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# The role of biomarkers in the early detection of pancreatic cancer

**Michael Goggins**[1,2,3]

[1]Department of Pathology, The Sol Goldman Pancreatic Cancer Research Center, The Johns Hopkins University School of Medicine, 1550 Orleans Street, Baltimore, MD 21231, USA

[2]Department of Medicine, The Sol Goldman Pancreatic Cancer Research Center, The Johns Hopkins University School of Medicine, Baltimore, MD, USA

[3]Department of Oncology, The Sol Goldman Pancreatic Cancer Research Center, The Johns Hopkins University School of Medicine, Baltimore, MD, USA

## Abstract

Pancreatic surveillance can detect early-stage pancreatic cancer and achieve long-term survival, but currently involves annual endoscopic ultrasound and MRI/MRCP, and is recommended only for individuals who meet familial/genetic risk criteria. To improve upon current approaches to pancreatic cancer early detection and to expand access, more accurate, inexpensive, and safe biomarkers are needed, but finding them has remained elusive. Newer approaches to early detection, such as using gene tests to personalize biomarker interpretation, and the increasing application of artificial intelligence approaches to integrate complex biomarker data, offer promise that clinically useful biomarkers for early pancreatic cancer detection are on the horizon.

### Keywords

Pancreatic cancer; Biomarker; Early detection; CA19–9; Pancreatic surveillance; Familial pancreatic cancer; Pancreatic intraepithelial neoplasia; Pancreatic cyst; Intraductal papillary mucinous neoplasm; Tumor marker gene test

## Introduction

In recent years, cohort studies have yielded convincing evidence that pancreatic surveillance can detect pancreatic cancer sufficiently early to achieve long-term survival [1, 2]. Pancreatic surveillance currently relies on the pancreatic imaging tests endoscopic ultrasound (EUS) and MRI/MRCP, tests that can image pancreatic masses or detect imaging biomarkers suggestive of precancerous neoplasia. Biomarkers have been investigated for decades for their potential as early detection tests. The primary target pathologies for early detection are Stage I pancreatic cancers and high-grade dysplasia in the absence of invasive cancer [3, 4]. Stage I pancreatic cancers accurately staged by surgical pathology are

✉Michael Goggins, mgoggins@jhmi.edu.

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associated with excellent long-term survival (5 year survival of over 80% in the NCI SEER database) [5]. A biomarker has been defined broadly including by the WHO as “almost any measurement reflecting an interaction between a biological system and a potential hazard, which may be chemical, physical, or biological. The measured response may be functional and physiological, biochemical at the cellular level, or a molecular interaction” [6].

The ultimate aim of an early detection biomarker is to detect cancer early enough to improve survival. Many candidate biomarkers have been evaluated as potential pancreatic cancer screening tests, but none have reached the high bar evidence of improved survival. Beyond cancer screening tests are biomarkers with narrower goals such as biomarkers that characterize an imaging abnormality. Table 1 lists examples of potential clinical uses of a biomarker for pancreatic cancer early detection. The performance characteristics needed for a biomarker depend on its intended use. Many years ago, the NCI’s Early Detection Research Network, recognizing that the evaluation of a biomarker especially one for cancer screening, is a complex process fraught with challenges, developed a framework for biomarker evaluation that involved five phases, the final one demonstrating that applying the test achieves a mortality benefit [7]. As more and more early detection tests come onto the market and are used outside of a clinical trial setting, it may become increasingly difficult to assess the full impact of many of these tests.

Pancreas surveillance with EUS and MRI/MRCP can detect small (often subcentimeter diameter) pancreatic masses once they have characteristics that distinguish them the surrounding pancreas parenchyma (e.g. hypoechoic, and often irregular borders by EUS) [8]. Cancers with less characteristic features, such as diffuse infiltrating and/or isoechoic cancers can be easily missed with current tests, and their detection could benefit from accurate imaging biomarkers. An example might be the detection of certain radiomic features which can now be combined into a test using artificial intelligence methods. A potentially greater challenge than detecting invasive cancer is establishing whether certain imaging abnormalities represent pancreatic dysplasia and if so trying to predict its grade, as is the case when characterizing the features of a pancreatic cyst, or determining the characteristics of a small mass lesion, such as a slightly hypoechoic lesion by ultrasound that could represent an area of parenchymal change arising from PanIN or from focal inflammatory changes. Most PanIN are too small to be detected by current imaging methods even when they have a significant effect on the local parenchyma. The most valuable pancreatic biomarker would likely be a highly accurate blood test for early-stage pancreatic cancer. Many of the cancer screening blood tests undergoing clinical evaluation are MCED tests whose performance characteristics are set for potential application in average risk populations. Another potential approach to improving the early detection of pancreatic cancer in the general population is interrogating electronic medical record data using machine learning methods. Beyond biomarkers that can indicate the presence of cancer are biomarkers that predict a significantly increased long-term risk of developing the disease, such as cancer susceptibility gene variants. Biomarkers of risk can help determine whether or not someone meets risk criteria for cancer surveillance.

The biomarker performance needed for clinical use should inform study design and biomarker evaluation. Since differences between populations can affect biomarker

performance, performance should be evaluated in study populations where it's use is intended. Promising candidate biomarkers for early detection ultimately need to be evaluated in large population studies. Several large MCED biomarker clinical trials have been established (the NIH's Cancer Screening Research Network, and the Grail NHS study) to evaluate MCED biomarkers. Research cohorts with banked biospecimens are also valuable as they enable evaluation of biomarkers in the pre-diagnostic setting.

One common limitation of many initial pancreatic cancer biomarker studies is the inclusion of patients advanced stage disease. Even when studies are designed to evaluate biomarker performance in early-stage disease, generally patients with Stage I/II pancreatic cancer are included, rather than Stage I cases alone, because so few Stage I cases are diagnosed. Diagnostic sensitivity generally improves with increasing tumor burden, and most biomarkers have very modest sensitivity for Stage I pancreatic cancer (e.g. ctDNA ~ 25–30%). A biomarker test with good all-stage or even Stage I/II performance, but poor performance for Stage I disease would likely not impact survival much as most patients even with Stage II pancreatic cancer currently die of their disease [5]. An additional challenge to evaluating early detection biomarkers is the accurate assessment of disease stage at diagnosis since most patients now receive upfront neoadjuvant therapy, and pancreatic cancer staging at diagnosis relies on imaging which often fails to detect lymph node involvement and so under-stages compared to the gold standard of surgical pathology.

An important characteristic needed of circulating biomarkers for early pancreatic cancer detection is high diagnostic specificity, even when applied to a high-risk population undergoing pancreas surveillance. Since a positive screening test is often followed with a more accurate test (e.g. pancreas-protocol CT), some clinicians may be willing to tolerate a higher sensitivity, lower specificity test, but when the disease incidence is low, (as is the case for high-risk populations where annual pancreatic cancer incidence is ~ 1/200 cancer) [1], the trade-offs generally favor the high-specificity test. The downstream testing needed to establish that a test is false-positive, with its potential risks, cancer worry and additional cost favor high specificity over sensitivity. For pancreatic surveillance of high risk individuals, probably the most important performance characteristic of a biomarker test is its sensitivity at high specificity (>/= 98%), with diagnostic cut-offs established in a retrospective case/control study and validated prospectively. Such a metric (Sensitivity@98% Specificity) is generally more clinically useful than the information providing in an AUC. Biomarkers need to have excellent analytic performance for clinical use. Important parameters of performance include measures of reproducibility such as co-efficient of variation and precision. Calibration is a useful measure of risk prediction tools, poor calibration between predicted and observed risk often reflecting differences in patient characteristics between study populations [9]. Considerations of reproducibility go well beyond analytic measures. Many factors can lead to failure of reproducibility and the related metric of repeatability which are often related to conceptual flaws in data analysis or interpretation, a topic well-known to statisticians, that ultimately reflects the underlying strength of evidence of the biomarker [10]. Another useful measure of biomarker performance is the area under the precision-recall curve ($AUC_{PR}$) [11] where precision refers to positive predictive value, and recall means diagnostic sensitivity. The positive predictive value is a function of the

prevalence of the disease in the population under study, so with a disease of low-prevalence, the $AUC_{PR}$ approximates a test's positive predictive value [12].

Many reviews of candidate pancreatic cancer biomarkers have been undertaken (such as [13]). Below I describe recent developments and progress in early detection biomarkers.

## Early detection biomarkers

### Imaging biomarkers

A wide variety of imaging biomarkers have been evaluated for their potential to detect pancreas pathology, including early pancreatic cancer. Multiple studies have found that radiology review of prediagnostic CT scans often finds subtle imaging abnormalities concerning for pancreatic cancer [14–16], even without the use of advanced imaging analysis. One such abnormality is a dilated main pancreatic duct [17]; main duct dilation can be benign transient finding, but can also be an early indicator of pancreatic pathology, such as from obstruction or mucin. Pancreatic atrophy is also a potentially concerning biomarker, albeit a relatively non-specific one since some pancreatic atrophy is a feature of aging [18], but can also arise secondary to main pancreatic duct obstruction [19]. The set of biomarkers that constitutes so-called "worrisome features" (e.g. large cyst, main pancreatic duct dilation and thickened cyst walls) are used to predict the grade of dysplasia of pancreatic cystic neoplasms, but do so with only modest accuracy [20–22]. As a result, only ~ 1/4 pancreas resections for IPMN contain high-grade dysplasia [23]. Better imaging biomarkers are needed. Another biomarker of pancreas pathology is the extent of pancreas fat [24], particularly fatty replacement within the gland, as opposed to fat deposition around the gland that reflects metabolic syndrome [25]. Pancreas fat can be accurately quantified using MRI [26] and semi-quantified using EUS. Small pancreatic ducts obstructed by PanIN or mucin causes lobulocentric atrophy of the surrounding pancreatic parenchyma that is replaced by pancreas fat [27]. The extent of pancreas fat has been quantified using digital pathology tools and has been shown to correlate with the extent of PanIN in high-risk individuals who have undergone pancreatic resection [28]. A variety of subtle quantitative imaging parameters broadly termed radiomics have been evaluated as potential early detection tools [29].

Artificial intelligence (AI) tools have been applied to assess the combined value of many of these imaging features as a composite test. In one study, machine learning models of radiomic features applied to prediagnostic CT scans heralded a diagnosis of pancreatic cancer and performed better than radiologists [30]. Loss of muscle and fat mass by CT [31–33], particularly muscle mass at the 2nd lumbar vertebrae which can be measured automatically with machine learning tools [32, 33], has been shown to herald a diagnosis pancreatic cancer by a year or more. Measures of fat and muscle mass as a useful clinical test may require having a prior CT scan for comparison. Applying AI tools to prediagnostic non-contrast CT was recently shown to be capable of detecting pancreatic cancer with excellent diagnostic performance [34]. Deep learning approaches to automate the detection of pancreatic imaging abnormalities including pancreatic cancer have shown promise [35], but challenges remain, given the normal variation in pancreas shape and other features [36]. Such studies await further evaluation but could significantly improve the detection of

pancreatic cancer in patients who get CT scans for other diagnostic indications. Compared to CT, fewer studies have applied artificial intelligence tools to MRI or EUS in large part because of the greater variability in MRI and EUS imaging protocols, but such studies could yield important insights. EUS finding of subtle parenchymal heterogeneity and other non-specific features [37, 38] originally described in patients with chronic pancreatitis [39], are often found in high-risk individuals, and while non-specific, these changes are thought to indicate parenchymal change surrounding the greater PanIN burden in these patients [40]. Such EUS features are difficult to quantify and interpret [41] and a more qualitative and quantitative analysis of these features could improve EUS parenchymal image interpretation, potentially improving risk stratification.

Molecular imaging has shown value in the detection of certain cancers and could have value in the evaluation of lesions of uncertain significance. One potential molecular target is the integrin α(v)β(6) [42]. This imaging test been used in clinical trials and can detect small metastases, but it's role in early detection has not been determined. Molecular imaging tests are difficult to employ in many diagnostic settings. More promising is that the α(v)β(6) integrin is being evaluated as a theranostic [43].

## Circulating biomarkers

A high performing circulating biomarker suitable for clinical use would likely have great impact on pancreatic cancer early detection efforts, in part because in some health care settings high-quality EUS and MRI tests are not widely available. But biomarker candidates evaluated to date all miss many early-stage pancreatic cancers detectable by imaging tests, and most have significant limitations with respect to false-positives. For high-risk individuals, where imaging is only modality shown to improve outcomes, a high-performing circulating early detection biomarker could still have clinical utility when combined with pancreatic imaging (such as alternating circulating blood test and imaging test every six months), and could be used to aid in the evaluation of worrisome imaging abnormalities.

**CA19-9, DUPAN-2 and the tumor marker gene test—**Serum CA19–9 (also known as sialyl Lewis$^a$ or sLe$^a$) has been evaluated extensively for many decades as a potential diagnostic, and while it lacks the performance characteristics needed for an early detection test on its own, (CA19–9's sensitivity is only ~ 50% among subjects with resectable-stage pancreatic cancer, and false-positive elevations of CA19–9 limit its potential as a screening test [44]), recent studies have shown its diagnostic performance can be improved by using a tumor marker gene test. Most false-positive CA19–9 tests arise because of genetic differences in individuals' capacity to synthesize CA19–9. *FUT3* encodes the enzyme that fucosylates glycan precursors to create CA19–9. Upstream in the pathway, FUT2 and other enzymes divert CA19–9 precursors to produce related molecules. Inactivating variants in *FUT3* and *FUT2* variants have a major effect on CA19–9 levels and these variants can be used to predict an individual's CA19–9 level. Four genetic groups best predict the level of CA19–9 in healthy individuals: These groups are from lowest to highest CA19–9 levels reference ranges are: (i) FUT3-null individuals who produce virtually no CA19–9; (ii) individuals with one FUT3-null allele with intact FUT2; (iii) individuals with two functional FUT3 alleles and intact FUT2; (iv) FUT2-null individuals that have at least one

*FUT3* functional allele) [45, 46]. Classifying patients in this manner improves the diagnostic performance of CA19–9 [45].

DUPAN-2, also known as sialyl Lewis$^c$ or sLe$^c$, is the immediate precursor to CA19–9 [47–49]. DUPAN-2 was first described as a pancreatic cancer antigen in 1982 [47], is elevated in patients with pancreatic cancer almost as often as CA19–9 [50, 51], and is used mainly in Japan to monitor pancreatic cancer disease burden, especially for patients who do not produce CA19–9 [51–54]. The same *FUT* variants that affect CA19–9 synthesis and metabolism affect DUPAN-2 levels. We have recently shown that individuals can be classified into three functional groups with respect to their circulating DUPAN-2 levels; those with intact FUT3, those who are FUT2-null with functional FUT3, and those who are both FUT2 and FUT3 null [55]. Grouping individuals this way and giving each group their own reference range significantly improves DUPAN-2 diagnostic performance for pancreatic cancer. And since DUPAN-2 complements CA19–9 performance, the combination of the two markers has high accuracy for early-stage pancreatic cancer, which is further improved using the tumor marker gene test. In our study of over 300 pancreatic cancer cases and over 600 controls, the combined test of FUT/CA19–9/DUPAN-2 achieved 62% sensitivity at ~ 98% specificity for Stage I pancreatic cancer (an AUC 0.919), and an AUC of 0.96 for patients with Stage I/II pancreatic cancer, significantly higher than without the FUT gene test [55]. This high diagnostic accuracy, particularly for Stage I disease is promising, suggesting it could be a clinically useful early detection test, though its diagnostic performance needs further prospective validation.

We also found an explanation as to why some patients with pancreatic cancer have detectable CA19–9 levels despite being FUT3-null. FUT3-null patients with pancreatic cancer who produced CA19–9 are generally FUT3-null and FUT2-null whereas FUT-3 null pancreatic cancer cases with functional FUT2 rarely have detectable CA19–9 [55].

**Circulating tumor DNA (ctDNA)—**CtDNA is used widely to monitor tumor burden in patients with cancer. Its potential as a diagnostic is being investigated, primarily as a multi-cancer early detection test. One advantage of the multi-cancer detection approach is that the pan-cancer incidence is considerably higher than it is for a single cancer, especially an uncommon one like pancreatic cancer. ctDNA biomarkers include mutated, methylated [56] or hydroxymethylated DNA [57], changes in DNA fragmentation [58], and tests of aneuploidy [59]. Two of the most extensively studied ctDNA-based biomarker tests are the multi-marker tests CancerSEEK [60], and the methylated DNA panel developed by GRAIL [61]. CancerSEEK is a multi-modality biomarker test that incorporates barcoding DNA and next-generation sequencing for mutations and aneuploidy, and a panel of protein markers that was used successfully in a clinical trial to identify asymptomatic early-stage cancers [60]. GRAIL has published several studies that demonstrate excellent performance. In their Pathfinder study of over 6000 subjects, 1.4% had a positive test, of whom 38% had cancer, corresponding to a false-positive rate of just under 1%, and approximately 1/2 of the subjects with a new cancer were Stage I/II [61]. ctDNA-based tests continue to undergo refinement. For example, tests that detect aneuploidy have shown superiority over mutation-based approaches [59]. Some groups have developed their own methylated DNA panels which show good diagnostic performance [62, 63].

Although ctDNA-based tests are promising multi-cancer detection tests, they have some limitations. One is that while most of the cancers detected are earlier-stage, few are Stage I, with overall performance varying by cancer-type. For pancreatic cancer where Stage I detection is paramount, less than half of patients with resectable-stage pancreatic ductal adenocarcinoma have detectable ctDNA [60] (and only ~ 25 to 30% with Stage I pancreatic cancer). Most circulating DNA arises from leukocytes [64], and some ctDNA alterations that might appear to be cancer-derived actually arise from leukocytes with clonal hematopoiesis or other abnormalities [65]. Another challenge is the low ctDNA signal in patients with cancer which is why ctDNA approaches for early detection have moved beyond mutation detection alone to approaches that detect aneuploidy. Novel preclinical strategies have been developed to try and improve ctDNA signals, such as blocking liver clearance of DNA [66].

Multi-cancer early detection (MCED) have potential utility in high risk cohorts, especially those with germline variants that give rise to hereditary cancer syndromes, since these patients are at increased risk for multiple cancers, some of which are not screened. However, it remains to be seen how well MCED tests can complement existing cancer screening tests.

**Other circulating biomarkers—**Many other circulating biomarkers have been evaluated as candidates for pancreatic cancer early detection [13]. Some biomarkers such as the cytokine GDF15/MIC-1 were initially identified by RNA profiling of cancers [67], others such as THBS2 were identified by analyzing the secreted proteins of in vitro models [68]. Circulating ApoAII fragments were identified as biomarkers through mass spectrometry profiling of plasma, and have undergone validation in primary care settings, where it shows elevations in patients without cancer [69, 70]. More recently, further testing using a test that detects reduced levels of ApoAII/AT and ApoAII/ATQ performed modestly better than CA19–9 for pancreatic cancer detection (Kashiro et al, 2024, PMID38261000). Circulating enzymatic activity of carboxypeptidase A (CPA) was evaluated as a candidate biomarker because of its pancreas specific expression [71]. Elevated CPA is a good biomarker of early in patients who have not developed pancreatic atrophy [72]. Multi-protein marker panels have been evaluated by many groups [73–75]. None of these biomarkers have better diagnostic performance than CA19–9, most add little when combined with CA19–9 [76], few have diagnostic characteristics needed for pancreatic surveillance on their own, and fewer still have shown an ability to detect very early stage (Stage I pancreatic cancer), or have good performance in the prediagnostic setting. CA19–9 has been shown to have some pre-diagnostic utility mainly in the year prior to diagnosis [77]. The UKTOCS study group employed machine learning methods in an attempt to overcome limitations of individual biomarkers [78], and found this approach improved biomarker performance, predicting pancreatic in prediagnostic serum samples with good accuracy.

Multiple studies have profiled circulating miRNAs and circRNAs and evaluated their diagnostic potential for pancreatic cancer, some marker panels showing excellent performance [79, 80]. Some of these studies have involved analysis of the contents of extracellular vesicles [79, 81–83]. However, although some of the miRNA biomarkers have been found in multiple studies, there is a lack a uniform list of the top candidates. Most of these circulating biomarkers have yet to be prospectively evaluated as clinical tests in the pancreas surveillance setting.

Other marker-types that have been evaluated for their cancer diagnostic potential include microbial DNA. Bacteria and fungi are found in pancreatic and other cancers [84, 85] and several studies have reported the detection of circulating microbial DNA including fungal DNA in cancer patients [86, 87]. However, concerns that the analysis pipeline misclassified microbial DNA [88] indicates that further investigation is needed to better understand whether circulating microbial DNA has diagnostic potential.

Other studies have reported microbial signatures in oral and fecal samples in patients with pancreatic cancer [89, 90] but it is not yet clear if these signatures are stable indicators of long-term cancer risk as opposed to diagnostic markers. Prospective studies are needed.

**New-onset diabetes—**Recognizing that the prevalence of diabetes is significantly increased in patients with newly-diagnosed pancreatic cancer, multiple studies have evaluated the prevalence of new-onset diabetes (NOD) as a potential early detection biomarker [91–95]. Between ~ 0.5% to almost 0.8% of NOD in older individuals will be due to pancreatic cancer, depending on the underlying prevalence of diabetes in the control population, with pancreatic cancer being diagnosed mostly within the first year of NOD and pancreatic cancer-related diabetes and becoming more likely as the cancer enlarges and causes atrophy of the gland [96]. More recent studies are attempting to identify additional biomarkers, such as declining serum lipids, that better predict which cases with NOD will have pancreatic cancer [96]. Studies have begun to prospectively identify cases of NOD and to determine if pancreatic cancer can be detected earlier [97, 98].

## Electronic health records

Many patients with pancreatic cancer initially present with vague symptoms. Such symptoms and other clinical features that may not individually raise concerns for cancer could do so in the context of other findings. For example, a recent analysis of the Harvard cohorts found evidence for age-dependent differences in the magnitude of common pancreatic cancer risk factors [99]. Another study from the group found evidence that patients with pancreatic cancer commonly show prediagnostic medication changes that reflect the emergence of diabetes, or a drop in blood pressure associated with weight loss [100]. Machine learning models are being applied to electronic health records to improve the early detection of pancreatic cancer and other diseases [101–103]. One study that demonstrated the potential of this approach utilized 6 million subjects in the Danish National Patient Registry as well as 3 million US Veterans Affairs and found good diagnostic performance for identifying pancreatic cancer within the Danish database within 3 years of diagnosis, though the model did not perform well when cross validated with the US dataset [102]. These studies offer promise that subjects with concerning findings in their EHR could be flagged for further investigation.

## Cyst fluid

Cyst fluid analysis has been used to determine the type of cysts and the likely grade of dysplasia, and is probably superior to biomarker measurements in blood for this purpose, although blood-based testing maybe more valuable for detecting evidence of an associated invasive cancer [83]. Multiple marker types have been evaluated as cyst fluid biomarkers

[104] including mutant DNA [105], methylated DNA [106], protein biomarkers [107, 108], mucins [109, 110], aneuploidy measurements [104], glucose levels [111], telomerase activity [112], telomere fusions [113], some of have been compared to the established cyst fluid biomarker, CEA, which has only modest diagnostic performance [114]. The most extensive clinical validation has involved mutant DNA and multi-modal biomarker panels [104]. The main diagnostic challenge when evaluating concerning pancreatic cysts is distinguishing neoplastic cysts with significant malignant potential such as IPMNs or MCNs with high-grade dysplasia and pancreatic neuroendocrine tumors, and cysts with little or no malignant potential such as serous cystadenomas or non-neoplastic cysts, and those cysts that need surveillance including IPMNs, MCNs with low-grade dysplasia [105, 115–117]. The multi-center CompCyst study evaluated multiple cyst fluid biomarkers including mutant DNA, measures of aneuploidy and protein biomarkers from over 800 patients, utilized machine learning approaches, and in a validation set of patients who underwent histologic resection would have significantly reduced the number of patients requiring surgery [104]. One of the most useful biomarkers is VEGF which distinguishes serous cystadenomas from other cysts [107]. Another prospective multi-center study [118] evaluated the role of next-generation sequencing of cyst fluid from over 1200 patients. The test panel predicted the presence of advanced neoplasia with high accuracy.

## Pancreatic juice

The inability of current pancreatic imaging tests to detect most PanIN, especially high-grade dysplasia in PanIN and very small pancreatic cancers, has led to efforts to find evidence of these lesions by biomarker profiling of pancreatic juice. Pancreatic juice can be collected endoscopically from the duodenum safely after secretin stimulation. Duodenal collections adds complexity to the sample as duodenal secretions can introduce confounding biomarkers, especially methylated DNA [119], and lowers the overall concentration of biomarkers of interest from the pancreas. Direct cannulation of the pancreatic duct to collect a more pure sample runs the risk of acute pancreatitis, and attempts to collect a more juice sample have yielded only modest success [120]. Despite these challenges, prior studies have found that patients who meet criteria for pancreas surveillance are more likely to have mutations in their pancreatic juice, especially *KRAS* mutations, even patients without imaging abnormalities; these mutations are thought to arise mainly from PanIN lesions and their detection increases with patient age [121, 122]. The *KRAS* and *GNAS* mutations commonly to IPMN are also frequently detected in pancreatic juice [121, 123, 124]. Next-generation sequencing has been used to detect other mutations in pancreatic juice, including *TP53* and *SMAD4* mutations which are associated with having pancreatic cancer and high-grade dysplasia [125, 126]. Collecting secretin stimulated pancreatic juice from the duodenum is safe, but the very low concentration of mutations in endoscopically-collected pancreatic juice is an obstacle to early detection as sophisticated NGS methods are needed to distinguish true mutations from background [121]. Although there is potential for pancreatic juice biomarker analysis to provide important information about the likelihood of dysplasia, including in patients who lack concerning findings by imaging [126], juice collection takes time [127] and apart from bicarbonate measurement to assess for pancreatic insufficiency, juice biomarker measurements has not yet been incorporated into routine clinical practice.

Other biomarkers of pancreatic cancer besides mutant DNA have been evaluated in initial studies, including methylated DNA [128, 129] and miRNAs [130].

### Urinary biomarkers

Several studies have investigated urinary biomarkers. One study that performed mass spectrometry profiling of urine identified a three protein marker urine panel, LYVE-1, REG1A, and TFF1 that exhibited good diagnostic accuracy (AUC 0.93 in validation) for stage I/II pancreatic cancer [131], performing less well in a subsequent study in a different study population [132]. Other biomarker types including miRNAs, exosomal markers, enzyme activity and other molecules have been measured in urine. It is not known that there is additional diagnostic value in sampling urine over blood.

### Biomarkers of risk

Moderate-to-high penetrant germline variants in many of the major cancer susceptibility genes significantly increase pancreatic risk [133, 134], and are used in the clinic along with family history of pancreatic cancer [135] to estimate an individual's lifetime risk and suitability for pancreas surveillance [3, 136]. Polygenic risk scores have potential as clinical tools for risk assessment in common diseases but don't yet have the diagnostic power to refine risk sufficiently for uncommon cancers such as pancreas, even when combined with other common risk factors [137, 138], though this could change with the identification of additional risk variants [139] and other factors. One such set of biomarkers are those that reflect metabolic dysfunction. Metabolomic profiling can measure hundreds of metabolites including microbial metabolites in the blood. One recent study found a metabolic profile that improved 5-year pancreatic cancer risk estimation [140].

## The future

Continued progress in biomarker development and cancer risk assessment is expected in the coming years providing opportunities. While few candidate early detection biomarker tests are ever shown to have clinical utility, when they do, their implementation into clinical practice can have significant resource implications. Many useful biomarker tests, particularly imaging-based biomarkers, will likely have limited uptake in resource-poor areas, where cost and lack of expertise prevent their widespread use. Research will be needed to evaluate how best to apply biomarker tests to individuals and populations with differing levels of pancreatic and other cancer risk.

## Conclusions

After decades of effort and many challenges associated with discovering suitable biomarkers that could improve the early detection of pancreatic cancer, there are signs of progress. The detection of early-stage pancreatic cancer, particularly Stage I disease is associated with long-term survival. Using a tumor marker gene test that accounts for common gene variants that influence the level of CA19–9 and DUPAN-2 significantly improves diagnostic accuracy. Machine learning approaches offer the possibility of yielding greater information

from biomarkers, particularly imaging-based biomarkers which remain the main diagnostic tools for pancreatic surveillance and the evaluation of suspected pancreatic cancer.

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## Data availability

No datasets were generated or analysed during the current study.

## Abbreviations

| | |
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| **EUS** | Endoscopic ultrasonography |
| **CT** | Computed tomography |
| **MRI** | Magnetic resonance imaging |
| **MRCP** | Magnetic resonance cholangiopancreatography |
| **PanIN** | Pancreatic intraepithelial neoplasia |
| **IPMN** | Intraductal papillary mucinous neoplasm |
| **AUC** | Area under the receiver operator characteristics curve |
| **AI** | Artificial intelligence |
| **MCED** | Multi-cancer early detection |
| **NOD** | New-onset diabetes |

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**Table 1**

Types of biomarkers for pancreatic cancer early detection

| Intended use | Type of biomarker | Examples | Primary target |
|---|---|---|---|
| Screening/surveillance | Blood tests | CA19–9, DUPAN-2, ctDNA | Stage I pancreatic cancer |
| | Electronic medical record | Weight loss, glucose, triglycerides, medication change | Earlier-stage pancreatic cancer |
| | Urinary biomarkers | Protein markers | Early-stage pancreatic cancer |
| | New-onset diabetes | Glucose | Early-stage pancreatic cancer |
| Pancreatic cyst evaluation (Neoplasia/grade) | Cyst fluid | Mutated DNA | Presence and grade of dysplasia |
| Endoscopic evaluation | Secretin-stimulated pancreatic juice | mutated DNA | PanIN, cystic neoplasia, pancreatic cancer |
| Evaluation of pancreatic imaging abnormalities | Imaging biomarkers | Dilated main pancreatic duct, fatty pancreas, atrophy, radiomics | Pancreatic cancer |
| | Molecular imaging | $\alpha^{v}$, $\beta^{7}$ Integrin | Pancreatic cancer |
| Risk assessment | Age, family history, smoking | # of first-degree and second-degree relatives with PANCREATIC CANCER | Pancreatic cancer |
| | Gene variants | BRCA1/2, ATM, CDKN2A, MLH1, MSH2 | Pancreatic cancer |
| | Plasma metabolomics | Metabolite panel | Pancreatic cancer |