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Liquid Biopsy in Pancreatic Ductal Adenocarcinoma and Precancerous Lesions

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ABSTRACT

Pancreatic ductal adenocarcinoma ranks seventh among all cancer-related causes of death and has an overall 5-year survival rate of no more than 15% across all stages. This high mortality rate is largely attributed to delayed diagnosis: due to late clinical manifestation and early metastasis, only about 5% of pancreatic ductal adenocarcinoma cases are detected at stage I. Another important issue is the risk of overtreatment in patients with benign or non-neoplastic pancreatic conditions that mimic pancreatic ductal adenocarcinoma, often resulting in unnecessary and invasive surgeries. A diagnostic approach capable of detecting pancreatic ductal adenocarcinoma with high sensitivity at early stages and distinguishing it from benign pancreatic diseases could improve survival rates and reduce the number of unwarranted high-risk procedures. One of the most promising technologies for early and noninvasive cancer detection is liquid biopsy. This term refers to a set of analytical methods designed to identify tumor-specific genetic, epigenetic, and antigenic alterations by analyzing tumor-derived materials in biological fluids such as plasma, bile, or urine. Liquid biopsy may be used not only for early detection of pancreatic ductal adenocarcinoma and its precursors in high-risk individuals but also for differential diagnosis. This review summarizes current research evaluating the diagnostic potential of liquid biopsy through the detection of extracellular tumor DNA and RNA, as well as circulating tumor cells in blood, pancreatic juice, and bile in patients with pancreatic neoplasms.

Keywords: circulating tumor DNA; microRNA; circulating tumor cells; liquid biopsy; pancreatic ductal adenocarcinoma; review.

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Жидкостная биопсия протоковой аденокарциномы и предраковых состояний поджелудочной железы

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АННОТАЦИЯ

Протоковая аденокарцинома поджелудочной железы занимает 7-е место по смертности среди всех онкологических заболеваний и характеризуется общей пятилетней выживаемостью на всех стадиях не более 15%. Столь высокая летальность связана с поздним выявлением данного новообразования — ввиду отсроченной клинической манифестации и раннего метастазирования лишь около 5% протоковых аденокарцином поджелудочной железы диагностируют на I стадии. Кроме того, актуальной остаётся проблема чрезмерного хирургического воздействия на пациентов, имевших доброкачественное или неопухолевое поражение поджелудочной железы, маскирующееся под протоковую аденокарциному. Применение диагностического подхода, способного с высокой чувствительностью выявлять протоковую аденокарциному на ранней стадии и дифференцировать её с доброкачественными заболеваниями поджелудочной железы, позволило бы увеличить выживаемость больных с данным новообразованием и снизить количество бессмысленных травматизирующих операций. Одной из наиболее перспективных технологий для ранней и неинвазивной диагностики злокачественных новообразований является жидкостная биопсия. Под данным термином понимают комплекс аналитических подходов, направленных на выявление характерных для опухоли генетических, эпигенетических и антигенных изменений при анализе опухолевых дериватов в биологических жидкостях организма (таких как плазма, желчь, моча и т. д.). Жидкостная биопсия может быть использована не только для раннего выявления протоковой аденокарциномы поджелудочной железы и её предшественников у пациентов, находящихся в группах риска, но и для её дифференциальной диагностики. В настоящем обзоре рассмотрены работы, посвящённые оценке диагностического потенциала жидкостной биопсии по внеклеточной опухолевой дезоксирибонуклеиновой кислоте и рибонуклеиновой кислоте, а также циркулирующих опухолевых клеток в крови, панкреатическом соке и желчи у пациентов с новообразованиями поджелудочной железы.

Ключевые слова: циркулирующая опухолевая ДНК; микроРНК; циркулирующие опухолевые клетки; жидкостная биопсия; протоковая аденокарцинома поджелудочной железы; обзор.

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BACKGROUND

Pancreatic cancer ranks 12th in incidence and 7th in cancer-related mortality worldwide [1]. Pancreatic Ductal Adenocarcinoma (PDAC) accounts for 90% of all pancreatic malignancies [1]. The relative 5-year survival rate remains below 10%–15% [1, 2], primarily due to late-stage diagnosis [1]. Despite advancements in diagnostic modalities—including computed tomography, magnetic resonance imaging, endoscopic ultrasound, ultrasound-guided biopsy, and serum biomarkers (CA 19-9 and carcinoembryonic antigen)—only approximately 5% of tumors are detected at stage I [1]. Approximately 70%–80% of PDAC cases are identified at the stage of local invasion or distant metastasis [1].

Mass screening for PDAC is deemed ineffective due to its low prevalence [2]. A more practical approach involves the surveillance of individuals with predisposing conditions. *Chronic pancreatitis* (CP) is one of the most significant risk factors, increasing the likelihood of PDAC by 2.7–16-fold [2]. However, in patients with CP, elevated CA 19-9 levels and altered pancreatic architecture hinder the early detection of PDAC through serum markers or imaging techniques [3]. Although histopathological examination remains the cornerstone of PDAC diagnosis, it presents challenges in CP patients, particularly when biopsy samples are limited and lack features such as perineural or vascular invasion, making the diagnosis highly dependent on the pathologist's expertise [4]. Precursor lesions such as intraductal papillary mucinous neoplasms (IPMNs) and pancreatic intraepithelial neoplasia (PanIN) with high-grade dysplasia substantially increase the risk of PDAC [5]. Notably, the 5-year survival rate for patients with IPMN or PanIN exceeds 85%, suggesting that early detection could significantly reduce PDAC mortality [6, 7]. However, due to their small size, deep anatomical location, and absence of clinical symptoms, these lesions are rarely detected by current laboratory or imaging techniques [8]. Biopsy followed by histopathological analysis is considered the gold standard for diagnosing pancreatic tumors [9]. Endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) demonstrates sensitivity and specificity of up to 93.1% and 100%, respectively [8, 9]. Nonetheless, EUS-FNA faces limitations in assessing the basement membrane, identifying non-glandular tissue architecture, and differentiating PDAC from its precursors, which often share similar morphological characteristics [5, 9].

Currently, the most effective treatment for PDAC involves a combination of chemotherapy and surgical resection [10]. EUS-FNA is mandatory before initiating to chemotherapy and is also recommended for patients with potentially resectable tumors. Nevertheless, surgery is sometimes performed without prior histological confirmation [10]. In 5%–10% of such cases, resections based solely on clinical and radiological findings reveal benign or non-neoplastic pancreatic lesions [9, 11], for which less invasive or conservative management would have been more appropriate [12]. Conversely, pancreatic neoplasms may present as CP or pancreatic pseudocysts, resulting in diagnostic delay [13]. While histological evaluation of biopsy samples remains the most reliable method for confirming malignancy [9, 13], the procedure is invasive, painful, and carries risks such as tumor cell dissemination and life-threatening complications [10, 11].

Thus, although histopathology remains a key diagnostic tool for identifying PDAC and its precursors, there is a growing need for adjunctive methods that are non- or minimally-invasive and capable of accurately differentiating PDAC, premalignant lesions, and benign pancreatic conditions. Increasing attention is being directed toward the development of liquid biopsy [19], which involves the analysis of tumor-derived materials (extracellular tumor nucleic acids, circulating tumor cells) in body fluids. This review aims to evaluate the characteristics of liquid biopsy in diagnosing PDAC, its precursor lesions, and benign pancreatic diseases. The present study was conducted to analyze the diagnostic and prognostic potential of various PDAC-related biomarkers in patients' body fluids. Literature was retrieved from *eLIBRARY.RU*, *CyberLeninka*, *PubMed*, and *Google Scholar*. The review included original articles published between 2010 and 2024. Search terms included *жидкостная биопсия* / *liquid biopsy*, *протоковая аденокарцинома поджелудочной железы* / *pancreatic ductal adenocarcinoma*, *внеклеточная опухолевая ДНК* / *cell-free DNA*, *внеклеточная опухолевая РНК* / *cell-free RNA*, *метилирование* / *methylation*, and *циркулирующие опухолевые клетки* / *circulating tumor cells*.

MOLECULAR CHARACTERISTICS OF PANCREATIC DUCTAL ADENOCARCINOMA

PDAC progresses through a series of transformations involving both genetic and epigenetic regulation of tumor cells, accompanied by changes in their antigenic profile (Fig. 1). A primary goal in the development of liquid biopsy is to identify specific alterations that are not only common among patients but also indicative of tumor presence [2].

Genetic Alterations

A hallmark of PDAC is the presence of mutations in codons 12, 13, and 61 of the *KRAS* gene, identified in 90%–95% of PDACs and 30%–80% of precursor lesions [2, 7]. Other frequently observed genetic alterations include mutations in *CDKN2A*, *TP53*, and *SMAD4*, found in approximately 70%–80%, 50%–70%, and 50% of patients, respectively. These mutations are considered relatively late events in PDAC oncogenesis and involve a wider range of mutational hotspots. Less common mutations occur in genes such as *ARID1A*, *RNF43*, *TGFBR2*, *LRP1B*, *PREX2*, *GNAS*, and *DNMT3A*, each detected in around 10% of PDAC patients [7, 14, 15]. Notably, mutations in codon 201 of *GNAS* are observed in 6% of PDACs but are present in 19%–75% of IPMNs [16]. Another feature of IPMNs is the

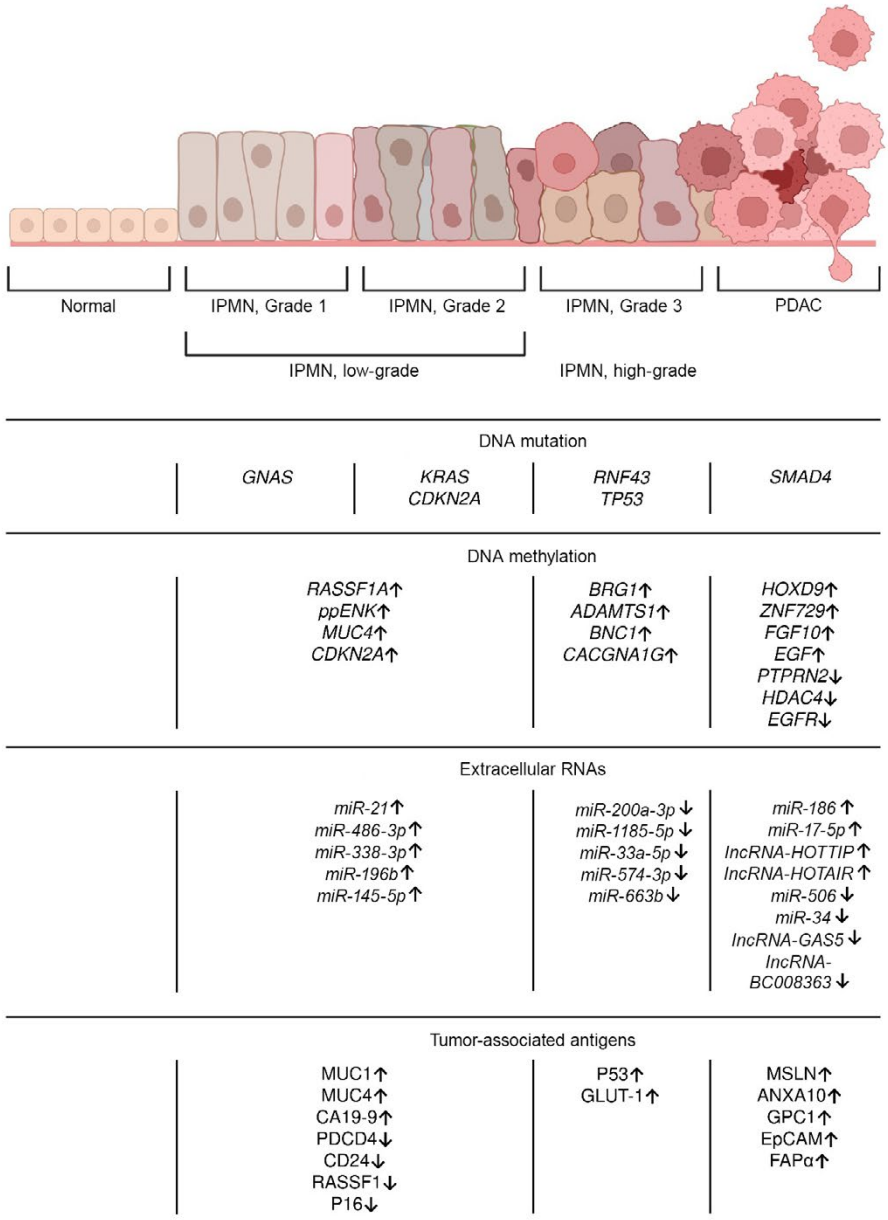

Fig. 1. Major genetic, epigenetic, and antigenic alterations contributing to the oncogenesis of pancreatic ductal adenocarcinoma. IPMN, intraductal papillary mucinous neoplasm; PDAC, pancreatic ductal adenocarcinoma.

presence of *RNF43* mutations, found in 0%–10% of low-grade lesions and 20%–75% of high-grade lesions [16].

Epigenetic Alterations

PDAC is marked by extensive changes in epigenetic regulation, including aberrant gene methylation and dysregulated expression of non-coding RNAs (ncRNAs) [17, 18]. Commonly hypermethylated tumor suppressor genes include *HOX*, *ZNF729*, *PRKCB*, *KLRG2*, *FGF10*, and *EGF*. In contrast, hypomethylation is frequently observed in oncogenes such as *PTPRN2*, *HDAC4*, *SERPINB5*, and *EGFR*. Both hypo- and hypermethylation have been reported in *NOTCH*. The genes *ppENK* and *CDKN2A* are typically hypermethylated, while *MUC4* is hypomethylated in both PDAC and its precursor lesions [17, 18]. The frequency of *APC*, *WNK2*, and *CACNA1G* hypermethylation increases with the degree of dysplasia in PanIN [17, 18].

NcRNAs play a critical role in post-transcriptional gene regulation. The major classes include microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs), with miRNAs being the most extensively studied. NcRNAs upregulated in PDAC include *miR-21*, *miR-186, miR-17-5p, miR-196b; lncRNAs HOTTIP, HOTAIR, PVT1;* and *circRNA 0007367*. Conversely, tumor-suppressive ncRNAs, such as *miR-506, miR-34, miR-142, miR-216b, miR-217; lncRNA GAS5;* and *BC008363*, are typically downregulated in PDAC cells [5, 19, 20]. In precursor lesions, upregulated ncRNAs include *miR-21, miR-486-3p, miR-338-3p, miR-196b* [9, 20]. In high-grade IPMNs, downregulation of miR-200a-3p,

miR-1185-5p, miR-33a-5p, miR-574-3p, miR-663b has been reported [21].

Antigenic and Morphologic Changes in the Tumor Tissue

Histologically, PDAC is characterized by irregular glandular structures embedded within a dense desmoplastic stroma and, in some cases, may display sarcomatoid features with minimal stromal separation [4]. The presence of mucin, detectable through *Alcian blue* or *mucicarmine staining*, is a key diagnostic marker of mucinous cystic neoplasms of the pancreas [9].

PDAC cells demonstrate altered surface antigen expression, a feature leveraged in the identification of circulating tumor cells. Antigens significantly overexpressed in PDAC compared to normal pancreatic tissue include mesothelin (MSLN), annexin A10 (ANXA10), and glypican-1 (GPC1) [22]. Additionally, PDAC cells express epithelial cell adhesion molecule (EpCAM), fibroblast activation protein alpha (FAPα), and the products of mutant *KRAS* and *TP53*. MUC1, MUC4, MUC5, and CA 19-9 are also commonly expressed in PanIN and IPMN lesions [9, 23, 24]. Reduced expression of p16 and metastin, along with the loss of membranous and the emergence of cytoplasmic E-cadherin, are further characteristic of PDAC cells [4].

DIAGNOSTIC POTENTIAL OF LIQUID BIOPSY FOR PDAC AND PRECURSOR LESIONS

Liquid biopsy enables malignancy detection by analyzing tumor-derived components in body fluids. This section outlines various techniques used to identify and quantify these components in patients with PDAC and individuals at high risk of developing the disease [25–34].

Cell-Free DNA

Cell-free DNA (cfDNA) is released into circulation either through cell death or active secretion. Under physiological conditions, its concentration in body fluids typically does not exceed 40 ng/mL. In malignancies, however, cfDNA levels can rise significantly due to tumor-associated hypoxia and the acidic microenvironment, both of which contribute to cellular stress and apoptosis [25, 26]. Consequently, cfDNA has emerged as a potential biomarker for oncologic processes. Yet, in early-stage disease, cfDNA concentrations may remain similar to those found in patients with benign pancreatic disorders or healthy individuals [27]. Therefore, early cancer detection depends on the selective identification of the tumor-derived fraction of cfDNA, known as circulating tumor DNA (ctDNA). This hypothesis is based on the authors' interpretation. Due to altered genetic material and dysregulated cellular processes in tumor cells, ctDNA fragments are, on average, 20–30 base pairs shorter than normal cfDNA, which may aid in their distinction [27]. Unfortunately, in early-stage disease, the proportion of ctDNA is relatively low, which limits the effectiveness of size-based detection methods [27]. As a result, detection strategies that target specific genetic and epigenetic alterations in ctDNA are considered more appropriate.

Although ctDNA can be isolated from various body fluids, plasma remains the most extensively studied source. Given the limited number of mutational hotspots and their high prevalence among patients with PDAC, single-nucleotide variants in the *KRAS* gene are considered the most suitable target for mutation-based diagnostics (Table 1). The sensitivity and specificity of PDAC detection in stages III–IV range from 70% to 90% [28, 29]; however, Kirchweger et al. reported a sensitivity of only 64.3% [30]. In stages I–II, the amount of detectable ctDNA is significantly lower, reducing diagnostic sensitivity to 30%–35%, despite the high specificity of 99.5%–100% [31–33]. Berger et al. demonstrated that *GNAS* mutations in plasma could identify IPMN patients with a sensitivity and specificity of 50% and 100%, respectively ($p < 0.0001$). Differentiation between PDAC and IPMN based on these mutations was also achieved with 50% sensitivity and 100% specificity [29]. Another key diagnostic challenge is distinguishing PDAC from CP and benign pancreatic neoplasms. Wang et al. showed that *KRAS* mutation analysis could distinguish PDAC from benign tumors with 35.2% sensitivity and 88.6% specificity [34].

Pancreatic juice, being in closer proximity to the tumor mass than plasma, contains circulating tumor DNA (ctDNA) at concentrations over 100 times higher [35]. Next-generation sequencing (NGS) of pancreatic juice has demonstrated nearly 100% sensitivity in detecting both PDAC and IPMN [36, 37]. In our previous experimental study, we also assessed the diagnostic potential of liquid biopsy using plasma and bile. ctDNA analysis of bile achieved approximately 90% sensitivity, compared with 60% for plasma. Bile outperformed plasma in both absolute ctDNA concentration (248.6 [6.743; 1068] copies/mL vs. 3.26 [0; 19.225] copies/mL, $p < 0.001$) and relative ctDNA concentration (0.045% [0; 0.413] vs. 1.74% [0.2; 11.11], $p = 0.002$) [38].

Methylation analysis of cfDNA also shows promise as a diagnostic tool. Frequently investigated targets include *ADAMTS1*, *ADAMTS2*, *BNC1*, and *BMP3* (Table 2). Methylation of individual genes enables PDAC diagnosis with a sensitivity of 50%–80% and specificity greater than 80% [39, 40]. Combined analysis of *ADAMTS1* and/or *BNC1* methylation allows for the detection of stage I and II PDAC with up to 90%–95% sensitivity [41]. Moreover, high-throughput methylation profiling using broader gene panels achieves a diagnostic sensitivity of 80%–90% [42]. These panel-based methylation assays have shown over 90% sensitivity and specificity in distinguishing PDAC from CP through liquid biopsy [43, 44].

In the study conducted by Majumder et al., methylation analysis of pancreatic juice achieved a sensitivity of 70% and a specificity of 86% for detecting stage I–II PDAC [45]. Similarly, Yokoyama et al. demonstrated that methylation profiling of *MUC* gene family members in pancreatic juice identified intestinal-type IPMN with 100% sensitivity and 88% specificity [46].

Table 1. Diagnostic performance of genetic alterations in circulating tumor DNA for pancreatic ductal adenocarcinoma and precursor lesions.

Study	Participants (n)	Body fluid	Method	Targets	Results
Wu et al., 2014 [28]	PDAC: 36, Control: 24	Plasma (0.2 mL)	qPCR	*KRAS* (G12V/D/R, G13S/D)	Sensitivity: 72.2%, Specificity: 100%
Berger et al., 2016 [29]	PDAC: 24, IPMN: 21, Control: 38	Plasma*	ddPCR	*GNAS* (R201C/H), *KRAS* (G12V/D)	IPMN vs. Control: Sensitivity 80.95%, Specificity: 84.21%; PDAC vs. Control: Sensitivity 83.33%, Specificity: 92.11%
Kirchweger et al., 2022 [30]	PDAC: 70	Plasma (10 mL)	ddPCR	*KRAS* (G12V/D/C/A/S G13D/R/V Q61H/K/L Q61R, 183A > T, Q61H, 183A > C,	Sensitivity: 64.3%
Cohen et al., 2017 [31]	PDAC: 221, Control: 182	Plasma (7.5 mL)	Safe-SeqS	*KRAS* G12V/D/R/A/C Q61H	Sensitivity: 29.9%, Specificity: 99.5%
Affolter et al., 2021 [32]	PDAC: 14, Control: 4	Plasma (5 mL),	NGS	118-gene panel	Sensitivity: 35.7%, Specificity: 100%
Watanabe et al, 2022 [33]	PC untreated: 71, Post-treatment: 74	Plasma (4 mL)	NGS	52-gene panel	Sensitivity: 56% (untreated), 36% (treated)
Wang et al., 2022 [34]	PDAC: 105, Benign pancreatic tumors: 44	Plasma (2.5 mL)	ddPCR	*KRAS* (G12V/D/R)	Sensitivity: 35.2%, Specificity: 88.6%
Volckmar et al., 2019 [36]	IPMN: 12, Pseudocysts: 3	Pancreatic juice (0.5 mL)	NGS	*KRAS* G12V/D/R, G13D/L Q61H *GNAS* R201C/H/S	Sensitivity: 100%, Specificity: 100%
Choi et al., 2019 [37]	PDAC: 21,	Pancreatic juice*	NGS	Mutation panel *(KRAS, TP53)*	*KRAS* sensitivity: 86% *TP53* sensitivity: 29%
Jain et al., 2024 [38]	PDAC: 95, IPMN + Benign: 18, Control: 38	Plasma (5 mL), Bile (5 mL)	ddPCR	*KRAS* G12A/C/D/R/S/V, G13D, Q61H(183A > C)/ Q61H (183A > T)/ K/L/R	Plasma (PDAC vs control): Sensitivity: 61.0%, Specificity: 100% Plasma (PDAC vs other tumors): Sensitivity: 61.0%, Specificity: 94.0% Bile: Sensitivity: 90.0%

Note (for Tables 1–4): *PDAC*, pancreatic ductal adenocarcinoma; *PC*, pancreatic cancer; *CP*, chronic pancreatitis; *IPMN*, intraductal papillary mucinous neoplasm; *ddPCR*, droplet digital polymerase chain reaction; *Safe-SeqS*, safe-sequencing system; *NGS*, next-generation sequencing. *Data not provided in the publication.

Extracellular RNA

Unlike cfDNA, extracellular RNA (exRNA) is primarily released through active secretion rather than as a result of cell death [20]. In oncologic conditions, various non-coding RNAs (ncRNAs) are present in body fluids at concentrations that can differ significantly—sometimes by several orders of magnitude—from those observed in healthy individuals, making them promising diagnostic biomarkers [47–50].

Among the various types of exRNA, microRNAs (miRNAs) are the most extensively studied in PDAC diagnostics, particularly *miR-10b, miR-19b-3p, miR-21, miR-25-3p, and miR-210* (Table 3). When a single miRNA is analyzed in serum, the diagnostic sensitivity and specificity can range from 70% to 90% [47]. However, liquid biopsy studies more often focus on miRNA panels, which may achieve sensitivity and specificity rates of up to 90%–95% [48]. In a study by Lai et al., plasma analysis of *miR-10b*, *miR-21*, *miR-30c*, and *miR-181a*

Table 2. Diagnostic performance of cell-free DNA methylation analysis in detecting pancreatic ductal adenocarcinoma and precursor lesions.

Study	Participants (n)	Body fluid	Method	Targets	Results
Shinjo et al., 2020 [39]	PC: 47, Control: 14	Plasma (1 mL)	ddPCR	*ADAMTS2*, *HOXA1*, *PCDH10*, *SEMA5A*, *SPSB4*	Sensitivity: 49.0%, Specificity: 86.0%
Yi et al., 2013 [40]	PC: 42, Control: 26	Plasma*	qPCR	*ADAMTS1*, *BNC1*	*ADAMTS1*: Sensitivity: 48.0%, Specificity: 92.0%; *BNC1*: Sensitivity: 79.0%, Specificity: 89.0%
Eissa et al., 2019 [41]	PDAC: 39, Control: 95, CP: 8	Plasma (2 mL)	qPCR	Promoters of *ADAMTS1* and *BNC1*	PDAC vs. Control: Sensitivity: 97.3%, Specificity: 91.6%; PDAC vs. CP: Sensitivity: 87.5%, Specificity: 91.6%;
Wu et al., 2022 [42]	PDAC: 74, CP: 25, Control: 65	Plasma (5 mL),	Sequencing	Panel of 56 markers	PDAC vs. Control: Sensitivity: 82%, Specificity: 88%; PDAC vs. CP: AUC 85.0%
Liggett et al., 2010 [43]	PC: 30, CP: 30	Plasma (0.2 mL)	qPCR	17-gene methylation panel	Sensitivity: 91.2%, Specificity: 90.8%
Wu et al., 2023 [44]	PDAC: 8, CP: 8	Plasma (2 mL)	NGS	6 methylation sites in *PRKCB*, 4 in. *KLRG2*	AUC 100%
Majumder et al., 2021 [45]	PDAC + High-grade IPMN (Group 1): 38, Benign + Control (Group 2): 73	Pancreatic juice (1 mL)	qPCR	*C13orf18*, *FER1L4*, *BMP3*	Group 1 vs. Group 2: Sensitivity 83%, Specificity: 86% Stage I–II PDAC vs. Group 2: Sensitivity 70%, Specificity: 86%
Yokoyama et al., 2014 [46]	PDAC: 15, IPMN (intestinal type): 8, Control: 2	Pancreatic juice*	qPCR	*MUC1*, *MUC2*, *MUC4*	PDAC vs. Control: Sensitivity: 87%, Specificity: 80%; IPMN vs. Control: Sensitivity: 100%, Specificity: 88%;

Note: (for Tables 2–4): *qPCR* indicates quantitative real-time PCR; *AUC*, area under the receiver operating characteristic curve. *Data not reported in the publication.

achieved 100% sensitivity and specificity, although the sample size was limited (29 PDAC patients and 6 healthy volunteers) [49]. Thus, miRNAs remain the most commonly investigated exRNAs in this context, although interest in other ncRNAs is growing. For example, in a study by Xu et al., a circular RNA (circRNA) panel composed of *circ-0060733*, *circ-0006117*, *circ-0064288*, *circ-0007895*, and *circ-0007367* demonstrated 84% sensitivity and 71% specificity for PDAC detection [50].

Plasma miRNA profiling also shows promising potential in differentiating PDAC from CP, with reported sensitivity and specificity reaching 81.5% and 93.3%, respectively [51–53]. However, miRNA analysis of pancreatic juice (*miR-16, miR-21*, and *miR-25*) does not appear to significantly outperform plasma-based analysis for distinguishing PDAC from CP, achieving sensitivity and specificity of 84.2% and 81.5%, respectively [54]. In contrast, distinguishing PDAC from other pancreatic tumors remains more challenging. For instance, Cao et al. reported a sensitivity and specificity of only 64.8% and 64.9%, respectively, using plasma-based miRNA analysis [51].

In the context of IPMN, Permuth-Wey et al. found that a 30-miRNA panel in plasma could detect IPMN with a sensitivity and specificity of 78.6% and 62.5%, respectively [55]. Studies by Kuratomi et al. and Vicentini et al. also demonstrated that miRNA content varies between plasma or pancreatic juice samples from patients with IPMN, CP, and healthy

Table 3. Diagnostic performance of non-coding RNA analysis in the detection of pancreatic ductal adenocarcinoma and its precursor lesions.

Study	Participants (n)	Body fluid	Method	Targets	Normalization	Results
Que et al., 2013 [47]	PDAC: 22, Benign + Ampullary CA + CP + Control: 47	Serum (1 mL)	qPCR	miR-21, miR-17-5p, miR-155, miR-196a	U6	*miR-17-5p:* Sensitivity: 72.7%, Specificity: 92.6%; *miR-21*: Sensitivity: 95.5%, Specificity: 81.5%
Zou et al., 2019 [48]	PDAC: 30, Control: 30	Plasma (0.2 mL)	qPCR	Panel: *let-7b-5p, miR-192-5p, miR-19a-3p, miR-19b-3p, miR-223-3p, miR-25-3p*	miR-34	Sensitivity: 93.3%, Specificity: 96.0%
Lai et al., 2017 [49]	PDAC: 29, Control: 6	Plasma (0.25 mL)	qPCR	miR-10b, miR-21, miR-30c, miR-181a	miR-425-5p	*miR-10b/21/30c/181a*: Sensitivity 100%, Specificity: 100%
Xu et al., 2024 [50]	PDAC: 88, Control: 46	Plasma (0.2 mL)	qPCR	Hsa_circ_0060733, 0006117, 0064288, 0007895, 0007367	β-Actin	Sensitivity: 84.0%, Specificity: 71.0%
Cao et al., 2016 [51]	PDAC: 156, Other tumors: 85, CP: 57	Plasma (0.625 mL)	qPCR	Panel 1*: miR-486-5p, 126-3p, 106b-3p;* Panel 2: *miR-486-5p, 126-3p, 106b-3p, 938, 26b-3p, 1285*	U6, miR-16	PDAC vs CP (Panel 1): Sensitivity: 82.7%, Specificity: 84.4% PDAC vs. other tumors (Panel 2): Sensitivity: 64.8%, Specificity: 64.9%
Guo et al, 2021 [52]	PDAC: 27, CP: 15	Plasma (1 mL)	qPCR	miR-95-3p, miR-26b-5p	Detected miRNA set per sample	Sensitivity: 81.5%, Specificity: 93.3%
Vicentini et al., 2020 [53]	PDAC: 58, IPMN: 20, CP: 15	Plasma (0.4 mL)	FISH, qPCR	Panel of 800 miRNAs	U6	*
Nesteruk et al., 2022 [54]	PDAC: 54, Control: 118	Pancreatic juice (0.2 mL) Plasma (0.2 mL)	qPCR	PJ*: miR-16, 21, 25;* Plasma: *miR-210*, CA 19-9	Mean signal in the control group	Sensitivity: 84.2%, Specificity: 81.5%
Permuth-Wey et al., 2015 [55]	IPMN: 42, Control: 24	Plasma (0.5 mL)	Direct multiplex RNA expression assay	Panel of 30 miRNAs	Housekeeping mRNAs *ACTB, B2M, GAPDH, RPL19, RPLP0*	Sensitivity: 78.6%, Specificity: 62.5%
Kuratomi et al., 2021 [56]	IPMN: 13	Pancreatic juice (0.5 mL)	NGS	miR-10a-5p, 106b-5p, 197-3p, 664a-3p, let-7d-3p	ncRNA levels in the normal tissue	*

Note: (for Table 4): *FISH* indicates fluorescence *in situ* hybridization. *Data not reported in the publication.

Table 4. Diagnostic performance of circulating tumor cell analysis in detecting pancreatic ductal adenocarcinoma and precursor lesions.

Study	Participants (n)	Body fluid	Method	Targets	Results
Freed et al., 2023 [24]	PDAC: 68, Control: 11	Blood (2 mL)	FCM	CD45– DAPI+ CK (++/+/–)/VIM (+/–) EpCAM+/FAPα+	Sensitivity: 97.6%, Specificity: 100%
Liu et al., 2017 [57]	PDAC: 95, Control: 48	Blood (2 mL)	FISH, IFA	CD45– DAPI+ CEP8 > 2	Sensitivity: 75.8%, Specificity: 68.7%
Ankeny et al. 2016 [58]	PDAC: 72, Control: 28	Blood (4 mL)	IFA	CD45– DAPI+ CK+/CEA+	Sensitivity: 75.0%, Specificity: 96.4%
Dotan et al., 2016 [59]	PC: 50	Blood (7.5 mL)	FISH	CD45– DAPI+ CK+ MUC-1	Sensitivity: 48%
Kuvendjiska et al., 2023 [60]	IPMN: 27, Control: 5	Blood (6 mL)	IFA	EpCAM+ L1CAM+ VIM+ PDX1+	Sensitivity: 37%, Specificity: 100%
Buscail et al., 2019 [61]	PDAC: 22, IPMN: 8, Control: 20	Blood (7.5 mL)	IFA	CD45- DAPI+ CK+ EpCAM+	PDAC vs. IPMN: Sensitivity 30%, Specificity 100%; PDAC vs. Control: Sensitivity 50%, Specificity: 90%
Buscail et al., 2019 [61]	PDAC: 22, Control: 20	Blood (7.5 mL)	IFA	GPC1	Sensitivity: 50%, Specificity: 90%
Kitagawa et al., 2023 [62]	PDAC: 9, Control: 13	Pancreatic juice*	Cytologic analysis	—	Sensitivity: 77.8%, Specificity: 100%
Tag-Adeen et al., 2018 [63]	IPMN: 29	Pancreatic juice*	Cytologic analysis	—	Sensitivity: 60%, Specificity: 79%
Miyamoto et al., 2020 [64]	Malignant IPMN: 15, Benign IPMN: 23	Pancreatic juice*	Cytologic analysis	—	Sensitivity: 40.0%, Specificity: 100%

Note: *IFA*, immunofluorescence assay; *FCM*, flow cytometry. *Data not reported in the publication.

individuals, although specific diagnostic accuracy metrics were not reported [53, 56].

Circulating Tumor Cells

Circulating tumor cells (CTCs) constitute a heterogeneous population of viable and apoptotic cells shed from primary or metastatic tumor sites. As CTCs retain both phenotypic and genotypic characteristics of the tumor, their detection in plasma represents a promising diagnostic strategy for PDAC [24, 57–61].

While most liquid biopsy studies in PDAC have concentrated on ctDNA and exRNA, several investigations into CTCs have produced clinically relevant findings. Common detection methods include fluorescence *in situ* hybridization (FISH), immunofluorescence, and cytologic evaluation. CTCs are typically identified based on their phenotype: CD45, DAPI+, CK+, and EpCAM+ (Table 4). Detection of CTC in peripheral blood allows for PDAC diagnosis with a sensitivity ranging from 50% to 90% and a specificity approaching 100% [24, 57–59]. Detection rates increase with disease progression, from 60% in stages I–II to 97% in stage IV [57, 58]. In a study by Kuvendjiska et al., detection of IPMN using blood-based L1CAM+, VIM+, and PDX1+ markers yielded a sensitivity of 37% and a specificity of 100% [60]. Buscail et al. assessed the diagnostic utility of CTC analysis in differentiating PDAC from IPMN; however, the sensitivity did not exceed 30% [61].

The sensitivity of liquid biopsy for detecting PDAC through CTC analysis in pancreatic juice is 77.8% [62]. In a study by Tag-Adeen et al., cytological evaluation of pancreatic secretions for IPMN diagnosis demonstrated a sensitivity and specificity of up to 60% and 79%, respectively [63]. In another study by Miyamoto et al., differentiation between low- and high-grade IPMN was achieved with a sensitivity of 40% [64].

DISCUSSION

Histopathological evaluation of biopsy material remains the gold standard for PDAC diagnosis, allowing for the detection of even small lesions with high sensitivity and specificity [13]. However, diagnostic accuracy is highly dependent on the pathologist's expertise and the adequacy of the sample obtained [4, 9]. Liquid biopsy may serve as a complementary diagnostic approach. This technology has demonstrated high diagnostic performance (with sensitivity and specificity reaching up to 90%–95%) for advanced PDAC (stages III–IV), based on the analysis of extracellular nucleic acids and CTCs in various body fluids (Tables 1–4). Nonetheless, considerable limitations remain in its application for early-stage PDAC, precursor lesions such as IPMN, and in differentiating these conditions from CP or other pancreatic neoplasms.

The low abundance of biomarker in early-stage disease likely contributes to the reduced sensitivity of detection. For instance, plasma cfDNA concentrations often does not exceed 30 ng/mL, whereas pancreatic juice may contain up to 2600 ng/mL [35]. Several studies suggest that pancreatic secretions provide superior sensitivity for detecting early PDAC and for differentiating it from CP and IPMN (Tables 1–4). However, the collection of pancreatic juice via endoscopic retrograde cholangiopancreatography is expensive, labor-intensive, and highly invasive [36, 37]. The procedure also carries risks of cholangitis, pancreatitis, and bleeding, limiting its routine clinical use [65]. In our previous study, bile—also located in close proximity to tumor tissue—was found to contain higher ctDNA concentrations than plasma [38]. Routine sampling of bile is feasible in approximately 40% of patients with tumors in the pancreatic head, where biliary obstruction necessitates drainage. However, bile analysis is unsuitable for early detection, as obstruction typically manifests in advanced stages of disease. Additionally, it is unlikely to be useful for diagnosing tumors located in the pancreatic body or tail.

The performance of liquid biopsy also depends on the volume of the substrate and the analytical technique used. In the referenced studies, nucleic acids were extracted from 0.2–10 mL of plasma and 0.2–1 mL of pancreatic juice (Tables 1–4), and variability in DNA extraction kits may have influenced the results [66]. Real-time PCR (qPCR) is less sensitive at low DNA concentrations and more prone to interference by PCR inhibitors when compared with droplet digital PCR (ddPCR) or NGS, especially for detecting single mutations in plasma [67, 68]. However, in the analysis of biomarker panels or pancreatic secretions, qPCR generally performed comparably to ddPCR and NGS (Tables 1–4).

Bisulfite conversion, commonly used in DNA methylation analysis, can degrade between 50%–90% of nucleic acids, limiting sensitivity to around 50% [39, 40, 69]. Gene panel-based epigenetic profiling may enhance sensitivity to 82% [42]. The use of methylation-sensitive restriction enzymes—which are highly specific for target sequences—can preserve DNA and raise sensitivity to 91% [43, 70]. However, not all clinically relevant methylation sites are located within regions suitable for the current available restriction enzymes [71].

CTCs are typically identified via cytologic analysis, a method that is operator-dependent and may account for the observed variability in sensitivity (30%–80%, Table 4). Freed et al. employed flow cytometry to achieve an exceptionally high sensitivity of 97.6%, although staging data for the patients were not reported [24]. Other studies report that cytologic detection of CTCs yields greater than 90% sensitivity in stage IV PDAC [58], yet data remain insufficient for early-stage disease.

Finally, the accuracy of liquid biopsy is influenced by both the number and diversity of biomarkers analyzed. Panel-based approaches generally outperform single-target tests (Tables 1–3). For instance, the combined analysis of ctDNA/miRNA and CA 19-9 enhances the detection of early-stage PDAC, with sensitivity reaching 70%–80% and specificity up to 95% [28, 50]. However, several potentially valuable biomarkers were not evaluated in the reviewed studies, such as ctDNA mutations in *CDKN2A*, *TP53*, and *SMAD4* (which are present in 30%–70% of PDAC cases) and *GNAS* mutations (common in IPMN) [16]. Additionally, markers like MUC1, MUC2, and MUC4 were not considered for CTC identification.

While this review primarily focused on the diagnostic role of liquid biopsy, its applications extend beyond diagnosis. For example, liquid biopsy may assist in prognostication. Patients with MUC1-positive CTCs exhibit significantly lower median survival rates (2.7 months [95% CI, 0.1–7.6]) compared to those with MUC1-negative CTCs (9.6 months [95% CI, 3.9–12.8]; $p = 0.044$) or no detectable CTCs (8.8 months [95% CI, 6.0–10.9]; $p = 0.014$) [59]. Certain genetic alterations, such as *KRAS G12V* and *G12D* mutations, are linked to poor prognosis and can be identified through liquid biopsy in unresectable tumors [34]. The combination of miR-335-5p and miR-340-5p has been proposed as a marker for metastatic potential [52]. In a study by Ankeny et al., patients with ⩾3 CTCs per 4 mL of blood were 6.39-fold more likely to experience metastasis at initial assessment compared to those with lower CTC counts [58]. Moreover, ctDNA and miRNA levels generally decrease following effective surgery or chemotherapy, providing a means for monitoring treatment response [30, 49].

CONCLUSION

Liquid biopsy for pancreatic diseases offers a promising tool for the diagnosis, prognosis, and therapeutic monitoring of PDAC. Despite its current limitations in detecting early-stage PDAC and IPMN, as well as in differentiating these conditions from benign pancreatic diseases, liquid biopsy shows substantial potential for clinical application. However, it is not yet capable of replacing conventional diagnostic methods, particularly histopathologic evaluation. For the technology to progress, standardized protocols and larger randomized trials are essential, including evaluations of its clinical and economic impact on healthcare systems.

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ADDITIONAL INFORMATION

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