Clinical Chemistry 59:1
41–46 (2013)

Pancreatic Cancer

Moderators: Caitlin C. Chrystoja[1] and Eleftherios P. Diamandis[1,2,3*]
Experts: Randall Brand,[4] Felix Rückert,[5] Randy Haun,[6] and Rafael Molina[7]

Pancreatic cancer (PC)[8] is the 10th most common cancer type and the fourth leading cause of cancer-related deaths. The vast majority of PCs are pancreatic adenocarcinomas. Diagnosis of small tumors at an early stage or dysplastic premalignant lesions that can be surgically resected offers patients the best chances for survival and can increase 5-year survival rates from approximately 5% to 20%–30%, or even higher, at specialized treatment centers. The early stages of PC are usually asymptomatic, and the aggressive nature of this disease, in combination with our limited capability for early detection, contribute to the very low percentage of patients (approximately 20%) diagnosed with resectable disease. Large numbers of early diagnoses are due to incidental findings during abdominal imaging procedures.

The most common diagnostic procedures for PC are based on imaging technologies, including computed tomography (CT), endoscopic ultrasound, and magnetic resonance imaging (MRI), among others. Other, more invasive procedures include endoscopic retrograde cholangiopancreatography (ERCP), which also enables tissue biopsy. Unfortunately, the indication for the use of these methods is after the patient has symptoms, when the disease is likely at a late stage. Given that this cancer is relatively rare (the incidence is approximately 12 cases per 100 000/year in the US), screening the general population for the disease is not recommended, because it is not cost-effective. Screening high-risk populations, such as those with a lifetime risk >10%–15%, may be feasible. A number of genetic syndromes are associated with a higher incidence of pancreatic carcinoma, but most cases are sporadic.

The only marker used clinically for PC at present is carbohydrate antigen 19.9 (CA19.9), a biomarker that was discovered approximately 30 years ago. CA19.9 is a sialylated Lewis A–active pentasaccharide detected primarily on the surface of mucins in the serum of PC patients. Although increased CA19.9 concentrations have been associated with advanced stages of the disease, they have also been associated with benign and inflammatory diseases, such as obstructive jaundice and pancreatitis, as well as other malignancies of the gastrointestinal system. The low diagnostic specificity and sensitivity of CA19.9 for early-stage disease (approximately 50%) and the absence of this antigen in the approximately 10% of the population who are Lewis genotype negative, underlines the necessity for the discovery of new cancer biomarkers for this disease.

In this Q&A, we discuss various aspects of PC with 4 experts in the field. These aspects include risk factors, diagnostic procedures, the need for new biomarkers, current therapies, and future prospects.

Are there any known risk factors, genetic or environmental, for developing pancreatic adenocarcinoma?

Randall Brand: Yes. Most risk factors, including obesity, diabetes, *Helicobacter pylori* infection, and male gender, confer a modest risk (<2-fold). The greatest environmental risk factor is smoking (approximately 3-fold risk). The greatest risk factors for PC development are genetic factors, including mutation carriers of known genetic syndromes such as Peutz–Jeghers or familial

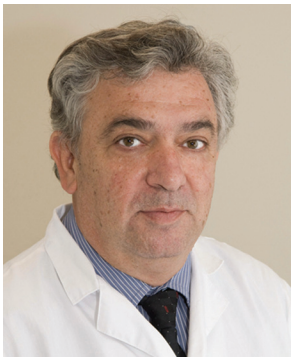
[1] Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Ontario, Canada; [2] Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada; [3] Department of Clinical Biochemistry, University Health Network, Toronto, Ontario, Canada; [4] Professor of Medicine, Division of Gastroenterology, Hepatology and Nutrition, University of Pittsburgh Medical Center, Pittsburgh, PA; [5] Surgical Department, University Hospital Mannheim, Mannheim, Germany; [6] Associate Professor, Department of Pharmaceutical Sciences, Winthrop P. Rockefeller Cancer Institute, University of Arkansas for Medical Sciences, Little Rock, AR; [7] Laboratory of Clinical Biochemistry, Unit of Cancer Research, Barcelona, Spain.
* Address correspondence to this author at: Mount Sinai Hospital, Joseph and Wolf Lebovic Centre, 60 Murray St., Box 32, Floor 6, Rm. L6–201, Toronto, Ontario, M5T 3L9 Canada. Fax 416-619-5521; e-mail ediamandis@mtsinai.on.ca.
Received September 20, 2012; accepted October 11, 2012.
[8] Nonstandard abbreviations: PC, pancreatic cancer; CT, computed tomography; MRI, magnetic resonance imaging; ERCP, endoscopic retrograde cholangiopancreatography; CA19.9, carbohydrate antigen 19.9; FAMMM, familial atypical multiple mole melanoma; HNPCC, hereditary nonpolyposis colorectal cancer; MRCP, magnetic resonance cholangiopancreatography; FDA, US Food and Drug Administration; EGTM, European Group on Tumor Markers.

atypical multiple mole melanoma (FAMMM), or individuals with 2 or more cases of PC in their family (with at least a pair of first-degree relatives) without a known mutation, also known as "familial PC."

Felix Rückert: Chronic inflammation seems to be a risk factor for PC. Patients with hereditary pancreatitis, as well as patients with chronic pancreatitis, have an increased risk of PC. Other risk factors include smoking, male gender, and old age.

Randy Haun: PC develops more frequently in older individuals, and there is a slightly higher incidence of PC in men than women. The risk in African Americans is higher than whites, though the underlying reason for these gender and racial disparities are not clear. Tobacco use, both smoking and smokeless tobacco, has been clearly associated with an increased relative risk of developing PC and represents one risk factor that can be reduced directly through behavior modification. Similarly, obese individuals have an increased risk compared to normal-weight individuals, as well as those with long-standing diabetes and chronic pancreatitis. Thus, avoiding tobacco use and maintaining a healthy weight can reduce one's risk of developing this particularly deadly form of cancer. Other factors, including coffee consumption, heavy alcohol use, and diets high in fat or processed meats have been linked to PC in some studies, but these associations have not been supported in all studies.

A family history of PC as well as some genetic syndromes may increase the risk of PC. These inherited gene mutations include *p16/CDKN2A*[9] (cyclin-dependent kinase inhibitor 2A) (familial melanoma), *PRSS1* [protease, serine 1 (trypsin 1)] (familial pancreatitis), *BRCA2* (breast cancer 2, early onset) (hereditary breast and ovarian cancer), *STK11* (serine/threonine kinase 11) (Peutz–Jeghers syndrome), and several genes involved in DNA mismatch repair that are defective in hereditary nonpolyposis colorectal cancer (HNPCC), also known as Lynch syndrome.



Rafael Molina: PC is an established hereditary tumor entity that is responsible for approximately 3%–10% of PC patients. Other factors may be patients with hereditary pancreatitis or patients with certain germline mutations in *BRCA2*, *p16*, or Peutz–Jeghers syndrome. Smoking is another important risk factor related to PC (relative risk, 3.7).

Why is PC so lethal?

Randall Brand: PC spreads early, even when the primary tumor is small (<2 cm in size). In the majority of PC cases, it is not possible to identify an advanced precursor lesion. The one exception is for the subset of PC cases that arise from mucinous cystic lesions.

Felix Rückert: In my opinion, 2 facts are crucial. Firstly, PC shows an aggressive, infiltrative tumor growth with frequent residual disease after resection. Because of that, some even refer to PC as "systemic disease." Secondly, PC shows a high resistance to chemotherapy. The reason for this is the strong desmoplastic reaction and the rare vascularization of the tumor tissue. This leads to an insufficient concentration of chemotherapeutics within the tumor tissue. Additionally, PC cells are resistant to apoptosis.

Randy Haun: The poor survival of patients diagnosed with PC is largely attributable to the detection of the disease at a locally advanced or metastatic stage, when treatment options are limited and essentially palliative. Symptoms of PC can be vague, such as back pain, or perceived as other minor ailments, such as indigestion, and may delay the patient from seeking medical attention in a timely manner. Once the disease is diagnosed, surgery is the best treatment option, but this is limited to the <20% of patients with localized disease. For the majority of PC patients, therefore, chemotherapy (with or without radiation therapy) is

[9] Human genes: *CDKN2A* (also known as *p16*), cyclin-dependent kinase inhibitor 2A; *PRSS1*, protease, serine 1 (trypsin 1); *BRCA2*, breast cancer 2, early onset; *STK11*, serine/threonine kinase 11; *KRAS*, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; *TP53*, tumor protein p53; *SMAD4*, SMAD family member 4.eb

the only treatment option, but such therapy elicits only a limited therapeutic response, which may reflect the chemoresistance of the tumors to the cytotoxic agents, as well as poor delivery of the drugs to the tumor due to the dense fibrotic stroma (desmoplasia) that develops around the tumor.

Rafael Molina: Patients with PC often present with nonspecific symptoms, experience delays in referral to specialized diagnostic services, or undergo imaging tests with suboptimal sensitivity for identifying small masses. The prognosis of PC is poor because most patients already have advanced disease at diagnosis and curative treatment (surgery) is possible only in 20% of patients.

How is PC diagnosed today?

Randall Brand: In most instances, PC is diagnosed by abdominal imaging. Most of the time, this consists of a CT scan of the abdomen. Endoscopic ultrasound is useful for equivocal cases and has the advantage of being able to obtain a tissue diagnosis by fine-needle aspiration.

Felix Rückert: The first diagnostic step should always be an ultrasound of the abdomen. Ultrasound is a fast and effective imaging modality to confirm the suspicion of PC and diagnose liver metastasis. However, the gold standard for diagnosis in Germany is the abdominal CT and/or MRI with magnetic resonance cholangiopancreatography (MRCP). Although endoscopic ultrasound is also effective, it is mainly performed in specialized centers and is therefore not available for many of the patients. As described later, tumor markers are also helpful when there is suspicion of cancer.

Randy Haun: In addition to obtaining a patient's history, a physical exam of the abdomen may reveal an enlarged gallbladder resulting from a blocked bile duct or enlarged liver if the cancer has spread to these organs. The largely retroperitoneal position of the pancreas prevents its direct palpation for masses. In individuals with suspected pancreatic malignancy, imaging studies using abdominal ultrasonography, endosonography, ERCP, MRI, and/or CT scan can be used to identify pancreatic lesions and dilated ducts. A definitive diagnosis often relies on the pathologic examination of a tissue biopsy (e.g., endoscopic ultrasound–guided biopsy or fine-needle aspiration, ERCP aspiration, or brushing).

Rafael Molina: In patients with suspicious signs, imaging techniques are used for diagnosis, including: transcutaneous ultrasound, CT scan, MRI, and endoscopic ultrasound.

Is screening the general population an effective way of detecting early and potentially curable disease? If not, why? Who should be screened?

Randall Brand: Due to the low incidence of PC, it is impossible to screen the general population for this cancer. Surveillance (screening in a high-risk population) should be reserved for those patients with an increased lifetime risk of developing PC of at least 10%. Presently, this just consists of those patients with a hereditary predisposition for PC development.

Felix Rückert: PC has a relatively low incidence of about 6 per 100 000 in Germany. Even if a tumor marker had a sensitivity of 100% and a specificity of 99% in a population of 100 000, there would be 1006 positive tests, of which only 6 would be true positives, and the remaining 1000 would be false positives (positive predictive value = 0.6%). This problem can be avoided by selecting subgroups where the incidence of PC is higher, e.g., patients with risk factors or with pancreatic lesions.

Randy Haun: Due to the lack of a sensitive and specific biomarker for detecting PC, there is no cost-effective way to screen the general (asymptomatic) population. Patients at increased risk for developing PC—those with hereditary factors (e.g., 2 or more first-degree relatives with PC) or genetic syndromes (e.g., hereditary pancreatitis, HNPCC syndrome)—may be candidates for PC screening and surveillance. This may include genetic testing for mutations associated with increased risk for PC and/or imaging studies (e.g., endoscopic ultrasound, CT scans, ERCP, and MRI). Recent evidence also suggests that older individuals (>50 years) with new-onset diabetes have an increased risk of PC and should also be considered for further screening.

Rafael Molina: PC screening programs are available, but because of the relatively low incidence, current efforts are focused on early detection only in patients at high risk for the development of the disease.

How are current biomarkers of this disease clinically used? What do guidelines recommend? Do they make any difference in disease outcomes? Do you use them routinely?

Randall Brand: Excluding cystic neoplasms of the pancreas, the only commercially available biomarker used for PC is CA19.9. To the best of my knowledge, CA19.9 is not in any formal guidelines, but many experts suggest that monitoring CA19.9 concentrations may be

useful as a marker of response to therapy or that extremely increased concentrations may reflect that the patient's tumor is unresectable (used in our center for this purpose). There is no role for CA19.9 concentrations in screening for PC.

Felix Rückert: In the last 20 years, more than 18 tumor markers were clinically tested. However, only CA19.9 has sufficient sensitivity and specificity. CA19.9 is recommended by the German S3 guideline "Exocrine Pancreatic Cancer" for differential diagnosis of pancreatic lesions and for follow-up after resection or during chemotherapy. CA19.9 seems to correlate with tumor load; a laparoscopy is recommended in patients with high CA19.9 to exclude peritoneal metastases before resection of the tumor. In our department, CA19.9 is routinely used. It is especially helpful in patients with known chronic pancreatitis when there is suspicion of cancer.

Randy Haun: Currently, serum CA19.9 is the only US Food and Drug Administration (FDA)-approved biomarker in clinical use for the management of PC. Serum CA19.9 concentrations are routinely used to monitor tumor recurrence or progression during PC treatment. CA19.9 is also used as a preliminary diagnostic tool in symptomatic patients suspected of having PC, but only as a precursor to more informative imaging studies. Other serum antigens (e.g., carcinoembryonic antigen and CA125) are also used to monitor response to therapy or disease burden but are not FDA approved for PC. With their inability to detect PC at an early, treatable stage and the overall poor efficacy of current treatment regimens for PC, these biomarkers have not appreciably affected the overall outcome of this disease.

Rafael Molina: CA19.9 is the tumor marker of choice today in clinical practice. The main problem with the use of CA19.9 is its specificity, ranging from 60% to 90%. Additionally, abnormal levels may be observed in several benign diseases, especially in jaundice, where CA19.9 may reach 1000 U/mL.

The European Group on Tumor Markers (EGTM) considers CA19.9 to have little diagnostic value, especially in the early stages of the disease, but this biomarker may be of interest as an adjunct to radiological methods, mainly in cases without jaundice. The National Comprehensive Cancer Network suggests that CA19.9 results can be used as an aid to differentiate patients with inflammatory pancreatic diseases from those with pancreatic adenoma, although negativity does not exclude malignancy (mainly Lewis A genotype patients), and false positives in patients with jaundice need to be taken into account.

The National Academy of Clinical Biochemistry and the EGTM recommend that serial determinations of CA19.9 can be used with imaging techniques to assess therapeutic response, especially in the case of palliative treatment. The American Society of Clinical Oncology suggests that there are insufficient data to recommend the routine use of tumor markers to evaluate the response to treatment but that levels of tumor markers can be determined at the start of treatment in advanced or metastatic cases and every 1 to 3 months during treatment. The detection of increases in CA19.9 indicates progression, which should then be confirmed with other techniques.

We are using CA19.9 as aid in the diagnosis in patients with suspicious signs of PC. We are using different cutoffs as suspicion criteria: >100 U/mL in patients without liver diseases, >300 U/mL in patients with liver diseases, and >1000 U/mL in patients with jaundice. CA19.9 may also be useful in the differential diagnosis of PC and neuroendocrine tumors, benign tumors, or intraductal papillary mucinous tumors of the pancreas (levels lower than the cutoff previously indicated).

Are new and improved biomarkers in the pipeline?

Randall Brand: Another set of biomarkers is available to identify patients who would benefit from aggressive evaluation of PC with more invasive or costly studies. The initial commercial applications of biomarkers include a diagnostic biomarker for symptomatic patients and biomarkers that can be used to better target therapy (personalized medicine). There is a lot of active research in regards to the latter 2 applications.

Felix Rückert: It would be convenient to have access to a cheap and reliable tumor marker. With a "perfect" tumor marker, screening of the normal population would be possible, and more patients could be diagnosed at a resectable stage, thereby prolonging survival of many patients. Hopefully, such a candidate might be found soon.

Randy Haun: Numerous technologies have been employed to identify markers that can distinguish malignant disease from benign pancreatic disease and/or normal tissue, including but not limited to the profiling of proteins, mRNA, DNA, microRNA, and metabolites from tissues, sera, and pancreatic juice and cysts. Although an abundance of interesting candidate markers have arisen from these studies, currently none have been validated for clinical use. The limited progress in biomarker discovery is disappointing, but as panels of biomarkers with improved performance over individual markers (e.g., CA19.9 alone) are assembled, the

outlook may improve. Novel technologies (e.g., multiple reaction monitoring using mass spectrometry) are being developed for the quantification of multiple targets in complex biological samples, which should accelerate the identification, quantification, and validation of panels of biomarkers over the "one marker, one assay" paradigm used currently to validate most serum protein biomarkers. Similarly, previous biomarker studies often focused attention on identifying changes in overall protein or mRNA levels and did not examine subtle, but perhaps important, changes in posttranslational modification of proteins (e.g., disease-specific glycosylation variants) or mRNA structures (e.g., disease-specific splicing variants). Now, investigators are delving deeper into these structural variants and uncovering enticing new findings. It does not seem unreasonable to imagine that a specific and sensitive assay for PC might include the determination of the levels of a panel of serum proteins and metabolites, combined with the detection of specific glycosylation patterns of other serum proteins. Defining such complex diagnostic patterns across different biomarker-discovery platforms may be a daunting task but may provide the most clinically useful results.

Rafael Molina: It is important to look for new tumor markers that can be easily detected, have high sensitivity and specificity, and are informative with respect to early diagnosis and tumor resectability. Therefore, an ideal marker should be identifiable in blood, fecal material, or bile, though the last is less accessible. To the best of my knowledge, there is not any new tumor marker with these characteristics. However, different studies have reported microRNA profiles that may be useful for early diagnosis of PC with fine-needle biopsies.

Has whole-genome sequencing shed any light in terms of disease pathogenesis or progression? For example, are there any known pathways that are disturbed in this disease?

Randall Brand: Much is known about the molecular biology of PC. A landmark study by Jones and coworkers has demonstrated that 12 cellular signaling pathways and processes had at least 1 gene genetically altered in at least 70% of tumors sequenced. However, performance of whole-genome sequencing has had limited clinical impact at this time.

Felix Rückert: Whole-genome sequencing opened the doors for high-throughput gene expression analysis. We used this technique to identify defects in the apoptosis pathway in PC. A very interesting study was performed by S. Jones (*Science*, 2010). By means of a comprehensive genetic analysis, different core signaling pathways in PC could be identified, which led to a better understanding of tumor pathophysiology. However, recent data suggest that carcinogenesis is not dependent only on intracellular mutations. Tissue-based theories of carcinogenesis, such as our "feedback model," postulate that stromal cells contribute largely to malignant transformation in PC and therefore render this process even more complex.

Randy Haun: Recent whole-genome and whole-exome sequencing efforts of patient tumors have provided valuable insights into the cellular signaling pathways that are altered during the development of PC. In addition to 4 genes that had previously been recognized to be mutated in PC [*KRAS* (v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog), *TP53* (tumor protein p53), *p16/CDKN2A*, and *SMAD4* (SMAD family member 4)], other gene mutations that were present in the majority of the cancers examined have been identified. The genes responsible for these genetic alterations could be assigned to a set of 12 cellular signaling pathways, including the regulation of the G_1/G_S cell cycle transition and signaling through the KRAS, transforming growth factor β (TGF-β), hedgehog, integrin, c-jun N-terminal kinase (JNK), and Wnt/Notch pathways. Of particular note is the observation that although the majority of the tumors possess dysfunctions in these signaling pathways, the precise genes mutated within these pathways varied widely between individual tumors. This should inform the development of new therapeutics toward disrupting the overall function of these aberrantly regulated pathways (e.g., targeting components at the convergence of different signaling pathways or that interfere with several signaling molecules within a pathway), rather than inhibiting the activity of a particular component along the pathway. The insights gained by whole-genome/-exome sequencing should also be helpful for the identification of therapeutically targetable mutations.

Are there any promising new therapies or other developments on the horizon?

Randall Brand: Personalizing our approach to PC treatment using the patient's own tumor may be one promising approach. Our growing knowledge of PC stem cells and the role of the immune response are 2 other areas that warrant further investigation.

Felix Rückert: As mentioned above, the desmoplastic reaction of the tumor stroma impedes tumor perfusion and therefore accumulation of chemotherapeutics. In my opinion, a very promising way is to antagonize the development of the desmoplastic reaction by hedgehog

inhibitors. However, recent clinical studies using these hedgehog inhibitors were disappointing. A combination chemotherapy regimen consisting of oxaliplatin, irinotecan, fluorouracil, and leucovorin (FOLFIRINOX) is now used in patients with metastasized disease. Survival is 11 months, and the response rate is 31%, which is better than gemcitabine. However, it is a very demanding regimen for patients, and the intent is still palliative. It seems that there is no magic bullet for PC so far.

Randy Haun: As whole-genome/-exome sequencing technologies develop into platforms that enable individualized patient genome sequencing in a cost-effective manner, the potential of personalized medicine may be realized. For patients with locally advanced or metastatic disease, targeted treatment strategies may be guided by their mutational profile such that specific treatment regimens are employed only in patients with dysfunction in the target of the particular therapeutics [analogous to using only Herceptin (trastuzumab) for the treatment of HER2-positive breast cancer]. Similarly, for patients with potentially resectable disease, surgical assessments may include identifying mutations that select patients with low recurrence risk and higher survival rates.

Many combination therapies are being examined, both as primary treatment for locally advanced and metastatic disease and after failure of standard chemotherapeutic regimens (i.e., gemcitabine), or as neoadjuvant therapies. Some of these investigational drugs target the signaling pathways that have been found to be aberrantly regulated in PC (e.g., combination therapies of inhibitors of Notch or hedgehog signaling with gemcitabine). Success of these trials may depend in part on the patient population (i.e., whether the patients recruited to the study exhibit a mutation in the signaling component targeted by the drug) and/or whether the drug targets a downstream mediator of the signaling pathway such that it disrupts the dysfunctional signaling, regardless of which upstream component is mutated.

As our understanding of the pancreatic tumor microenvironment has improved, it is becoming evident that merely devising new therapeutics that target the tumor itself may not be sufficient for treating PC. Recent studies aimed at improving drug delivery to the tumor by targeting the stroma (e.g., inhibiting hedgehog signaling or targeting extracellular matrix proteins associated with the stroma) in addition to targeting the tumor cells with gemcitabine have elicited promising results. Similarly, eliminating the rapidly dividing tumor cells may lead to debulking of the tumor but may leave a niche of specialized cancer cells with the capacity to self-renew and asymmetrically divide. These putative cancer stem cells would thus lead to tumor recurrence; thus, they have become the targets for the development of novel therapeutics.

Rafael Molina: The treatment options in PC remain limited. However, there are new drugs and new promising strategies as adjuvant chemotherapy in patients with resectable tumors (gemcitabine) that double the 5-year survival rate, from 10% with surgery alone to 25%. Neoadjuvant chemoradiotherapy may downstage the borderline resectable disease and make resection possible, which could translate to a survival benefit. Improved management of pancreatic resections for cancer with more extensive and less-invasive surgical techniques has increased the number of patients who are candidates for effective surgical treatment.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.*

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:*

Employment or Leadership: E.P. Diamandis, *Clinical Chemistry*, AACC.
Consultant or Advisory Role: R. Brand, Asuragen, Inc., and Quest Diagnostics.
Stock Ownership: None declared.
Honoraria: None declared.
Research Funding: R. Brand, Early Detection Research Network (NIH), Coulter Foundation, and Somalogic, Inc.
Expert Testimony: None declared.
Patents: None declared.

Previously published online at DOI: 10.1373/clinchem.2012.196642