

## EDITORIAL

# Molecular and Clinical Markers of Pancreas Cancer

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### Summary

Pancreas cancer has the worst prognosis of any solid tumor but is potentially treatable if it is diagnosed at an early stage. Thus there is critical interest in delineating clinical and molecular markers of incipient disease. The currently available biomarker, CA 19-9, has an inadequate sensitivity and specificity to achieve this objective. Diabetes mellitus, tobacco use, and chronic pancreatitis are associated with pancreas cancer. However, screening is currently only recommended in those with hereditary pancreatitis and genetic syndromes which predispose to cancer. Ongoing work to identify early markers of pancreas cancer consists of high throughput discovery methods including gene arrays and proteomics as well as hypothesis driven methods. While several promising candidates have been identified none has yet been convincingly proven to be better than CA 19-9. New methods including endoscopic ultrasound are improving detection of pancreas cancer and are being used to acquire tissue for biomarker discovery.

Pancreas cancer has the darkest prognosis of any gastrointestinal cancer with the mortality approaching the incidence [1]. While the overall five year survival is less than 4%, those recognized early, with tumor involving only the pancreas have a 25-30% five-year survival after surgery [2]. Given the limited treatment options there has been considerable focus on clinical and molecular harbingers of early disease.

### Currently Available Tools to Evaluate for Pancreas Cancer

There are no population wide screening tests for pancreas cancer. The best established marker is CA 19-9 which is a sialylated Lewis antigen of the MUC1 protein with an overall sensitivity of 80% and specificity of 90% [3]. Unfortunately, CA 19-9 may be positive in patients with non malignant diseases including cirrhosis, chronic pancreatitis, cholangitis, as well as other gastrointestinal cancers [4]. Patients with certain blood types are incapable of expressing the antigen recognized by CA 19-9 [3]. Furthermore, only 65% of those with resectable pancreas cancer have elevated CA 19-9 levels [5]. Additionally, CA 19-9 is a

poor screening test. At the Samsung Medical Center in South Korea 70,940 asymptomatic patients were screened using CA 19-9. However, among 1,063 with elevated levels only 4 had pancreas cancer and only 2 had resectable disease [6]. Nonetheless, CA 19-9 is widely used to evaluate patients with suspected pancreas cancer and those undergoing treatment.

Most cases of pancreas cancer are diagnosed by the discovery of a mass by computed tomography (CT) or magnetic resonance imaging. Endoscopic ultrasound (EUS), in which a high frequency ultrasound transducer is positioned in close proximity to the gland using an endoscope, has improved detection [7]. Additionally, EUS guided fine needle aspiration has improved the accuracy of diagnosing small lesions (less than 3 cm) from 66.7% for percutaneous methods to 86.1% [8]. EUS-FNA of the pancreas is associated with less malignant seeding compared to percutaneous methods and the region which is traversed is also typically removed during surgical resection [9]. However, the ability of EUS to detect malignancy in the setting of chronic pancreatitis is limited due to acoustic artifact and the yield of FNA is diminished by compromised visualization as well as desmoplastic tissue changes [10, 11, 12]. Emerging techniques such as elastography which measures the distensibility of tissue may improve the performance of EUS in this arena [13]. Analysis of molecular markers in FNA tissue shows promise and is likely to be the strategy of the future [14].

Precursor lesions of pancreas cancer include intraductal papillary mucinous neoplasms (IPMN), mucinous

**Key words** CA-19-9 Antigen; Diabetes Mellitus; Endosonography; Molecular Epidemiology; Pancreatic Neoplasms; Tobacco; Tumor Markers, Biological

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**Document URL** <http://www.joplink.net/prev/201011/18.html>

cysts, and pancreatic intraepithelial neoplasia (PanIN). Cystic lesions can be detected and sampled by EUS as well as radiographic methods and are the primary target for screening which is performed in high risk families. The evolution of cysts into malignancy is thought to involve the enlargement of the cysts, thickening of the lining, and the invagination of popcorn like dysplastic nodules into the cyst [15]. PanIN is microscopic and thus difficult to detect [16]. Akin to the Vogelgram seen for colonic neoplasia, PanIN progresses through stages 1-3 marked by worsening histologic dysplasia and the accumulation of additional genetic mutations [17, 18].

There are now molecular markers to help differentiate which cysts are mucinous and later to help determine which might be malignant. In the breakthrough cooperative pancreatic cyst study, lesions were sample by needle aspiration and a panel of potential markers in the fluid analysis was evaluated. Brugge *et al.* demonstrated that carcinoembryonic antigen (CEA) at a level greater than 192 ng/mL optimally predicted that a cyst was mucinous [19]. The pancreatic cyst fluid DNA analysis (PANDA) investigators reported that a DNA quality and mutational analysis can improve the differentiation of malignant from benign mucinous cysts [20]. Analysis of EUS-FNA tissue for *K-ras* point mutations can also help differentiate pseudo-tumoral chronic pancreatitis from pancreas cancer thus improving the accuracy of cytopathology from 86% to 90% [14]. Due to increased risk of complications, inability to evaluate the parenchyma, and relatively low yield of cytologic brushings, endoscopic retrograde cholangiopancreatography (ERCP) and percutaneous transhepatic cholangiography have less of a role in the diagnostic evaluation for pancreas cancer, though they are important in the management of biliary obstruction in patients with locally advanced and metastatic disease who are eligible for chemotherapy. A new panel of markers assessing for the absence of tumor suppressor genes may improve the yield of cytologic brushings [21].

### Genetic and Familial Pancreas Cancer

While there is no population screening for pancreas cancer, surveillance programs are widely used to evaluate individuals with genetic syndromes strongly associated with pancreatic malignancy and those with extensive family history. Patients with Peutz-Jeghers syndrome harbor the *STK11/LKB1* mutation. In addition to developing buccal hyperpigmentation and hamartomatous polyps throughout the gastrointestinal tract, these patients have an increased risk of multiple types of cancer. They have an extraordinarily high risk of pancreas cancer, 132 times that of the general population [22]. The most common germline mutation associated with pancreas cancer is the familial breast and ovarian cancer mutation (*BRCA2*) though the relative risk of disease is only 3.5. Others associated with increased risk of pancreas cancer include the familial atypical multiple mole melanoma (FAMMM)

syndrome associated with the *p16/CDKN2A* mutation and the ataxia telangiectasia syndrome [23, 24].

Individuals with hereditary pancreatitis develop recurrent pancreatitis in childhood often resulting in advanced chronic pancreatitis by adolescence. The best understood mutation is in the cationic trypsinogen gene (*PRSS1*). In these patients trypsin is resistant to autolysis, one of the cardinal mechanisms to down regulate premature pancreatic enzyme activity [22]. Mutations in the serine protease inhibitor, Kazal type 1 (*SPINK1*) gene, an intra-pancreatic inactivator of trypsin is also associated with familial pancreatitis [25]. Finally, mutations in the cystic fibrosis gene (*CFTR*) are correlated with pancreatitis. Patients with genetic pancreatitis are at a 50 fold increased risk of pancreas cancer.

Additionally, many patients present with a very strong family history of pancreas cancer without a known genetic abnormality. This risk increases with the number of family members involved; 4.5 fold for one first degree relative, 8.4 for two, and 32 fold for three [26]. The palladin gene which encodes a cytoskeletal protein has been implicated in at least one pancreatic cancer kindred [17].

Screening for pancreas cancer is now performed for patients with the high risk genetic syndromes described above as well as those with multiple family members who have pancreas cancer. Experts recommend that patients with a greater than ten fold increased risk in pancreas cancer undergo screening which includes those with hereditary pancreatitis, Peutz-Jeghers syndrome, and FAMMM [24]. This also includes those who have two family members with pancreas cancer of the same lineage with one of these being a first degree family member or in those with three family members in the same lineage. Additionally, patients who have the *BRCA1*, *BRCA2*, *p16* mutations and a first or second degree relative with pancreas cancer are recommended to undergo screening. Typically this is performed by performing cross sectional imaging or EUS (often alternating annually) beginning at age 30 for those with Peutz-Jeghers and 40 for those with familial cancer and hereditary pancreatitis [27, 28]. Magnetic resonance imaging is often favored over CT to minimize the risk of long term radiation. The aim of these test are to identify small masses, pancreatic cysts (primarily IPMN have been reported), and PanIN [28]. Patients with abnormalities then undergo EUS-FNA or ERCP and subsequently resection [27, 29]. Canto *et al.* report that amongst a cohort of 76 patients followed for 3 years with annual EUS and CT scan that 8 lesions were detected including 6 IPMN, 1 PanIN3, and 1 cancer associated with a cyst; 2 lesions were missed by CT alone [27].

### Proposed and Confirmed Clinical Risk Factors for Pancreas Cancer

With the exception of those with genetic cancer syndromes or concerning family history there are few known risk factors for pancreas cancers. The best

established risk factors include diabetes mellitus, tobacco use, and chronic pancreatitis. For patients who have these clinical risk factors there are no screening recommendations. However it is important to educate these patients, particularly smokers, regarding the risk of pancreas cancer.

More than 50% of patients with pancreas cancer have diabetes mellitus which is a stronger association than that observed for pregnancy or obesity [30]. Meta-analysis demonstrated that patients who have diabetes for greater than five years have a two fold relative risk of pancreas cancer though other studies indicate that long term diabetes may not be a strong risk factor for pancreas cancer [30, 31]. A growing body of data suggests that new onset diabetes mellitus may be a consequence of the development of pancreas cancer and may resolve following removal of malignant tissue [32]. Chari *et al.* demonstrated that patients who present with new onset diabetes within the past 3 years have a 7.94 (95% CI: 1.61-12.74) observed to expected ratio of pancreas cancer [33]. Furthermore, the pancreas cancer risk associated with onset of diabetes mellitus correlates inversely with the duration of disease; i.e. the more recent the onset the stronger the correlation with pancreas cancer [34, 35]. Additionally, in comparison to controls with new onset diabetes mellitus type II without malignancy, patients with new onset diabetes prior to pancreas cancer have higher fasting glucose levels in the 12 months prior to diagnosis [36].

Fuchs *et al.* have demonstrated in 118,339 health care providers in the Nurses Health Study and 118,339 physicians in the Health Professional Study that the relative risk of pancreas cancer for current smokers was 2.5 fold, this decreased by 50% in two years, and returned to baseline 10 years after quitting smoking [37]. The risk of cancer also increases in parallel with the quantity of tobacco products consumed [38]. Overall in this population 25% of pancreas tumors were a consequence of smoking. Chronic pancreatitis has also been strongly associated with pancreas cancer. In a large retrospective cohort of 2,015 subjects with chronic pancreatitis the incidence ratio for cancer was 14.4 in patients with a five year history of chronic pancreatitis [39]. There was no difference between patients with alcoholic *versus* non alcoholic chronic pancreatitis.

Additionally, it has been proposed that obesity is a risk factor for pancreas cancer. Stolzenberg-Solomon *et al.* report that patients with a body mass index (BMI) of 35 kg/m<sup>2</sup> compared to 18.5-25 kg/m<sup>2</sup> have a hazard ratio of 1.45 (95% CI: 1.04-2.02) for the development of pancreas cancer, though this association weakens to a hazard ratio of 1.33 (95% CI: 0.95-1.86) when diabetes is accounted for [40]. However, other data suggest that obesity may not be an independent risk factor thus this remains a topic of ongoing investigation [41]. Alcohol and coffee have been proposed but do not appear to be risk factors for pancreas cancer [38].

While surveillance is performed in those with genetic syndromes, hereditary pancreatitis, and a strong family history there are no clear guidelines for those with clinical risk factors. Given that new onset diabetes may precede the clinical presentation of pancreas tumors, it has been proposed that CT scans in these patients could detect early lesions [42]. A prospective study employing cross sectional imaging in patients over 50 with recent onset diabetes showed that 6 out of 115 had tumors [43]. Unfortunately, five of the tumors were large and none were resectable, thus the authors did not advocate the widespread application of this strategy. In addition to population wide anti-tobacco campaigns, patients with chronic pancreatitis and those with genetic syndromes that predispose to pancreas cancer should be counseled to avoid smoking to reduce their already considerable risk [23]. Patients with hereditary pancreatitis who smoke are at an additional 3 fold risk of pancreas cancer, thus increasing from 50 fold to 150 fold [25].

### Ongoing Quest for Molecular Markers

The Holy Grail for pancreas cancer investigators is to identify early markers which predict the development of pancreas cancer, uncover early resectable disease, and guide therapy. As previously described CA 19-9 levels are inadequate to identify early pancreas cancer in the population. Universal cross sectional imaging is impractical and would be associated with high cost and potential radiation related morbidity.

There are two major approaches to molecular marker discovery. In the high throughput "shotgun" strategies thousands of contenders are screened simultaneously. In the traditional hypothesis driven approach, interactions between molecules known to be important to pancreas cancer development are studied to identify novel molecules and pathways.

#### High Throughput Approach: Strategies and Yield

Several advanced technologies are being employed to evaluate for markers of pancreas cancer. The underlying principle is to analyze tissue from those with pancreas cancer compared to normal tissue. DNA arrays involve the use of microchips to which are appended the "negative" sequences to thousands of portions of genes [44]. Tissue from those with and without pancreas cancer is processed to yield RNA which is used to generate cDNA sequences which are differentially marked using fluorescence tags. The cDNA is then exposed to the microchips which anneals to the corresponding negative sequences. Differences in the fluorescence pattern between the pancreas cancer and control can be used to rapidly identify differential gene expression.

Another critical high throughput technique is proteonomics. The potential advantage of proteonomics is that proteins levels may be more clinically relevant as gene expression does not necessarily correlate with the quantity and nature of the proteins they encode [45, 46, 47]. As is the case in

gene arrays tissue from those with and without pancreas cancer are compared. While there are several methods, most rely on an initial fractionation step whether by two dimensional electrophoresis or more sophisticated methods such as protein chip technology [48]. In most cases the samples are then analyzed by mass spectroscopy in which the components are separated based on molecular weights and the quantities of different mass size can be compared between malignant and control samples [49, 50, 51]. Subsequently, the proteins are identified by iterative techniques and sequencing [51]. In order to quantitatively compare levels between samples from patients with pancreas cancer and healthy controls, isotope-coded affinity tagging may be used [52]. In this process proteins (cysteine residues) from the cancer and control samples are labeled with heavy and light isotopes of a reagent and the samples are then combined and analyzed simultaneously by mass spectrometry [50]. Peaks corresponding to the protein from the two groups appear immediately adjacent (due to the slight difference in weight between the isotopes) and their ratio allows quantification of the differences in that particular protein in patients with cancer as compared to controls.

High throughput analysis can also be performed to look at RNA. MicroRNA are relatively short stable non-coding RNA sequences which bind to target RNA and prevent translation into protein [53]. Aberrant microRNA expression has been found to be important in the development of leukemia due to its role in gene translation [54]. Arrays containing probes for hundred of known microRNA's are being used to study their role in pancreas cancer with encouraging results [54]. Major challenges include correctly identifying the genes with which specific microRNA interact [53]. Messenger RNA has also been evaluated using microarray technology [55].

Subsequently, expression of potentially important genes and protein levels (as well as epigenetic markers) from patients with pancreas cancer, control, and those with benign pancreatic disease, particularly chronic pancreatitis, must be compared among groups of patients. While high throughput techniques have generated several important candidates much work remains [56]. Problems with high throughput methods include reproducibility and in particular identification of markers which can be measured by readily available clinical laboratory methods [46, 48, 52, 57, 58].

#### Candidate Markers

High throughput methods have identified a number of candidate molecular markers. In subsequent validation studies several have not been found to be more discriminating than CA 19-9 including hepatocarcinoma - intestine - pancreas / pancreatitis - associated protein (HIP-PAP), ostepontin, tissue inhibitor of metalloproteinase 1 (TIMP 1), DUPAN-2, CA 242, CA 72-4, CA 195, MMP-7, cathepsin D, integrin B1, and plasminogen [59, 60, 61, 62, 63, 64].

In a trial comparing 50 pancreas cancer patients to 50 chronic pancreatitis and to 50 healthy controls, macrophage inhibitory cytokine-1 (MIC-1) had a greater accuracy (92%) to distinguish pancreas cancer from normal patients than CA 19-9 (71%) but did not perform better in differentiating patients with pancreas cancer from those with chronic pancreatitis (67%) compared to CA 19-9 (67%) [59]. S100A6 level were measured in the pancreatic juice of 26 patients with pancreas cancer, 37 with IPMN, and 30 with chronic pancreatitis. S100A6 did significantly discriminate between those with chronic pancreatitis and those with cancer and IPMN, but did not discern between cancer and IPMN [64]. CEACAM1 which is part of the CEA family performs better than CA 19-9 in discriminating between those with pancreas cancer and normal controls and its level increases in patients with PanIN-3 versus PanIN-1 or PanIN-2 [65]. Nonetheless, it does not differentiate effectively between those with chronic pancreatitis and cancer.

After initially being identified by microarray of pancreas cancer tissue the presence of microRNA (miR)-155 has been demonstrated in precursor lesions [2, 66]. MiR-155 was present in PanIN-2 and to an even greater extent in PanIN-3 indicating that increased expression of the microRNA correlated with development of pancreas cancer via the PanIN pathway [2]. Its expression was also demonstrated to be 11.5 fold greater in IPMN tissue than control pancreas specimens [66]. Microarray technology has been used to identify messenger RNA transcripts in saliva which may assist in differentiating those with and without pancreas cancer [55].

Other potential candidates identified primarily by high throughput methods which are under investigation include alpha-1,4-N-acetylglucosaminyltransferase, cyclin I, GD12, annexin A2, annexin A8, claudin 18, insulin-like growth factor I (IGF-I), growth factor I binding protein-I (IGFBP-1), insulin-like growth factor-binding protein 2 (IGFBP-2), MBD3L2, DPML, ACRV1, insulin-like apolipoprotein A-I, REG4, and transthyretin [1, 44, 51, 52, 55, 57, 60, 67, 68, 69, 70, 71]. MicroRNA 16, 143, 155, 196a, 217, and 223 also are potential candidates under active investigation [54].

#### Hypothesis Driven Research

Hypothesis driven research has concentrated on several pathways important to the development of pancreas cancer including stimulation of proto-oncogenes, inactivation of tumor suppressor gene, dysregulation mechanisms including aberrant methylation and telomerase activity, and the role of proteins known to be important in gastrointestinal cancers including the mucin family. Proto-oncogenes that promote abnormal proliferation are thought to be important in pancreas cancer. K-ras, which encodes a signal transduction protein, has been found to have a mutation in codon 12 in greater than 90% of pancreas cancer cases [72]. However, it is frequently detected in the serum and

pancreatic juice only in those with advanced disease [73, 74]. A prospective study showed that only 38.1% of those with pancreas cancer were seen to have mutated *K-ras* samples in pancreatic juice and bile [75]. Additionally, *K-ras* mutations are seen in chronic pancreatitis and in smokers; as high as 39% of heavy smokers have *K-ras* mutations [76]. Recently Shi *et al.* employed a quantitative assay to detect single nucleotide *K-ras* mutations which may enable better discrimination between mutations seen in those with malignancy *versus* benign disease based on quantitative levels in pancreatic juice, bile, and serum [73, 77].

Tumor suppressor genes including SMAD4, APC, and p53 have also been of interest. When used alone p53 is not sensitive enough to function as a tumor marker; in one series only 56% of pancreas carcinoma were found to have the mutation [78]. The leading mechanism for inactivation of tumor suppressor gene is chromosomal loss. One method of detection is to measure the presence of microsatellites, which are well described DNA sequence repeats. The loss of one of the pair of microsatellite repeats disappears, termed loss of heterozygosity correlates with the silencing of the nearby tumor suppressor genes. As part of the PANDA study a group of investigators found that a panel of makers including loss of heterozygosity and *K-ras* could be used to differentiate malignant from nonmalignant mucinous cysts [20].

Another mechanism through which tumor suppressor genes may be silenced is by hypermethylation of key gene promoter regions (CpG islands) [79]. It has been demonstrated that more than 1% methylation of 2 of 5 key tumor suppressor genes (Cyclin D2, FOX E1, NPTX2, ppENK, and TFP12) occurred in 82% of patients with pancreas cancer compared to none of the controls [80]. Progressive methylation abnormalities have been correlated with dysplastic progression in PanIN lesions [79].

Telomerase is an enzyme which stabilizes chromosomes by placement of repeat sequences at their ends. It has a particularly important role in proliferating cells including lymphocytes, germ cells, and malignant cells. Ohuchida *et al.* have demonstrated that the relative telomerase activity from the pancreatic juice of those with cancer is elevated relative to those with chronic pancreatitis and other benign diseases [63].

CA 19-9 is an antigen expressed by the MUC1 protein. Like other members of the mucin family it is a glycosylated extracellular protein implicated in a number of malignancies [4]. MUC1 levels are elevated in pancreas cancer. It also expresses an aberrant antigen profile in this setting [62]. Other MUC1 antigens of interest in those with pancreas cancer include CA 15-3 and CA 27.29. The PAM4 antibody against MUC1 is more specific for pancreas cancer than antibodies to other MUC1 antigens which are seen in other tumors [62]. Gold *et al.* demonstrated in a group of 43 healthy individuals, 87 patients with pancreatitis, and 53 patients with pancreas cancer that

PAM4 antibody is more sensitive (71% *versus* 59%) and specific (96% *versus* 63%) than CA 19-9. This improvement was largely a result of less false positive results for patients with chronic pancreatitis when the PAM4 was used, 5%, compared to CA 19-9, 37%.

A combination of hypothesis driven and high throughput methods is resulting in the development of additional potential biomarkers. In a groundbreaking study published in *Science* a combination of approaches including gene sequencing and microarrays were used to delineate twelve core pathways including *K-ras*, hedgehog, and TGF- $\beta$  signaling among others [81]. The associated genes identified to be involved in these processes have potential as markers of pancreas cancer.

### Diabetes and Molecular Markers

The correlation of new onset diabetes and pancreas cancer also represents fertile ground in the search for molecular markers [82]. Surgeons have noted that peripheral insulin sensitivity improves after resection of malignant tissue raising the question of whether there is a substance secreted by the tumor which might cause diabetes. In 1994 it was found in a group of patients with pancreas cancer that those who also had diabetes had significantly elevated levels of the protein amylin relative to those with cancer but no diabetes, those with diabetes but no cancer, as well as those without either disease [83]. Furthermore, those who underwent resection were found to have normalization of amylin levels. Amylin is a protein co-secreted with insulin and which inhibits glucose uptake and glycogen synthesis by skeletal muscles [84]. Thus amylin was proposed as a serum marker of pancreas cancer associated with glucose intolerance.

However, prospective studies by Chari *et al.* demonstrated that amylin had a sensitivity of only 39% and specificity of 93% as a tumor marker, significantly less than CA 19-9 [85]. Amylin is elevated in chronic pancreatitis as well as biliary obstruction and other gastrointestinal cancers [86]. Nonetheless, while enthusiasm for amylin has decreased, *in vitro* data has demonstrated that the media from pancreas cancer cell lines injected into mice can lead to impaired glucose tolerance suggesting the presence of a yet undiscovered humoral factor [87]. Co-culture of the tumor media with hepatocytes and myocytes appears to impact glucose metabolism, implicating that the substance may interact with these tissues [88, 89, 90]. Both proteomic and hypothesis driven approaches are being used in the quest for such a serum factor [91, 92]. Other groups are investigating changes in pancreatic function in the setting of malignancy. Kolb *et al.* demonstrated that in pancreas cancer associated diabetes as opposed to diabetes mellitus type II, islet cells express increased glucagon and decreased insulin [93]. They propose that an insulin to glucagon ratio greater than 7.4 ng/mU can differentiate pancreas cancer associated diabetes mellitus from diabetes

mellitus type II with a sensitivity and specificity of 77% and 69%, respectively.

### Markers of Therapy

Additionally, markers used to predict therapeutic response are being evaluated. Gemcitabine is the mainstay of modern chemotherapy for pancreas cancer. It is transported into cells by the human equilibrative nucleoside transporter 1 (hENT-1) protein. High hENT-1 protein expression has been found to be strongly predictive of treatment response to chemotherapy and survival [94, 95]. Evidence suggests that levels of the most established tumor marker, CA 19-9 also predicts therapeutic response. In a cohort of 424 patients with pancreas cancer who underwent resection, those with a preoperative CA 19-9 less than 1,000 had a median survival of 2.3 years versus 1 year for those with a CA 19-9 greater than 1,000 [96]. Necrotic pancreatic cancer cells undergoing apoptosis express the inactivated complement component iC3b which is important for phagocytosis [97]. Marten *et al.* have demonstrated that soluble levels of iC3b predict recurrence 4 months prior to image confirmed recurrence [98]. Potentially, this marker may also have role in those at high risk for cancer including those with familial cancer syndromes.

### Approach to the Gland

Potential molecular markers are sought in the pancreatic tissue, juice as well as other body fluids including serum and urine. To minimize invasive procedures ideal markers should optimally be detected in the serum and other body fluids. An important consideration is that pancreatic tumor cells and secreted molecules are found in markedly higher concentrations in the pancreas and pancreatic juice compared to the serum [99, 100]. CA 19-9 and CEA levels in the pancreatic juice are 30-1,000 times higher than in serum [101]. It has been reported that one potential marker, the HIP/PAP protein, is 1,000 times more concentrated in the pancreatic juice compared to serum [102]. Additionally, molecules and protein in the serum are overwhelmed by high concentrations of albumin, transferrin, and immunoglobulins. Thus it is logical to first obtain pancreas tissue to identify differential markers of pancreas cancer and then look for their presence further from the gland. EUS-FNA can be used to readily and safely obtain pancreas tissue to enable this process and has a burgeoning role in biomarker discovery [103].

### **Conclusions**

While the landscape of pancreas cancer is currently bleak, several auspicious developments are ongoing. While there is no screening test for pancreas cancer, those with genetic syndrome, hereditary pancreatitis, and those with strong family history may benefit from surveillance by EUS and cross sectional imaging. New onset diabetes, tobacco use, and chronic pancreatitis have been demonstrated to be risk factors. These

findings are important for patient education and represent a fertile territory for biomarker discovery. Both hypothesis driven and high throughput searches for molecular markers to predict disease, early diagnosis, and treatment response are underway. Challenges include differentiation of cancer from chronic inflammatory diseases of the pancreas and achieving reproducible results among diverse patients. Minimally invasive methods including EUS-FNA to acquire tissue may facilitate these important efforts.

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**Conflict of interest** The authors have no potential conflict of interest

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