SCIREA Journal of Clinical Medicine

http://www.scirea.org/journal/CM

August 21, 2019

Volume 4, Issue 5, October 2019

Dilemma and solution in diagnosing pancreatic cancer

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Abstract

Pancreatic cancer is projected to become the second most common cause of cancer-related death in the United States in 2030, overtaking deaths from breast and colon cancers. Although increased screening has led to improvements in the detection of certain cancers such as colorectal cancer, the same is not true for pancreatic cancer. The diagnosis of pancreatic cancer, especially early-stage pancreatic cancer, is still challenging for clinicians. In spite of progresses in our understanding of the carcinogenesis of pancreatic cancer, improved resolution of imaging equipment, and development of screening methods based on molecular biology, the prognosis of patients with pancreatic cancer is still poor. The most promising method of increasing the survival rate of patients with pancreatic cancer is early-stage detection. However, the diagnosis of pancreatic cancer is difficult because of nonspecific symptoms in early cases and the lack of effective diagnostic tools. We look forward to new biomarkers with good sensitivity and specificity or new combinations of existing biomarkers

to improve sensitivity or specificity for pancreatic cancer diagnosis. Advances in the field of imaging technique should make high-resolution equipment available in the future, and we hope that new imaging modalities combined with various contrasts would increase diagnostic effectiveness for pancreatic cancer.

Keywords: pancreatic cancer; biomarker; imaging

Pancreatic cancer is among the five most lethal malignancies in developed countries (1), and survival has not substantially increased in the past 30 years (2). The incidence of pancreatic cancer in China has increased because of the change in diet and lifestyle over the last decades. Pancreatic cancer is primarily a disease of patients older than 60 years of age. Approximately half of patients diagnosed with pancreatic cancer in the United States are older than 75 years of age, and only 13% of patients are diagnosed before the age of 60 (3). Less than 10% of pancreatic cancer cases can be accounted for by hereditary genetic factors. Germline mutations in PRSS1, STK11, CDKN2a, BRCA2, or mismatched repair genes may account for up to 20% of inherited pancreatic cancers (4). Several genetic syndromes have been associated with increased risk of pancreatic cancer, including hereditary pancreatitis, hereditary non-polyposis colorectal cancer, Peutz–Jeghers syndrome, and familial atypical multiple-mole melanoma (5). Pre-existing chronic pancreatitis has been associated with a 10-20-fold increased risk of pancreatic cancer. In general, the cumulative 25-year risk for patients with chronic pancreatitis is approximately 4% (6). Certain studies have linked the development of diabetes to pancreatic cancer. The researchers concluded that diabetes, although not causally related to pancreatic cancer, may be a complication or an early marker of pancreatic cancer (7). Smoking has been consistently and convincingly linked to a marked increased risk of pancreatic cancer (8). With the abovementioned risk factors in mind, a full detailed history should be obtained from each patient. The physician needs to assess presenting signs and symptoms that may suggest an underlying pancreatic neoplasm. Tumors in the head of the pancreas often present symptoms caused by the compression of the common

bile duct. Biliary duct obstruction frequently causes jaundice, and patients may have dark urine. Elevated bilirubin levels may also cause pruritus, which can be quite significant and clinically symptomatic. Patients with tumors in the body and tail of the pancreas are likely to show nonspecific pain and weight loss without jaundice. Patients may also experience anorexia, fatigue, and weight loss. Other symptoms can include new-onset diabetes or malabsorption. On physical exam, weight loss and jaundice are the most common findings. Some jaundiced patients may have a distended, palpable but non-tender gallbladder (Courvoisier's sign). Tumors in the head of the pancreas usually results in elevated total bilirubin, alkaline phosphatase, γ-glutamyl transpeptidase, and hepatic aminotransferases. By contrast, patients with lesions in the tail of the pancreas frequently have a normal biochemical profile.

The anatomic location of the pancreas makes early detection of pancreatic cancer very difficult, and delayed diagnosis means poor prognosis for patients with pancreatic cancer. Thus, overlooking a malignant lesion in the pancreas is a great concern for both the physician and the patient. The lack of specific symptoms at early tumor stages and the high biological aggressiveness of the tumor contribute to the high mortality rate associated with pancreatic cancer. Nearly 45,000 individuals develop pancreatic cancer in the United States annually, with almost equal number of deaths from the disease (2). When diagnosed with pancreatic cancer, only 15%–20% patients have the opportunity to undergo curative Whipple procedure, and most patients who undergo surgery die within the first year (9). In general, the five-year survival is less than 5%. Early diagnosis of pancreatic cancer is the only way to increase the survival rate. A number of precursors to pancreatic cancer have recently been characterized including mucinous cystic neoplasms and intraductal papillary mucinous neoplasms (IPMNs). These lesions have potential malignancy and present distinct morphology. With the understanding and detection of theses precursor lesions, it is possible that more patients could be diagnosed with pancreatic cancer in early stage. An ideal diagnostic tool should be able to identify every malignant lesion but keep the number of false positive results to a minimum. However, very few satisfactory diagnostic tools are available for the early detection of pancreatic cancer. Two main approaches are usually used to diagnose pancreatic cancer:

based on molecule and based on imaging.

Based on molecule

Biomarkers in body fluids

Improved screening for early diagnosis through the detection of diagnostic serum markers provides the best hope of increasing the rate of curatively resectable pancreatic cancer. Serum tumor markers have been widely adopted in clinical practice to diagnose carcinomas because of convenience and simplicity. Although many serum markers have been reported to be elevated in patients with pancreatic cancer, most of these markers have not been implemented into clinical routine because of low sensitivity or specificity. Considerable effort has been devoted to the discovery and development of blood-based biomarkers capable of detecting pancreatic cancer at early, preclinical stages. Carbohydrate antigen 19-9 (CA 19-9), carcino-embryonic antigen (CEA), and carbohydrate antigen 125 (CA125) are the most promising serum markers, but none of them is sufficiently sensitive or specific in the early detection of pancreatic cancer individually.

As the only Food and Drug Administration-approved biomarker for pancreatic cancer, CA 19-9 is increased in the sera of patients with pancreatic cancer (10). However, in certain cases that do not show antibody response against CA19-9, the tested results may be negative even when the tumor burden is high (11). CA 19-9 has demonstrated modest effectiveness when applied as a diagnostic tool in symptomatic individuals on an outpatient basis with a median sensitivity of 79% (range 70–90%) and median specificity of 82% (range 68%–91%); however, it is not useful in the mass screening of asymptomatic subjects (12). The elevation of CA19-9 could also be associated with pancreatitis and obstructive jaundice derived from benign conditions.

Since the 1970s, CEA has been used to diagnose pancreatic cancer. In the past years, CEA has been replaced by certain serum markers with higher sensitivity and specificity, such as CA19-9. However, a recent meta-analysis indicated that elevated serum CEA level, as a vital supplementary to CA19-9, can play an important role in the clinical diagnosis of pancreatic

cancer patients (13).

CA125 is a cell-surface glycoprotein that functions in promoting cancer cell growth in ovarian cancer initially (14). Certain studies have indicated that CA125 has an important function in the diagnosis of pancreatic cancer and shows superiority in predicting the resectability of pancreatic cancer (15). CA125 is independently associated with a poor outcome in pancreatic cancer (16).

In the last decades, many serum biomarkers have been tested for pancreatic cancer detection, some of which show higher specificity and sensitivity than CA19-9. These biomarkers include growth differentiation factor (GDF-15) (17), tumor-derived exosomes (18), REG4 (19), serum phosphoproteins extracellular signal-regulated kinase (p-ERK1/2) (20), CEACAM1, a proliferation-inducing ligand (21), and laminin, gamma 2 (22). Further validation studies including a large number of cases are required for the clinical utility of these biomarkers.

Combined measurement of multiple serum tumor markers may improve the diagnostic accuracy of pancreatic cancer. A novel system for multiplex detection of pancreatic biomarkers CA19-9, MMP7 and MUC4 in sera samples was reported with high sensitivity using surface enhanced Raman spectroscopy (23). Osteopontin and TIMP-1 were found to be effective in the early detection of pancreatic cancer (24). A recent systematic review and meta-analysis showed that a CA125-based diagnostic panel was better at diagnosing pancreatic cancer than a test using CA125 or CA 19-9 alone (25). A three-biomarker panel including CA19-9, CEA, and TIMP-1 provided a sensitivity and specificity of 76% and 90%, respectively, for the classification of pancreatic cancer from benign pancreatic disease (26). In the same study, a panel that comprised CA 19-9, ICAM-1, and osteoprotegerin provided a sensitivity of 88% and specificity of 90% for the discrimination of pancreatic cancer from healthy controls. Another study discovered a new panel of pancreatic cancer biomarkers using a mass spectrometry-based pipeline. The novel biomarker panel combining with CA19-9 significantly improved the sensitivity (95%) and specificity (94.1%), outperforming CA19-9 alone, for the diagnosis of pancreatic cancer (27).

Some researchers have found that elevated plasma levels of branched-chain amino acids (BCAAs) are associated with a greater than twofold increased risk of future pancreatic cancer

diagnosis. This elevated risk was independent of known predisposing factors, with the strongest association observed among subjects with samples collected 2 to 5 years before diagnosis, when occult disease is probably present. The study also showed that plasma BCAAs are elevated in mice with early-stage pancreatic cancer driven by mutant K-ras expression but not in mice with K-ras-driven tumors in other tissues, and that breakdown of tissue protein accounts for the increase in plasma BCAAs that accompanies early-stage disease. These findings suggested that increased whole-body protein breakdown is an early event in development of pancreatic cancer (28).

Several reports have shown that aberrant miRNA production is an early event in the development of pancreatic cancer. MiRNAs 21, 155, 16, 196a, 1290, 221, 375 (low in pancreatic cancer), and 18a were identified by miRNA expression profiling or other methods as potential biomarkers of pancreatic cancer alone or in combination with CA19-9. A meta-analysis of three blood-based miRNA studies reported that the median specificity and sensitivity were 91% and 96%, respectively (29). Abnormal long non-coding RNA expression profiles could be promising biomarkers for diagnosing pancreatic cancer (30). A recent study suggested that circular RNA may be a biomarker in the diagnosis of pancreatic cancer (31). Although RNA detection is not used as a routine test for pancreatic cancer diagnosis, investigations have indicated that they may be promising biomarkers in the near future.

A meta-analysis of a large number of pancreatic microarray datasets has identified more than 800 genes that are significantly up-regulated in pancreatic cancer. This study has yielded new targets for cancer genes and potential biomarkers for pancreatic cancer (32). However, the list of identified genes is so long that the next screening and confirming works are complicated and time consuming.

Novel mathematical analyses that use multiple markers can increase sensitivity without sacrificing specificity (33). Therefore, a computer-based diagnostic and referral system may potentially increase the quality of care by minimizing human error. The search for new models to evaluate the diagnostic potential of combined measurement of multiple serum markers in pancreatic cancer has attracted much attention in the previous years. Artificial neural network (ANN) is a computational model that simulates the process of learning in

response to external brain stimulation. The principle involves adaptation to changing environment through continuous learning via trial and error (34). Yang et al. evaluated an ANN model in the diagnosis of pancreatic cancer using multiple serum markers. In their retrospective analysis, 913 serum specimens were analyzed for CA19-9, CA125, and CEA. The three tumor marker values were used as inputs into an ANN and randomized into a training set of 658 (70.31% were malignant) and a test set of the remaining 255 samples (70.69% were malignant). The samples were also evaluated using a logistic regression (LR) model. The ANN-derived composite index was superior to each of the serum tumor markers alone and the LR model. The areas under receiver-operating characteristic curves was 0.905 (95% confidence interval (CI) 0.868–0.942) for ANN, 0.812 (95% CI 0.762–0.863) for the LR model, 0.845 (95% CI 0.798–0.893) for CA19-9, 0.795 (95% CI 0.738–0.851) for CA125, and 0.800 (95% CI 0.746–0.854) for CEA. The ANN analysis of multiple markers yielded a high level of diagnostic accuracy (83.53%) compared with LR (74.90%). This study showed that the performance of the ANN model in the diagnosis of pancreatic cancer is better than those of the single tumor marker and the LR model (35).

Pancreatic secretion (juice) is a natural place to look for mutant genes shed from precursor lesions in the pancreas and can easily be obtained by stimulating pancreatic juice secretion with secretin and then collecting the juice with an endoscope. Certain researchers have reported that pancreatic juice could be obtained repeatedly via an endoscopic nasopancreatic drainage (ENPD) tube and that this method was useful for making a definitive diagnosis of pancreatic cancer (36). Diagnosis by cytology and brush cytology using an ENPD tube guided by endoscopic retrograde cholangiopancreatography (ERCP) has also been reported but with variable rates of detection (37). Molecular analysis of cells in the pancreatic juice includes an examination of the K-ras codon 12 point mutation, the p53 mutation, CD44 expression, and telomerase activity. Proteomics can also be used to differentiate pancreatic cancer from pancreatitis (38). However, the diagnostic potential of most of these methods is yet to be established. Precancerous lesions such as IPMNs involve the pancreatic duct system, and mutant DNA from these lesions is therefore likely to be shed into the pancreatic juice. GNAS mutations were detected in pancreatic juice samples from patients with a clinically normal

pancreas who only later developed IPMN, indicating the power of molecular-based tests (39). Studies have shown the importance of DNA methylation of cyclin D2, ppENK, and NPTX2 in the pancreatic juice for the diagnosis of pancreatic cancer (40, 41). Mucins (MUC) have a crucial function in carcinogenesis and tumor invasion in pancreatic cancer. MUC1 (pan-epithelial membrane mucin), the first cloned mucin, is an important human tumor antigen, second only to WT1 in cancer antigen pilot prioritization using a ranking based on predefined and pre-weighted criteria (42). High MUC1 expression is observed in pancreatic cancer and is related to a poor outcome (43). Yokoyama et al. reported that evaluating the DNA methylation status of MUC1 in the pancreatic juice can provide important information for the diagnosis of human pancreatic cancer (44).

Kisiel tried to select discriminant methylated genes and to assess accuracy of these genes and mutant K-ras in stool to detect pancreatic cancer. The study demonstrated that stool assay of a methylated gene marker can detect pancreatic cancer. Among candidate methylated markers discriminant in tissue, BMP3 alone performed well in stool. Combining methylated BMP3 and mutant K-ras increased stool detection over either marker alone (45). One study detected urinary matrix metalloproteases (uMMPs) and urinary tissue inhibitor of metalloproteases (uTIMPs) in the urine of patients with pancreatic cancer and assessed the usefulness of the molecules in diagnosis of pancreatic cancer. The results indicated that the detection of uMMP-2 and uTIMP-1 may have diagnostic value in the detection of pancreatic cancer and that uTIMP-1 may be useful in distinguishing between pancreatic cancer and neuroendocrine tumors (46). A recent study showed that detection of volatile organic compounds in urine could discriminate healthy individuals from pancreatic cancer patients (47). These results demonstrated that urine analysis shows promise as a non-invasive approach for identification of patients with pancreatic cancer. Zhang conducted a prospective sample collection and retrospective blinded validation to evaluate the performance and translational utilities of salivary transcriptomic biomarkers for the noninvasive detection of pancreatic cancer. The results of the study showed that the salivary biomarkers possess discriminatory power for the detection of resectable pancreatic cancer with high specificity and sensitivity (48).

Based on imaging

Imaging techniques

Several imaging modalities are used in the diagnosis of pancreatic cancer: trans-abdominal ultrasound, computed tomography (CT), magnetic resonance imaging (MRI), ERCP, endoscopic ultrasound (EUS), and positron emission tomography (PET)–CT. Dynamic contrast-enhanced CT is the most widely used imaging technique to diagnose pancreatic cancer and has been considered the most comprehensive tool for the diagnosis and surgical staging of pancreatic cancer (49). Despite all the advances with multi-detector helical CT scan, differential diagnosis of mass-forming chronic pancreatitis, pancreatic cancer, and autoimmune pancreatitis based on CT image only is still difficult (50,51). For pancreatic cancer, CT and MRI are still the primary image diagnostic tools, with 91% and 84% sensitivity and 85% and 82% specificity, respectively (52), which means that with the use of these imaging techniques, 10%–20% of all malignant tumors may be missed (false negatives). CT, MRI, and EUS have all been used to detect curable lesions in the pancreas. All three imaging modalities have been compared in a prospective study of 225 asymptomatic high-risk individuals by Canto and colleagues (53). EUS detected a pancreatic abnormality in 43% of patients, in contrast with MRI and CT, which identified lesions in 33% and 11%, respectively. Five of the lesions identified on EUS were resected, of which three were IPMNs with high-grade dysplasia. Cystic precursor lesions can clearly be detected by the existing imaging technique, and some of these lesions are curable, high-grade precursor lesions. EUS seems to be the most sensitive modality. A study indicated that MRI and EUS have been shown to have the highest accuracy in detecting pancreatic cancer (54). ERCP has no clinical role in the diagnosis and staging of pancreatic cancer. Indirect findings such as combined dilation of the bile and the pancreatic duct, abrupt cutoff in the main pancreatic duct, or a solitary long stricture of the pancreatic duct could raise suspicion of malignant disease but may also be observed in chronic pancreatitis.

EUS has become the standard technique for diagnosing pancreatic cancer. EUS can provide improved information on the pancreas because of overcoming the limitations associated with the use of trans-abdominal ultrasound. EUS with or without fine needle aspiration (FNA) has a higher diagnostic yield than PET, CT, and trans-abdominal ultrasound for detecting early

pancreatic cancer. Given that EUS has the inherent advantage of a high-frequency transducer placed in close proximity to the cancer, EUS may diagnose pancreatic cancer with more accurate sensitivity and specificity (55). For pancreatic cancer less than 2–3 cm, the sensitivity rate of EUS was 99% compared with 55% of CT (56). The incorporation of contrast-enhanced images of EUS makes the differentiation of pancreatic cancer from chronic pancreatitis and neuroendocrine tumors possible (57). EUS is a diagnostic modality with high negative predictive value and allows clinicians to reliably exclude pancreatic cancer. EUS is considered a safe procedure with complication rates as low as 1.1%–3% (58). EUS has the ability to provide FNA, which is essential in the evaluation of patients with solid pancreatic lesions. FNA has been widely used to establish the diagnosis of pancreatic cancer (59). However, diagnosing pancreatic cancer on cytology specimen is not always easy. The sensitivity of diagnosing pancreatic cancer with FNA on morphology can be as low as 50% (60). Certain researchers recommended on-site cytopathology to provide opportunity for real-time interpretation of samples to increase the diagnostic accuracy of FNA (61). Alsohaibani reported that on-site cytopathology improves the diagnostic yield of EUS–FNA independent of the number of needle passes undertaken for tissue sampling (62). Some molecular markers have been reported to increase the detection sensitivity on FNA specimens such as MUC4, S100p, and XIAP (63). A study suggested that CRABP-II is highly and specifically expressed in pancreatic cancer, and it could be used as a diagnostic marker to distinguish pancreatic cancer from other benign pancreatic conditions in cytology specimens (64).

MRI with MRCP is an option for diagnosis of pancreatic cancer and is the preferred method for young patients without radiation. To improve the sensitivity of diagnosing pancreatic cancer with MRI, several modified scanning methods have been suggested. With pre-gadolinium and early gadolinium-enhanced fat-suppressed T1-weighted images, pancreatic cancer is detected more easily (65, 66). Pancreatic cancer is generally seen as a focal mass of low T1 signal intensity and shows progressive delayed enhancement (67). When the cancer is located within the pancreatic head, the pancreatic body and tail sometimes lose the normal high T1 signal. This phenomenon is produced by the obstruction of the main

pancreatic duct, leading to inflammation, fibrosis, and atrophy. In this situation, the early contrast-enhanced images may show a low signal intensity mass with rim enhancement superimposed on a background of slightly enhanced, chronically inflamed, pancreatic parenchyma. If the mass is located within the pancreatic tail, it is usually well shown on the unenhanced fat suppressed T1-weighted images. Diffusion-weighted imaging has been shown to have a high sensitivity (96.2%) and specificity (98.6%) for detecting pancreatic cancers and may allow early detection of pancreatic cancer (68, 69). Diffusion-weighted imaging may also help in detecting liver and lymph node metastases, which may be missed on other sequences. Not every lymph node seen on diffusion-weighted imaging is malignant because both benign and malignant lymph nodes can show restricted diffusion. Liu et al. investigated the diagnostic value of quantitative dynamic contrast-enhanced MRI (QDCE–MRI) of pancreatic cancer on a 3.0-T magnetic resonance and found that the parameters of QDCE–MRI are useful for the diagnosis of pancreatic cancer (70).

PET with F-18-fluorodeoxyglucose (^{18}FDG) has been proven to be of clinical value in several gastrointestinal cancer (71). It is based on the principle that the photons that result from the annihilation of the positrons emitted by specific radioisotopes (e.g., ^{18}fluorine) are detected by the PET scanner and subsequently computed to a tomogram. Another tool, the ^{18}FDG–PET–CT, combines in a single gantry both ^{18}FDG–PET and CT and produces one combined image (72). Several studies have assessed the efficacy of ^{18}FDG–PET in diagnosing pancreatic cancer. According to these reports, the sensitivity and specificity of ^{18}FDG–PET is up to 95% and 100%, respectively (73). Certain reports have shown that ^{18}FDG–PET is superior in sensitivity and specificity compared with CT alone (74, 75). The ^{18}FDG–PET–CT is considered to be more sensitive than conventional imaging modalities with regard to the detection of pancreatic cancer (76). However, a meta-analysis found that both ^{18}FDG–PET and ^{18}FDG–PET/CT offer no benefit over the current primary diagnostic tools in diagnosing pancreatic cancer. Nevertheless, the ^{18}FDG–PET–CT systems are still improving, and ^{18}FDG–PET–CT may have a role in the staging of pancreatic cancer and survival prediction and may add to other diagnostic information, like histology (77). The advent of simultaneous PET/MRI represents an exciting advancement in hybrid functional imaging with potential

applications in the imaging of pancreatic cancer. The advantages of PET/MRI include simultaneous acquisition to improve registration of fusion images, lower radiation dose, superior soft tissue contrast, and availability of multiparametric imaging. Studies are underway to evaluate the utility of PET/MRI in pancreatic cancer and further studies are warranted to better understand the role of this modality in pancreatic cancer (78).

Confocal laser endomicroscopy (CLE) is a technique used for the imaging of pancreatic cancer where a confocal probe is passed into the duct through the working channel of a duodenoscope. And another approach is needle-based CLE which can be specifically used in the pancreas to image cysts and solid masses providing a potential imaging technique for pancreatic cancer (79).

Summary

After clinical diagnosis, most cases with pancreatic cancer progress rapidly, and pancreatic cancer is usually resistant to conventional chemotherapy and radiotherapy. Therefore, early detection of pancreatic cancer is crucial for a possible curative resection and a good prognosis. A diagnostic tool, which has high sensitivity and specificity, would be expected to identify a high percentage of pancreatic cancer in early stage.

In modern clinical practice, all curable pancreatic cancers are detectable with existing imaging technologies, including EUS, MRI, and CT. The contribution of imaging modalities to the detection of precursor lesions of pancreatic cancer increases only as the resolution of imaging improves in the coming years. Diagnostic tools based on molecule have enormous potential in the future. The advantages of technology based on molecule being applied to biosamples, such as blood or stool, are its simplicity and non-invasiveness. Two advances highlight the enormous potential of approaches based on molecule as tools for the detection precursor lesions of pancreatic cancer. First, the entire exome sequencing of well-characterized cyst-forming precursor lesions has defined the genes targeted (mutated) in each of the different precursor lesions in the pancreas. Second, new technologies have been developed that can detect rare mutant alleles, even when these mutant alleles are admixed with a

1000-fold more wild-type alleles (80-82). The resolution of imaging and the sensitivity and specificity of screening tools based on molecule are certain to improve in the coming years, and the two technologies may even be combined as imaging based on molecule to resolve the dilemma in the detection of early pancreatic cancer.

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