E, Pantel K. Liquid biopsies. *Genes Chromosomes Cancer*. 2019;58(4):219–32.
- Alix-Panabières C, Pantel K. Liquid biopsy: from discovery to clinical application. *Cancer Discov*. 2021;11(4):858–73.

13. Del Villano BC, Brennan S, Brock P, Bucher C, Liu V, McClure M, et al. Radioimmunoassay for a monoclonal antibody-defined tumor marker, CA 19-9. *Clin Chem*. 1983;29(3):549–52.
14. Lee T, Teng TZJ, Shelat VG. Carbohydrate antigen 19-9 - tumor marker: Past, present, and future. *World J Gastrointest Surg*. 2020;12(12):468–90.
15. Goonetilleke KS, Siriwardena AK. Systematic review of carbohydrate antigen (CA 19-9) as a biochemical marker in the diagnosis of pancreatic cancer. *Eur J Surg Oncol*. 2007;33(3):266–70.
16. Chan A, Prassas I, Dimitromanolakis A, Brand RE, Serra S, Diamandis EP, et al. Validation of biomarkers that complement CA19.9 in detecting early pancreatic cancer. *Clin Cancer Res*. 2014;20(22):5787–95.
17. Qu D, Johnson J, Chandrakasan P, Weygant N, May R, Aiello N, et al. Doublecortin-like kinase 1 is elevated serologically in pancreatic ductal adenocarcinoma and widely expressed on circulating tumor cells. *PLoS ONE*. 2015;10(2): e0118933.
18. Scarà S, Bottoni P, Scatena R. CA 19-9: biochemical and clinical aspects. *Adv Exp Med Biol*. 2015;867:247–60.
19. Alix-Panabières C, Pantel K. Clinical Applications of Circulating Tumor Cells and Circulating Tumor DNA as Liquid Biopsy. *Cancer Discov*. 2016;6(5):479–91.
20. Alix-Panabières C, Pantel K. Circulating Tumor Cells: Liquid Biopsy of Cancer. *Clin Chem*. 2013;59(1):110–8.
21. Nikanjam M, Kato S, Kurzrock R. Liquid biopsy: current technology and clinical applications. *J Hematol Oncol*. 2022;15(1):131.
22. Gerlinger M, Rowan AJ, Horswell S, Math M, Larkin J, Endesfelder D, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N Engl J Med*. 2012;366(10):883–92.
23. Bingham C, Fernandez SV, Fittipaldi P, Dempsey PW, Ruth KJ, Cristofanilli M, et al. Mutational studies on single circulating tumor cells isolated from the blood of inflammatory breast cancer patients. *Breast Cancer Res Treat*. 2017;163(2):219–30.
24. Barradas AM, Terstappen LW. Towards the biological understanding of CTC: capture technologies, definitions and potential to create metastasis. *Cancers (Basel)*. 2013;5(4):1619–42.
25. Meng S, Tripathy D, Frenkel EP, Shete S, Naftalis EZ, Huth JF, et al. Circulating tumor cells in patients with breast cancer dormancy. *Clin Cancer Res*. 2004;10(24):8152–62.
26. Smit DJ, Schneegans S, Pantel K. Clinical applications of circulating tumor cells in patients with solid tumors. *Clin Exp Metastasis*. 2024. <https://doi.org/10.1007/s10585-024-10267-5>.
27. Wang Y, Yu X, Hartmann D, Zhou J. Circulating tumor cells in peripheral blood of pancreatic cancer patients and their prognostic role: a systematic review and meta-analysis. *HPB (Oxford)*. 2020;22(5):660–9.
28. Zhu Y, Zhang H, Chen N, Hao J, Jin H, Ma X. Diagnostic value of various liquid biopsy methods for pancreatic cancer: a systematic review and meta-analysis. *Medicine*. 2020;99(3): e18581.
29. Komar G, Kauhanen S, Liukka K, Seppänen M, Kajander S, Ovaska J, et al. Decreased blood flow with increased metabolic activity: a novel sign of pancreatic tumor aggressiveness. *Clin Cancer Res*. 2009;15(17):5511–7.
30. Catenacci DV, Chapman CG, Xu P, Koons A, Konda VJ, Siddiqui UD, et al. Acquisition of portal venous circulating tumor cells from patients with pancreaticobiliary cancers by endoscopic ultrasound. *Gastroenterology*. 2015;149(7):1794–803.e4.
31. Markou A, Lazaridou M, Paraskevopoulos P, Chen S, Świerczewska M, Budna J, et al. Multiplex gene expression profiling of in vivo isolated circulating tumor cells in high-risk prostate cancer patients. *Clin Chem*. 2018;64(2):297–306.
32. Fehm TN, Meier-Stiegen F, Driemel C, Jäger B, Reinhardt F, Naskou J, et al. Diagnostic leukapheresis for CTC analysis in breast cancer patients: CTC frequency, clinical experiences and recommendations for standardized reporting. *Cytometry A*. 2018;93(12):1213–9.
33. Stoecklein NH, Fluegen G, Guglielmi R, Neves RPL, Hackert T, Birgin E, et al. Ultra-sensitive CTC-based liquid biopsy for pancreatic cancer enabled by large blood volume analysis. *Mol Cancer*. 2023;22(1):181.
34. Keller L, Pantel K. Unravelling tumour heterogeneity by single-cell profiling of circulating tumour cells. *Nat Rev Cancer*. 2019;19(10):553–67.
35. Khoja L, Backen A, Sloane R, Menasce L, Ryder D, Krebs M, et al. A pilot study to explore circulating tumour cells in pancreatic cancer as a novel biomarker. *Br J Cancer*. 2012;106(3):508–16.
36. Rhim AD, Mirek ET, Aiello NM, Maitra A, Bailey JM, McAllister F, et al. EMT and dissemination precede pancreatic tumor formation. *Cell*. 2012;148(1–2):349–61.
37. Ankeny JS, Court CM, Hou S, Li Q, Song M, Wu D, et al. Circulating tumour cells as a biomarker for diagnosis and staging in pancreatic cancer. *Br J Cancer*. 2016;114(12):1367–75.
38. Kuznetsova A, Popova O, Panchenkov D, Dyuzheva T, Ivanov A. Pancreatic ductal adenocarcinoma: tumor microenvironment and problems in the development of novel therapeutic strategies. *Clin Exp Med*. 2023;23(3):619–43.
39. Thomas D, Radhakrishnan P. Tumor-stromal crosstalk in pancreatic cancer and tissue fibrosis. *Mol Cancer*. 2019;18(1):14.
40. Jacobetz MA, Chan DS, Neesse A, Bapiro TE, Cook N, Frese KK, et al. Hyaluronan impairs vascular function and drug delivery in a mouse model of pancreatic cancer. *Gut*. 2013;62(1):112–20.
41. Arneith B. Tumor Microenvironment. *Medicina (Kaunas)*. 2019;56(1):15.
42. Götze J, Nitschke C, Uzunoglu FG, Pantel K, Sinn M, Wikman H. Tumor-stroma interaction in PDAC as a new approach for liquid biopsy and its potential clinical implications. *Front Cell Dev Biol*. 2022;10: 918795.
43. Kalluri R. The biology and function of fibroblasts in cancer. *Nat Rev Cancer*. 2016;16(9):582–98.
44. Ao Z, Shah SH, Machlin LM, Parajuli R, Miller PC, Rawal S, et al. Identification of Cancer-Associated Fibroblasts in Circulating Blood from Patients with Metastatic Breast Cancer. *Cancer Res*. 2015;75(22):4681–7.
45. Jones ML, Siddiqui J, Pienta KJ, Getzenberg RH. Circulating fibroblast-like cells in men with metastatic prostate cancer. *Prostate*. 2013;73(2):176–81.
46. Richardson AM, Havel LS, Koyen AE, Konen JM, Shupe J, Wiles WG IV, et al. Vimentin Is Required for Lung Adenocarcinoma Metastasis via Heterotypic Tumor Cell–Cancer-Associated Fibroblast Interactions during Collective Invasion. *Clin Cancer Res*. 2018;24(2):420–32.
47. Ortiz-Otero N, Marshall JR, Lash B, King MR. Chemotherapy-induced release of circulating-tumor cells into the bloodstream in collective migration units with cancer-associated fibroblasts in metastatic cancer patients. *BMC Cancer*. 2020;20(1):873.
48. Schwarzenbach H, Hoon DS, Pantel K. Cell-free nucleic acids as biomarkers in cancer patients. *Nat Rev Cancer*. 2011;11(6):426–37.
49. Jaworski JJ, Morgan RD, Sivakumar S. Circulating cell-free tumour DNA for early detection of pancreatic cancer. *Cancers (Basel)*. 2020;12(12):3704.
50. Bettogowda C, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. *Sci Transl Med*. 2014;6(224):224ra24.
51. Newman AM, Bratman SV, To J, Wynne JF, Eclov NC, Modlin LA, et al. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. *Nat Med*. 2014;20(5):548–54.
52. Diehl F, Schmidt K, Choti MA, Romans K, Goodman S, Li M, et al. Circulating mutant DNA to assess tumor dynamics. *Nat Med*. 2008;14(9):985–90.
53. Lo YMD, Zhang J, Leung TN, Lau TK, Chang AMZ, Hjelm NM. Rapid clearance of fetal DNA from maternal plasma. *Am J Hum Genet*. 1999;64(1):218–24.
54. Cristiano S, Leal A, Phallen J, Fiksel J, Adleff V, Bruhm DC, et al. Genome-wide cell-free DNA fragmentation in patients with cancer. *Nature*. 2019;570(7761):385–9.
55. Keller L, Belloum Y, Wikman H, Pantel K. Clinical relevance of blood-based ctDNA analysis: mutation detection and beyond. *Br J Cancer*. 2021;124(2):345–58.
56. Zill OA, Greene C, Sebisano D, Siew LM, Leng J, Vu M, et al. Cell-free DNA next-generation sequencing in pancreaticobiliary carcinomas. *Cancer Discov*. 2015;5(10):1040–8.
57. Marin AM, Sanchuki HBS, Namur GN, Uno M, Zanette DL, Aoki MN. Circulating cell-free nucleic acids as biomarkers for diagnosis and prognosis of pancreatic cancer. *Biomedicine*. 2023;11(4):1069.
58. Jones S, Zhang X, Parsons DW, Lin JCH, Leary RJ, Angenendt P, et al. Core signaling pathways in human pancreatic cancers revealed by Global Genomic Analyses. *Science*. 2008;321(5897):1801–6.
59. Saiki Y, Horii A. Molecular pathology of pancreatic cancer. *Pathol Int*. 2014;64(1):10–9.
60. Waddell N, Pajic AM, Patch AM, Chang DK, Kassahn KS, Bailey P, et al. Whole genomes redefine the mutational landscape of pancreatic cancer. *Nature*. 2015;518(7540):495–501.

61. Bailey P, Chang DK, Nones K, Johns AL, Patch A-M, Gingras M-C, et al. Genomic analyses identify molecular subtypes of pancreatic cancer. *Nature*. 2016;531(7592):47–52.
62. Pelosi E, Castelli G, Testa U. Pancreatic cancer: molecular characterization, clonal evolution and cancer stem cells. *Biomedicines*. 2017;5(4):65.
63. Notta F, Chan-Seng-Yue M, Lemire M, Li Y, Wilson GW, Connor AA, et al. A renewed model of pancreatic cancer evolution based on genomic rearrangement patterns. *Nature*. 2016;538(7625):378–82.
64. Le Calvez-Kelm F, Foll M, Wozniak MB, Delhomme TM, Durand G, Chopard P, et al. KRAS mutations in blood circulating cell-free DNA: a pancreatic cancer case-control. *Oncotarget*. 2016;7(48):78827–40.
65. Conces M, Ni Y, Bazeley P, Patel B, Funchain P, Carraway HE. Clonal hematopoiesis of indeterminate potential (CHIP) mutations in solid tumor malignancies. *J Clin Oncol*. 2019;37(15):1507.
66. Hoermann G. Clinical significance of clonal hematopoiesis of indeterminate potential in hematology and cardiovascular disease. *Diagnostics (Basel)*. 2022;12(7):1613.
67. Qi T, Pan M, Shi H, Wang L, Bai Y, Ge Q. Cell-free DNA fragmentomics: the novel promising biomarker. *Int J Mol Sci*. 2023;24(2):1503.
68. Gorganzad L, Umer M, Islam MN, Nguyen N-T, Shiddiky MJA. Circulating tumor DNA and liquid biopsy: opportunities, challenges, and recent advances in detection technologies. *Lab Chip*. 2018;18(8):1174–96.
69. Newman AM, Lovejoy AF, Klass DM, Kurtz DM, Chabon JJ, Scherer F, et al. Integrated digital error suppression for improved detection of circulating tumor DNA. *Nat Biotechnol*. 2016;34(5):547–55.
70. Nicholson BD, Oke J, Virdee PS, Harris DA, O'Doherty C, Park JES, et al. Multi-cancer early detection test in symptomatic patients referred for cancer investigation in England and Wales (SYMPHONY): a large-scale, observational cohort study. *Lancet Oncol*. 2023;24(7):733–43.
71. García-Ortiz MV, Cano-Ramírez P, Toledano-Fonseca M, Aranda E, Rodríguez-Ariza A. Diagnosing and monitoring pancreatic cancer through cell-free DNA methylation: progress and prospects. *Biomark Res*. 2023;11(1):88.
72. Xi H, Spencer CD, Peiyong J. Emerging frontiers of cell-free DNA fragmentomics. *Extracellular Vesicles and Circulating Nucleic Acids*. 2022;3(4):380–92.
73. Snyder MW, Kircher M, Hill AJ, Daza RM, Shendure J. Cell-free DNA comprises an in vivo nucleosome footprint that informs its tissues-of-origin. *Cell*. 2016;164(1–2):57–68.
74. Moulie F, Chandrananda D, Piskorz AM, Moore EK, Morris J, Ahlborn LB, et al. Enhanced detection of circulating tumor DNA by fragment size analysis. *Sci Transl Med*. 2018;10(466):eaat4921.
75. Lo KW, Lo YM, Leung SF, Tsang YS, Chan LY, Johnson PJ, et al. Analysis of cell-free Epstein-Barr virus associated RNA in the plasma of patients with nasopharyngeal carcinoma. *Clin Chem*. 1999;45(8 Pt 1):1292–4.
76. Koprski MS, Benko FA, Kwak LW, Gocke CD. Detection of tumor messenger RNA in the serum of patients with malignant melanoma. *Clin Cancer Res*. 1999;5(8):1961–5.
77. Heredia-Soto V, Rodríguez-Salas N, Feliu J. Liquid biopsy in pancreatic cancer: are we ready to apply it in the clinical practice? *Cancers (Basel)*. 2021;13(8):1986.
78. Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, et al. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. *Cell Res*. 2008;18(10):997–1006.
79. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, et al. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci U S A*. 2008;105(30):10513–8.
80. Hunter MP, Ismail N, Zhang X, Aguda BD, Lee EJ, Yu L, et al. Detection of microRNA expression in human peripheral blood microvesicles. *PLoS ONE*. 2008;3(11):e3694.
81. Arroyo JD, Chevillet JR, Kroh EM, Ruf IK, Pritchard CC, Gibson DF, et al. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. *Proc Natl Acad Sci U S A*. 2011;108(12):5003–8.
82. Vickers KC, Palmisano BT, Shoucri BM, Shamburek RD, Remaley AT. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. *Nat Cell Biol*. 2011;13(4):423–33.
83. Griffiths-Jones S, Grocock RJ, van Dongen S, Bateman A, Enright AJ. miRBase: microRNA sequences, targets and gene nomenclature. *Nucleic Acids Research*. 2006;34(suppl_1):D140–4.
84. Bracken CP, Scott HS, Goodall GJ. A network-biology perspective of microRNA function and dysfunction in cancer. *Nat Rev Genet*. 2016;17(12):719–32.
85. O'Brien J, Hayder H, Zayed Y, Peng C. Overview of MicroRNA biogenesis, mechanisms of actions, and circulation. *Front Endocrinol (Lausanne)*. 2018;9:402.
86. van Rooij E. The art of microRNA research. *Circ Res*. 2011;108(2):219–34.
87. Cheung KWE, Choi SR, Lee LTC, Lee NLE, Tsang HF, Cheng YT, et al. The potential of circulating cell free RNA as a biomarker in cancer. *Expert Rev Mol Diagn*. 2019;19(7):579–90.
88. Peng C, Wang J, Gao W, Huang L, Liu Y, Li X, et al. Meta-analysis of the diagnostic performance of circulating MicroRNAs for pancreatic cancer. *Int J Med Sci*. 2021;18(3):660–71.
89. Schaaïj-Visser TB, de Wit M, Lam SW, Jiménez CR. The cancer secretome, current status and opportunities in the lung, breast and colorectal cancer context. *Biochim Biophys Acta*. 2013;1834(11):2242–58.
90. Anderson NL, Anderson NG. The human plasma proteome: history, character, and diagnostic prospects. *Mol Cell Proteomics*. 2002;1(11):845–67.
91. Nagayoshi Y, Nakamura M, Matsuoka K, Ohtsuka T, Mori Y, Kono H, et al. Profiling of autoantibodies in sera of pancreatic cancer patients. *Ann Surg Oncol*. 2014;21(Suppl 3):S459–65.
92. Robinson JL, Feizi A, Uhlén M, Nielsen J. A systematic investigation of the malignant functions and diagnostic potential of the cancer secretome. *Cell Rep*. 2019;26(10):2622–35.e5.
93. Veyssi re H, Bidet Y, Penault-Llorca F, Radosevic-Robin N, Durando X. Circulating proteins as predictive and prognostic biomarkers in breast cancer. *Clin Proteomics*. 2022;19(1):25.
94. Suhre K, McCarthy MI, Schwenk JM. Genetics meets proteomics: perspectives for large population-based studies. *Nat Rev Genet*. 2021;22(1):19–37.
95. O'Neill RS, Stoita A. Biomarkers in the diagnosis of pancreatic cancer: are we closer to finding the golden ticket? *World J Gastroenterol*. 2021;27(26):4045–87.
96. Kane LE, Mellotte GS, Mylod E, O'Brien RM, O'Connell F, Buckley CE, et al. Diagnostic accuracy of blood-based biomarkers for pancreatic cancer: a systematic review and meta-analysis. *Cancer Res Commun*. 2022;2(10):1229–43.
97. Faur  J, Lachenal G, Court M, Hirrlinger J, Chatellard-Causse C, Blot B, et al. Exosomes are released by cultured cortical neurones. *Mol Cell Neurosci*. 2006;31(4):642–8.
98. Richards KE, Zeleniak AE, Fishel ML, Wu J, Littlepage LE, Hill R. Cancer-associated fibroblast exosomes regulate survival and proliferation of pancreatic cancer cells. *Oncogene*. 2017;36(13):1770–8.
99. Ge R, Tan E, Sharghi-Namini S, Asada HH. Exosomes in cancer micro-environment and beyond: have we overlooked these extracellular messengers? *Cancer Microenvironment*. 2012;5(3):323–32.
100. Valadi H, Ekstr m K, Bossios A, Sj strand M, Lee JJ, L tvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol*. 2007;9(6):654–9.
101. Whiteside TL. Tumor-derived exosomes and their role in cancer progression. *Adv Clin Chem*. 2016;74:103–41.
102. Armacki M, Polaschek S, Waldenmaier M, Morawe M, Ruhland C, Schmid R, et al. Protein kinase D1, reduced in human pancreatic tumors, increases secretion of small extracellular vesicles from cancer cells that promote metastasis to lung in mice. *Gastroenterology*. 2020;159(3):1019–35.e22.
103. Chang C-H, Pauklin S. Extracellular vesicles in pancreatic cancer progression and therapies. *Cell Death Dis*. 2021;12(11):973.
104. Ruivo CF, Bastos N, Adem B, Batista I, Duraes C, Melo CA, et al. Extracellular vesicles from pancreatic cancer stem cells lead an intratumor communication network (EVNet) to fuel tumour progression. *Gut*. 2022;71(10):2043–68.
105. Zhang YF, Zhou YZ, Zhang B, Huang SF, Li PP, He XM, et al. Pancreatic cancer-derived exosomes promoted pancreatic stellate cells recruitment by pancreatic cancer. *J Cancer*. 2019;10(18):4397–407.
106. Liu C, He D, Li L, Zhang S, Wang L, Fan Z, et al. Extracellular vesicles in pancreatic cancer immune escape: Emerging roles and mechanisms. *Pharmacol Res*. 2022;183: 106364.

107. Melo SA, Luecke LB, Kahlert C, Fernandez AF, Gammon ST, Kaye J, et al. Glypican-1 identifies cancer exosomes and detects early pancreatic cancer. *Nature*. 2015;523(7559):177–82.
108. Patton MC, Zubair H, Khan MA, Singh S, Singh AP. Hypoxia alters the release and size distribution of extracellular vesicles in pancreatic cancer cells to support their adaptive survival. *J Cell Biochem*. 2020;121(1):828–39.
109. Jia E, Ren N, Shi X, Zhang R, Yu H, Yu F, et al. Extracellular vesicle biomarkers for pancreatic cancer diagnosis: a systematic review and meta-analysis. *BMC Cancer*. 2022;22(1):573.
110. Chang ST, Zahn JM, Horecka J, Kunz PL, Ford JM, Fisher GA, et al. Identification of a biomarker panel using a multiplex proximity ligation assay improves accuracy of pancreatic cancer diagnosis. *J Transl Med*. 2009;7:105.
111. Balasenthil S, Chen N, Lott ST, Chen J, Carter J, Grizzle WE, et al. A migration signature and plasma biomarker panel for pancreatic adenocarcinoma. *Cancer Prev Res (Phila)*. 2011;4(1):137–49.
112. Brand RE, Nolen BM, Zeh HJ, Allen PJ, Eloubeidi MA, Goldberg M, et al. Serum biomarker panels for the detection of pancreatic cancer. *Clin Cancer Res*. 2011;17(4):805–16.
113. Park HD, Kang ES, Kim JW, Lee KT, Lee KH, Park YS, et al. Serum CA19-9, cathepsin D, and matrix metalloproteinase-7 as a diagnostic panel for pancreatic ductal adenocarcinoma. *Proteomics*. 2012;12(23–24):3590–7.
114. Nie S, Lo A, Wu J, Zhu J, Tan Z, Simeone DM, et al. Glycoprotein biomarker panel for pancreatic cancer discovered by quantitative proteomics analysis. *J Proteome Res*. 2014;13(4):1873–84.
115. Nolen BM, Brand RE, Prosser D, Velikokhatnaya L, Allen PJ, Zeh HJ, et al. Prediagnostic serum biomarkers as early detection tools for pancreatic cancer in a large prospective cohort study. *PLoS ONE*. 2014;9(4):e94928.
116. Shaw VE, Lane B, Jenkinson C, Cox T, Greenhalf W, Halloran CM, et al. Serum cytokine biomarker panels for discriminating pancreatic cancer from benign pancreatic disease. *Mol Cancer*. 2014;13:114.
117. Nie S, Yin H, Tan Z, Anderson MA, Ruffin MT, Simeone DM, et al. Quantitative analysis of single amino acid variant peptides associated with pancreatic cancer in serum by an isobaric labeling quantitative method. *J Proteome Res*. 2014;13(12):6058–66.
118. Tang H, Singh S, Partyka K, Kletter D, Hsueh P, Yadav J, et al. Glycan motif profiling reveals plasma sialyl-lewis x elevations in pancreatic cancers that are negative for sialyl-Lewis A. *Mol Cell Proteomics*. 2015;14(5):1323–33.
119. Liu X, Zheng W, Wang W, Shen H, Liu L, Lou W, et al. A new panel of pancreatic cancer biomarkers discovered using a mass spectrometry-based pipeline. *Br J Cancer*. 2017;117(12):1846–54.
120. Mattila N, Seppänen H, Mustonen H, Przybyla B, Haglund C, Lassila R. Preoperative biomarker panel, including fibrinogen and FVIII, improves diagnostic accuracy for pancreatic ductal adenocarcinoma. *Clin Appl Thromb Hemost*. 2018;24(8):1267–75.
121. Peng HY, Chang MC, Hu CM, Yang HI, Lee WH, Chang YT. Thrombospondin-2 is a Highly Specific Diagnostic Marker and is Associated with Prognosis in Pancreatic Cancer. *Ann Surg Oncol*. 2019;26(3):807–14.
122. Staal B, Liu Y, Barnett D, Hsueh P, He Z, Gao C, et al. The sTRA Plasma biomarker: blinded validation of improved accuracy over CA19-9 in pancreatic cancer diagnosis. *Clin Cancer Res*. 2019;25(9):2745–54.
123. Zhou Q, Andersson R, Hu D, Bauden M, Sasor A, Bygott T, et al. Alpha-1-acid glycoprotein 1 is upregulated in pancreatic ductal adenocarcinoma and confers a poor prognosis. *Transl Res*. 2019;212:67–79.
124. Kim H, Kang KN, Shin YS, Byun Y, Han Y, Kwon W, et al. Biomarker panel for the diagnosis of pancreatic ductal adenocarcinoma. *Cancers (Basel)*. 2020;12(6):1443. <https://doi.org/10.3390/cancers12061443>.
125. Kim Y, Yeo I, Huh I, Kim J, Han D, Jang JY, et al. Development and multiple validation of the protein multi-marker panel for diagnosis of pancreatic cancer. *Clin Cancer Res*. 2021;27(8):2236–45.
126. Mehta S, Bhimani N, Gill AJ, Samra JS, Sahni S, Mittal A. Serum biomarker panel for diagnosis and prognosis of pancreatic ductal adenocarcinomas. *Front Oncol*. 2021;11: 708963.
127. Nené NR, Ney A, Nazarenko T, Blyuss O, Johnston HE, Whitwell HJ, et al. Serum biomarker-based early detection of pancreatic ductal adenocarcinomas with ensemble learning. *Commun Med (Lond)*. 2023;3(1):10.
128. Cao H, Zhu L, Li L, Wang W, Niu X. Serum CA724 has no diagnostic value for gastrointestinal tumors. *Clin Exp Med*. 2023;23(6):2433–42.
129. Jang SI, Lee HK, Chang EJ, Kim S, Kim SY, Hong IY, et al. Improved predictability of pancreatic ductal adenocarcinoma diagnosis using a blood immune cell biomarker panel developed from bulk mRNA sequencing and single-cell RNA-sequencing. *Cancer Immunol Immunother*. 2023;72(8):2757–68.
130. Makawita S, Dimitromanolakis A, Soosapillai A, Soles I, Chan A, Gallinger S, et al. Validation of four candidate pancreatic cancer serological biomarkers that improve the performance of CA19.9. *BMC Cancer*. 2013;13:404.
131. Tang H, Partyka K, Hsueh P, Sinha JY, Kletter D, Zeh H, et al. Glycans related to the CA19-9 antigen are elevated in distinct subsets of pancreatic cancers and improve diagnostic accuracy over CA19-9. *Cell Mol Gastroenterol Hepatol*. 2016;2(2):201–21.e15.
132. Yoneyama T, Ohtsuki S, Honda K, Kobayashi M, Iwasaki M, Uchida Y, et al. Identification of IGFBP2 and IGFBP3 as compensatory biomarkers for CA19-9 in early-stage pancreatic cancer using a combination of antibody-based and LC-MS/MS-based proteomics. *PLoS ONE*. 2016;11(8):e0161009.
133. Capello M, Bantis LE, Scelo G, Zhao Y, Li P, Dhillon DS, et al. Sequential validation of blood-based protein biomarker candidates for early-stage pancreatic cancer. *J Natl Cancer Inst*. 2017;109(4):djw266.
134. Balasenthil S, Huang Y, Liu S, Marsh T, Chen J, Stass SA, et al. A plasma biomarker panel to identify surgically resectable early-stage pancreatic cancer. *J Natl Cancer Inst*. 2017;109(8):djw341.
135. Kaur S, Smith LM, Patel A, Menning M, Watley DC, Malik SS, et al. A combination of MUC5AC and CA19-9 improves the diagnosis of pancreatic cancer: a multicenter study. *Am J Gastroenterol*. 2017;112(1):172–83.
136. Kim J, Bamlet WR, Oberg AL, Chaffee KG, Donahue G, Cao XJ, et al. Detection of early pancreatic ductal adenocarcinoma with thrombospondin-2 and CA19-9 blood markers. *Sci Transl Med*. 2017;9(398):eaah5583.
137. Park J, Choi Y, Namkung J, Yi SG, Kim H, Yu J, et al. Diagnostic performance enhancement of pancreatic cancer using proteomic multimarker panel. *Oncotarget*. 2017;8(54):93117–30.
138. Resovi A, Bani MR, Porcu L, Anastasia A, Minoli L, Allavena P, et al. Soluble stroma-related biomarkers of pancreatic cancer. *EMBO Mol Med*. 2018;10(8):e8741.
139. Dong D, Jia L, Zhang L, Ma N, Zhang A, Zhou Y, et al. Periostin and CA242 as potential diagnostic serum biomarkers complementing CA19.9 in detecting pancreatic cancer. *Cancer Sci*. 2018;109(9):2841–51.
140. Song J, Sokoll LJ, Pasay JJ, Rubin AL, Li H, Bach DM, et al. Identification of serum biomarker panels for the early detection of pancreatic cancer. *Cancer Epidemiol Biomarkers Prev*. 2019;28(1):174–82.
141. Jahan R, Ganguly K, Smith LM, Atri P, Carmichael J, Sheinin Y, et al. Trefoil factor(s) and CA19.9: a promising panel for early detection of pancreatic cancer. *EBioMedicine*. 2019;42:375–85.
142. Zhang J, Wang Y, Zhao T, Li Y, Tian L, Zhao J, et al. Evaluation of serum MUC5AC in combination with CA19-9 for the diagnosis of pancreatic cancer. *World J Surg Oncol*. 2020;18(1):31.
143. Peng H, Pan S, Yan Y, Brand RE, Petersen GM, Chari ST, et al. Systemic proteome alterations linked to early stage pancreatic cancer in diabetic patients. *Cancers (Basel)*. 2020;12(6):1534.
144. Choi YJ, Yoon W, Lee A, Han Y, Byun Y, Kang JS, et al. Diagnostic model for pancreatic cancer using a multi-biomarker panel. *Ann Surg Treat Res*. 2021;100(3):144–53.
145. Lee DH, Yoon W, Lee A, Han Y, Byun Y, Kang JS, et al. Multi-biomarker panel prediction model for diagnosis of pancreatic cancer. *J Hepatobiliary Pancreat Sci*. 2023;30(1):122–32.
146. Song J, Sokoll LJ, Chan DW, Zhang Z. Validation of serum biomarkers that complement CA19-9 in detecting early pancreatic cancer using electrochemiluminescent-based multiplex immunoassays. *Biomedicines*. 2021;9(12):1897.
147. Hinesstrosa JP, Kurzrock R, Lewis JM, Schork NJ, Schroeder G, Kamat AM, et al. Early-stage multi-cancer detection using an extracellular vesicle protein-based blood test. *Commun Med (Lond)*. 2022;2:29.
148. Firpo MA, Boucher KM, Bleicher J, Khanderao GD, Rosati A, Poruk KE, et al. Multianalyte serum biomarker panel for early detection of pancreatic adenocarcinoma. *JCO Clin Cancer Inform*. 2023;7: e2200160.
149. Byeon S, McKay MJ, Molloy MP, Gill AJ, Samra JS, Mittal A, et al. Novel serum protein biomarker panel for early diagnosis of pancreatic cancer. *Int J Cancer*. 2024;155:365–71.

150. Yi JM, Guzzetta AA, Bailey VJ, Downing SR, Van Neste L, Chiappinelli KB, et al. Novel methylation biomarker panel for the early detection of pancreatic cancer. *Clin Cancer Res*. 2013;19(23):6544–55.
151. Eissa MAL, Lerner L, Abdelfatah E, Shankar N, Canner JK, Hasan NM, et al. Promoter methylation of ADAMTS1 and BNC1 as potential biomarkers for early detection of pancreatic cancer in blood. *Clin Epigenetics*. 2019;11(1):59.
152. Ganepola GA, Rutledge JR, Suman P, Yiangpruksawan A, Chang DH. Novel blood-based microRNA biomarker panel for early diagnosis of pancreatic cancer. *World J Gastrointest Oncol*. 2014;6(1):22–33.
153. Makler A, Asghar W. Exosomal miRNA biomarker panel for pancreatic ductal adenocarcinoma detection in patient plasma: a pilot study. *Int J Mol Sci*. 2023;24(6):5081.
154. Yang Z, Huang J, Wu X, Zhou Y, Tang Y, Zhu Y, et al. Contribution of a circulating 2'-O-methylated MicroRNA panel to the diagnosis of pancreatic ductal adenocarcinoma. *J Cancer*. 2024;15(6):1583–92.
155. Madhavan B, Yue S, Galli U, Rana S, Gross W, Müller M, et al. Combined evaluation of a panel of protein and miRNA serum-exosome biomarkers for pancreatic cancer diagnosis increases sensitivity and specificity. *Int J Cancer*. 2015;136(11):2616–27.
156. Yuan W, Tang W, Xie Y, Wang S, Chen Y, Qi J, et al. New combined microRNA and protein plasmatic biomarker panel for pancreatic cancer. *Oncotarget*. 2016;7(48):80033–45.
157. Berger AW, Schwerdel D, Reinacher-Schick A, Uhl W, Algül H, Friess H, et al. A blood-based multi marker assay supports the differential diagnosis of early-stage pancreatic cancer. *Theranostics*. 2019;9(5):1280–7.
158. Reese M, Flammang I, Yang Z, Dhayat SA. Potential of exosomal microRNA-200b as liquid biopsy marker in pancreatic ductal adenocarcinoma. *Cancers (Basel)*. 2020;12(1):197.
159. Chen J, Wang H, Zhou L, Liu Z, Tan X. A combination of circulating tumor cells and CA199 improves the diagnosis of pancreatic cancer. *J Clin Lab Anal*. 2022;36(5): e24341.
160. Cohen JD, Javed AA, Thoburn C, Wong F, Tie J, Gibbs P, et al. Combined circulating tumor DNA and protein biomarker-based liquid biopsy for the earlier detection of pancreatic cancers. *Proc Natl Acad Sci U S A*. 2017;114(38):10202–7.
161. Fahrman JF, Bantis LE, Capello M, Scelo G, Dennison JB, Patel N, et al. A plasma-derived protein-metabolite multiplexed panel for early-stage pancreatic cancer. *J Natl Cancer Inst*. 2019;111(4):372–9.
162. Nakamura K, Zhu Z, Roy S, Jun E, Han H, Munoz RM, et al. An exosome-based transcriptomic signature for noninvasive, early detection of patients with pancreatic ductal adenocarcinoma: a multicenter cohort study. *Gastroenterology*. 2022;163(5):1252–66.e2.
163. Zhao R, Ren S, Li C, Guo K, Lu Z, Tian L, et al. Biomarkers for pancreatic cancer based on tissue and serum metabolomics analysis in a multicenter study. *Cancer Med*. 2023;12(4):5158–71.
164. Xu C, Jun E, Okugawa Y, Toyama Y, Borazanci E, Bolton J, et al. A circulating panel of circRNA biomarkers for the noninvasive and early detection of pancreatic ductal adenocarcinoma. *Gastroenterology*. 2023;166(1):178–90.E16. <https://doi.org/10.1053/j.gastro.2023.09.050>.
165. He J, Long J, Zhai C, Xu J, Bao K, Su W, et al. Codetection of proteins and RNAs on extracellular vesicles for pancreatic cancer early diagnosis. *Anal Chem*. 2024;96(17):6618–27.
166. Kim S, Park BK, Seo JH, Choi J, Choi JW, Lee CK, et al. Carbohydrate antigen 19-9 elevation without evidence of malignant or pancreatobiliary diseases. *Sci Rep*. 2020;10(1):8820.
167. Prior IA, Hood FE, Hartley JL. The frequency of ras mutations in cancer. *Can Res*. 2020;80(14):2969–74.
168. Cohen JD, Li L, Wang Y, Thoburn C, Afsari B, Danilova L, et al. Detection and localization of surgically resectable cancers with a multi-analyte blood test. *Science*. 2018;359(6378):926–30.
169. Jamshidi A, Liu MC, Klein EA, Venn O, Hubbell E, Beausang JF, et al. Evaluation of cell-free DNA approaches for multi-cancer early detection. *Cancer Cell*. 2022;40(12):1537–49.e12.
170. Liu MC, Oxnard GR, Klein EA, Swanton C, Seiden MV. Sensitive and specific multi-cancer detection and localization using methylation signatures in cell-free DNA. *Ann Oncol*. 2020;31(6):745–59.
171. Klein EA, Richards D, Cohn A, Tummala M, Lapham R, Cosgrove D, et al. Clinical validation of a targeted methylation-based multi-cancer early detection test using an independent validation set. *Ann Oncol*. 2021;32(9):1167–77.
172. Schrag D, Beer TM, McDonnell CH 3rd, Nadauld L, Dilaveri CA, Reid R, et al. Blood-based tests for multicancer early detection (PATHFINDER): a prospective cohort study. *Lancet*. 2023;402(10409):1251–60.
173. Gao Q, Lin YP, Li BS, Wang GQ, Dong LQ, Shen BY, et al. Unintrusive multi-cancer detection by circulating cell-free DNA methylation sequencing (THUNDER): development and independent validation studies. *Ann Oncol*. 2023;34(5):486–95.
174. Hartwig W, Hackert T, Hinz U, Gluth A, Bergmann F, Strobel O, et al. Pancreatic cancer surgery in the new millennium: better prediction of outcome. *Ann Surg*. 2011;254(2):311–9.
175. Cameron JL, Riall TS, Coleman J, Belcher KA. One thousand consecutive pancreaticoduodenectomies. *Ann Surg*. 2006;244(1):10–5.
176. Yang R, Du Y, Zhang M, Liu Y, Feng H, Liu R, et al. Multi-omics analysis reveals interferon-stimulated gene OAS1 as a prognostic and immunological biomarker in pan-cancer. *Front Immunol*. 2023;14:1249731.
177. Lampignano R, Neumann MHD, Weber S, Sploten V, Herdean A, Voss T, et al. Multicenter evaluation of circulating cell-free DNA extraction and downstream analyses for the development of standardized (Pre)analytical work flows. *Clin Chem*. 2020;66(1):149–60.
178. Klotten V, Neumann MHD, Di Pasquale F, Sprenger-Haussels M, Shaffer JM, Schlumpberger M, et al. Multicenter evaluation of circulating plasma MicroRNA extraction technologies for the development of clinically feasible reverse transcription quantitative PCR and next-generation sequencing analytical work flows. *Clin Chem*. 2019;65(9):1132–40.
179. Neves RPL, Ammerlaan W, Andree KC, Bender S, Cayrefourcq L, Driemel C, et al. Proficiency Testing to Assess Technical Performance for CTC-Processing and Detection Methods in CANCER-ID. *Clin Chem*. 2021;67(4):631–41.
180. PANCAID Consortium. PANcreatic CAncer Initial Detection via liquid biopsy (PANCAID). 2023. Available from: <https://pancaid-project.eu>.
181. Pantel K, Alix-Panabières C. Liquid biopsy and minimal residual disease - latest advances and implications for cure. *Nat Rev Clin Oncol*. 2019;16(7):409–24.
182. Pascual J, Attard G, Bidard FC, Curigliano G, De Mattos-Arruda L, Diehn M, et al. ESMO recommendations on the use of circulating tumour DNA assays for patients with cancer: a report from the ESMO Precision Medicine Working Group. *Ann Oncol*. 2022;33(8):750–68.
183. Pantel K, Lindbjerg Andersen C, Schuurin E, Malats N, Heitzer E, Vat L, et al. 237TIP GUIDE.MRD: a Consortium guiding multi-modal therapies against minimal residual disease (MRD) by liquid biopsy to assess implementation of circulating tumor DNA (ctDNA) in clinical practice to improve patient outcomes. *Ann Oncol*. 2023;34:5276–7.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.