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Validation of Biomarkers for Early Detection of Pancreatic Cancer: Summary of the Alliance of Pancreatic Cancer Consortia for Biomarkers for Early Detection Workshop

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Abstract

Pancreatic cancer is the fourth leading cause of cancer death in the United States and the five-year relative survival for patients diagnosed with pancreatic cancer is less than 10%. Early intervention is the key to a better survival outcome. Currently, there are no biomarkers that can reliably detect pancreatic cancer at an early stage or identify precursors that are destined to progress to malignancy. The National Cancer Institute in partnership with the Kenner Family Research Fund and the Pancreatic Cancer Action Network convened a Data Jamboree on Biomarkers workshop on December 5, 2016, to discuss and evaluate existing or newly developed biomarkers and imaging methods for early detection of pancreatic cancer. The primary goal of this workshop was to determine if there are any promising biomarkers for early detection of pancreatic cancer that are ready for clinical validation. The Alliance of Pancreatic Cancer Consortia for Biomarkers for Early Detection, formed under the auspices of this workshop, will provide the common platform and the resources necessary for validation. Although none of the biomarkers evaluated appeared ready for a largescale biomarker validation trial, a number had sufficiently high sensitivity and specificity to warrant additional research especially if combined with other biomarkers to form a panel.

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Introduction

Pancreatic cancer is the fourth leading cause of cancer death in the United States, with, pancreatic ductal adenocarcinoma (PDAC) representing over 90% of all pancreatic malignancies and the remaining are neuroendocrine tumors. The five-year survival for PDAC is only about 10%¹. The poor prognosis is primarily due to its advanced stage at diagnosis. Consequently, there is a critical need for biomarkers and/or imaging methods that can more accurately detect early stage disease.

If PDAC is detected at an early stage when it is resectable, the five-year survival (not adjusted for lead time) increases to 30–60%. However, surgical resection is only possible for about 15% of patients. A major focus of pancreatic cancer research is to develop biomarkers and imaging methods with sufficient sensitivity and specificity to accurately detect early-stage PDAC and thereby increase the proportion of patients who have their cancer detected at an early stage and improve the five-year survival. There is also interest in developing methods that can accurately detect pancreatic intraepithelial neoplasia 3 (PanIN-3) lesions as they are believed to be an important precursor of PDAC. Lower grade PanIN-1 and PanIN-2 lesions are very common and most are not likely to progress to cancer. A number of recent published articles on biomarkers and imaging methods for early PDAC and/or PanIN-3 detection were discussed and evaluated during this workshop.

An area of increasing clinical importance is treatment of pancreatic cysts, intraductal papillary mucinous neoplasm (IPMN) and mucinous cystic neoplasm (MCN). IPMNs and MCNs have a high prevalence and are detected in 0.6 to 1.8% abdominal computed tomography (CT) scans in the United States. One-third of patients with IPMNs have an associated invasive carcinoma and at the time of diagnosis there is a 40% to 50% chance of the IPMN already being cancerous. MCNs can progress to PDAC, and one-third of patients with resected MCNs have cancer^{2,3}. Resection of IPMNs or MCNs prior to the development of invasive cancer is considered curative, but there are substantial risks of morbidity and mortality associated with surgery. Currently, it is difficult to distinguish precancerous mucinous cysts from benign non-mucinous cysts, the timing and frequency of malignant progression within the mucinous cysts are unknown, and there is a need for biomarkers to accurately detect high-grade dysplasia and determine the risk of progression. A number of recent articles on biomarkers and imaging methods for managing pancreatic cysts were discussed and evaluated at this workshop.

WORKSHOP ORGANIZATION

Investigators from four National Cancer Institute (NCI)-supported consortia on pancreatic cancer detection, including the Pancreatic Cancer Detection Consortium (PCDC), the Early Detection Research Network (EDRN), the Consortium for the Study of Chronic Pancreatitis, Diabetes and Pancreatic Cancer (CPDPC) and the Consortium for Molecular Characterization of Screen-Detected Lesions Created (MCL), were invited to participate in the Data Jamboree on Biomarkers workshop. Other invited participants included NCI program directors, investigators supported by and members of the Kenner Family Research Fund and the Pancreatic Cancer Action Network (PanCAN), and investigators from industry.

Biostatisticians from the NCI, Division of Cancer Prevention, Biometry Research Group, and the EDRN also participated. The expected outcome of this meeting was to identify biomarkers and/or imaging methods that could be further tested and validated through the PCDC and/or the EDRN, as part of The Alliance of Pancreatic Cancer Consortia for Biomarkers for Early Detection.

Participants were assigned one or two published original research articles and/or commercially available products to evaluate their strengths and weaknesses in detecting PDAC and early lesions. Each article was evaluated for the following: 1) biological strengths; 2) supporting evidence from model systems (e.g., cell lines, mouse models); 3) supporting evidence from human studies; 4) power and statistical relevance; 5) clinical relevance; 6) practicality – is the assay portable and amenable to clinical settings; 7) clinical utility – for early detection, detecting recurrence, and monitoring progression.

The papers discussed at the workshop represents only a small subset of the articles published each year, but were selected for discussion as they were representative of the different classes of biomarkers being examined, the sources of biospecimens used, and for the reported performance characteristics of the biomarkers and/or imaging method. Because of time constraint, numerous other published reports could not be discussed, but the approach used to evaluate the articles discussed at this workshop can be used to evaluate other publications as well.

STATISTICAL CRITERIA

Prior to the workshop, statisticians from NCI's Biometry Research Group provided the following guidelines to help evaluate the biomarkers being discussed. They and the EDRN statistician also provided statistical evaluations of each biomarker or panel of biomarkers discussed. The focus of this statistical discussion is the early detection of pancreatic cancer. Based on various methodological papers⁴⁻⁶ the statisticians identified several criteria for evaluation of biomarkers for the early detection of pancreatic cancer.

Is the study design appropriate for the intended clinical application? The study design should be driven by the intended clinical application such that the target population and the setting to collect specimens to measure biomarkers are the same as the future use of the biomarker⁴⁻⁶.

Was the data split into separate training and validation samples? The purpose of the training sample is to search for promising biomarkers and combining markers into a classification model. The purpose of the validation sample is definitive evaluation of marker performance. The splitting into training and validation samples avoids the problem of overfitting in which a classification model that performs well in the training sample performs poorly when applied to new data because the classification model too closely fits random perturbations.

Were prediagnostic specimens used to evaluate a marker for early detection of cancer? A prediagnostic specimen is a specimen taken from person without symptoms, which is then stored and later evaluated for the biomarker. Along with the prediagnostic specimen is there follow up information on whether the person developed cancer? Many studies use specimens

from persons already diagnosed with cancer. Markers typically perform better in specimens collected from a person diagnosed with cancer (diagnostic sample) than in a specimen from a person not yet diagnosed with cancer (prediagnostic sample). However, diagnostic markers do not necessarily make good prediagnostic markers. One potential limitation of using prediagnostic sample is that the stage of the disease at the time of acquisition is not known.

What is the reported performance of the marker? The false positive rate (FPR), which is also known as 1 minus specificity, is the probability the marker is positive indicator of cancer in a person without cancer. The true positive rate (TPR), which is also known as the sensitivity, is the probability the marker is positive in a person with cancer. For studies of biomarkers for the early detection of pancreatic cancer, it is necessary to have a very small FPR (less than 0.01) and a moderate to high TPR (at least 0.6), i.e. sensitivity of at least 60% at a specificity of at least 99%, which corresponds to an area under the Receiver Operating Characteristic curve (AUC) of at least 0.80. This target of AUC at least 0.80 is useful for studies that reported AUC without presenting the FPR's or TPR's.

What was the comparison group? Typically, the comparison is either cancer versus healthy or cancer versus other disease condition. This comparison depends on the intended clinical application. For early detection, the comparison is usually between cancer and healthy but it could involve a high-risk group such as patients with pancreatitis. The ideal comparison group depends on the purpose of the analysis and is driven by the intended clinical use of the marker.

What was the sample size used for training and or validation? Due to its low prevalence, studies to validate methods for the early detection of asymptomatic PDAC are very difficult and require large number of subjects to power the study. But there are other clinical settings where biomarkers maybe more readily applicable. Studies involving high risk populations, such as people with a family history, germline mutations, pancreatic cysts or new-onset diabetes, should require less subjects to power the study and the performance requirements of the biomarker somewhat less demanding. The sample size required for validation depends on the biomarker's target values and precision for FPR and TPR.⁴ Based on this type of calculation, a sample size of 100 cases and 400 controls would be appropriate for many instances.

THE DATA JAMBOREE ON BIOMARKERS WORKSHOP

The workshop was divided into four sessions based on the type of biomarkers evaluated. Session 1 focused on genomic markers. Sessions 2 and 3 focused on proteomic markers. Session 4 focused on imaging methods and/or markers. After the presentation of five or six papers by the extramural investigators based on the criteria outlined earlier, a biostatistician presented the statistical evaluation of the biomarkers and/or imaging methods, followed by a panel discussion which included the presenters, other experts on PDAC and the biostatistician. The panelists discussed the potential utility of the biomarkers and/or imaging methods and provided suggestions as to whether they warranted further investigation and/or validation.

SESSION 1: GENOMIC BIOMARKERS

Genomic biomarkers include DNA mutation, epigenetic alteration, microsatellite instability, gene amplification and loss of heterozygosity. These biomarkers for early detection of pancreatic cancer were measurable in blood, cyst fluid, pancreatic juice and urine.

Blood-based Markers

The workshop participants discussed the potential utility of circulating tumor DNA (ctDNA) as a marker of early detection, including methodologies to distinguish false-positive results related to PCR errors from true ctDNA mutations that are present at very low concentrations. Several publications have evaluated ctDNA in patients with pancreatic cancer. The article by Bettegowda et al. used a molecular barcoding strategy known as Safe-Sequencing System (SafeSeqS) to identify the amplicons of individual DNA templates. This strategy is very effective in distinguishing assay-generated alterations that are read as mutations from true ctDNA mutations. Using SafeSeqS, the authors identified circulating mutated *KRAS* DNA in 48% of individuals with localized pancreatic cancer and in 85% of patients with advanced disease⁷. Others have reported detecting mutated *KRAS* ctDNA using digital-droplet PCR,^{8,9} which although a simpler assay, has the disadvantage that it cannot distinguish low level false-positive results related to PCR errors. PCR errors can occur at levels approaching the concentration of mutant DNA in circulation from patients with cancer.¹⁰ For this reason, it is particularly important that studies involving ctDNA employ extensive testing to determine the specificity of their assay.

The workshop participants also discussed a recent article that reported detecting mutated *KRAS* and *GNAS* ctDNA using digital droplet PCR in patients with IPMNs. This study included only a small number of patients and controls, and there was a lack of concordance between the mutations in IPMNs and those detected in circulation. Furthermore, the authors detected circulating *GNAS* mutations in 15/21 IPMN cases, however, circulating *KRAS* mutations were not detected, which is surprising since these mutations are found in similar percentages of IPMNs.¹¹

Although only ~40–50% of patients with resectable pancreatic cancer have detectable circulating mutations, the detection of these mutations could be valuable as part of an early detection strategy if the presence of these mutations is very specific for pancreatic cancer. ctDNA detection methods should utilize barcoding strategies or digital approaches that account for the potential of false positives. For pancreatic cancer, the major ctDNA biomarker is mutated *KRAS*, but any early detection test that used mutated *KRAS* would have to consider the possibility that these mutations could arise from many different cancers. Further studies are needed to determine whether patients without cancer harbor true circulating mutations. The challenges of reliably detecting low levels of ctDNA has limited its evaluation as a potential screening test for pancreatic and other cancers to date, but with advancement in detection methods and technologies, it is likely to become a useful test in the future.

Cyst Fluid Markers

While most pancreatic cysts are benign and produce no symptoms, some are precancerous or cancerous. Pancreatic cysts are often discovered incidentally from radiological scans of the abdomen for unrelated symptoms. The dilemma is in distinguishing the precancerous and cancerous cysts which require treatment from the benign low-grade dysplastic cysts that are usually over treated.

The workshop participants discussed an article evaluating a DNA panel for the assessment of pancreatic cysts.¹² These investigators evaluated a cyst fluid DNA test both for its ability to classify the type of cyst and to predict its grade of dysplasia. The test utilized SafeSeqS to detect mutated DNA in cyst fluid and found that over 90% of IPMNs and MCNs harbor mutations in *KRAS* or *GNAS* (IPMNs only), whereas serous cystadenomas frequently contain mutations in *VHL*. *KRAS* and *GNAS* mutations arise early in the natural history of IPMNs and MCNs, therefore, do not reliably predict the grade of neoplasia of a pancreatic cyst. Other mutated genes such as mutated *TP53* helped predict the neoplastic grade of a cyst, as did the presence of chromosomal copy number alterations. The participants discussed other studies that have evaluated cyst fluid DNA markers and found similar results. The SafeSeqS method is being incorporated into commercially available tests, which should help translate this test for use in clinical practice. Other biomarkers are currently being evaluated to determine if they can match or improve upon the diagnostic accuracy of a mutated gene panel. There is still room to develop more cost-effective and accurate cyst fluid tests that can be readily incorporated into routine clinical diagnostic laboratories.

Elevated telomerase activity is often associated with malignant cells. The participants discussed an article by Hata et al¹³ that evaluated the diagnostic performance of telomerase activity in cyst fluids. Telomerase activity was measured using the telomerase repeat amplification protocol (TRAP) with digital-droplet PCR (dd-TRAP). The accuracy of telomerase activity for diagnosing invasive cancer and high grade dysplasia was 88.6% in their validation trial. For the cysts fluids that were classified as having “worrisome features”, telomerase activity had high diagnostic performance (sensitivity 73.7%, specificity 90.6%). The results from this multivariate analysis indicate that telomerase activity independently identified the presence of invasive cancer and high-grade dysplasia. Limitations of this study are that most of the samples analyzed were obtained from surgical resected specimens and that activity was reduced in previously thawed samples. A prospective validation study is needed to determine the predictive power of telomerase activity from patients whose cysts pose diagnostic and management dilemmas.

Pancreatic Juice Markers

Pancreatic juice is a potential source of cancer-specific biomarkers due to the proximity of pancreatic ducts to tumor tissue.

The workshop participants discussed two articles that evaluated pancreatic juice biomarkers for their potential utility as pancreatic screening tests. Many high-risk patients undergo endoscopic ultrasound (EUS) as their primary screening test, and pancreatic juice collected non-invasively from the duodenum represents a potentially valuable source of biomarkers.

Pancreatic juice samples obtained from patients undergoing pancreatic screening, even those without pancreatic cysts, often contain mutations in *KRAS* and other genes.¹⁴ These mutations are thought to be shed from PanINs. A recent study that employed digital next-generation sequencing to detect a panel of mutations in pancreatic juice found that overall pancreatic juice mutation concentrations, and in particular, *SMAD4* and *TP53* mutations were very useful in distinguishing patients with pancreatic cancer from those with IPMN and normal pancreata.¹⁵ *TP53* and *SMAD4* mutations are known to arise relatively late in the progression of PanINs, typically with the emergence of PanIN-3/high-grade dysplasia or at the transition to invasive cancer. Of particular interest was the detection of mutated *SMAD4* and *TP53* in pancreatic juice samples obtained from patients undergoing pancreatic surveillance more than a year prior to the diagnosis of pancreatic cancer. Further studies are needed to determine how specific the detection of *SMAD4* and *TP53* mutations are for the presence of high-grade dysplasia, PanIN-3 and invasive cancer.

Another promising biomarker strategy using pancreatic juice samples is to measure aberrantly methylated DNA. Many articles in the literature have reported methylated DNA alterations that arise during tumorigenesis. One of the challenges of methylated DNA biomarkers is that some non-cancer tissues also undergo low-level DNA methylation changes, particularly rapidly proliferating tissues such as the duodenum. Kisiel et al identified differentially methylated DNA present in pancreatic ductal adenocarcinomas and developed a test to detect the aberrantly methylated DNAs in pancreatic fluid.¹⁶ Further studies are needed to evaluate whether such a test could be used to evaluate the pancreas of patients undergoing pancreatic screening and surveillance.

SESSIONS 2 AND 3: PROTEOMIC BIOMARKERS

Many of the biomarkers currently used for early cancer detection are proteins or glycoproteins, e.g. PSA for prostate, AFP for hepatocellular carcinoma, and CA 125 for ovarian cancer. Many researchers are trying to develop protein biomarkers or panels of biomarkers for resectable PDAC or PanIN-3 lesions. Many proteomic approaches used to discover biomarkers allow for the simultaneous measurement of multiple biomarkers. When combined with limited numbers of samples, overfitting the data is a major concern. As noted earlier, this can be addressed by using independent discovery/training and validation sets with panel of biomarkers before being assayed in the validation set. Unfortunately, this approach is not always followed.

CA 19-9

The only established pancreatic cancer biomarker is CA 19-9, a modified Lewis(a) blood group antigen that is a component of glycoproteins and mucins. Although useful for monitoring for recurrence, CA 19-9 does not have the sensitivity and specificity required for early detection¹⁷. In a study by Haab et al,¹⁸ the pancreatic cancer reference set collected by the EDRN was used to characterize CA 19-9 as a diagnostic marker for pancreatic cancer. This reference set was collected under rigorous standards by multiple institutions with patient populations including a large cohort of resectable cancers and the inclusion of the key control groups¹⁹. Because the previous studies using kits for CA 19-9 detection have

divergent results, likely owing to differences in the specificities of the antibodies or differences in the assay platforms, the authors compared the difference between two antibody-based assay kits. The two kits performed similarly in differentiating cancer from healthy controls with an area under the curve (AUC) of 0.77, but the values did not always agree for individual patients. The findings from this study were comparable to previous studies on CA 19-9. While this study showed clinical relevance for use of the EDNR reference set for diagnostic biomarkers validation and set a benchmark for CA 19-9, the use of prediagnostic specimens would be needed for evaluating CA19-19 the early detection of cancer.

The workshop participants also discussed an article by Nolen et al²⁰ that evaluated biomarkers in prediagnostic samples collected by the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial in which incidental PDAC was diagnosed at various intervals after sample collection. Using a Luminex platform, 67 previously identified biomarkers were assayed in samples from 325 healthy patients and 79 patients that later developed pancreatic cancer. In this training-validation study where half of the samples were used for training, CA 19-9, CEA, NSE, bHCG, CEACAM1 and PRL were significantly altered in sera obtained from cases more than one year prior to diagnosis (the AUC for CA 19-9 + CEA was 0.66); however, no biomarkers were identified that performed better than CA 19-9 alone. For cases obtained 1–12 months prior to diagnosis, the AUC for CA 19-9 + CEA was 0.71. O'Brien et al²¹ reported that measurements of CA 19-9 in prediagnostic samples collected for the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS) could detect 68% (at a specificity of 95%) of the PDAC 0–12 months prior to diagnosis.

Glycoproteins

Because alterations in glycosylation regulate the development and progression of cancer, glycosylated proteins can be good cancer biomarkers. Indeed, many of the currently used cancer biomarkers, including, CEA, AFP, PSA and CA125, are glycoproteins. The participants discussed a glycoprotein biomarker panel reported by Nie et al.²² Using a lectin-array and quantitative proteomics, these authors identified a panel of biomarkers with glycosylation alterations that could lead to differential diagnosis of pancreatic cancer from high risk groups. The panel consists of AACT, THBS1 and HPT. The performance these glycoproteins were verified using lectin-ELISA and ELISA assays. This panel performed better than CA 19-9 alone (AUC = 0.89) in distinguishing pancreatic cancer from normal controls (AUC = 0.95), diabetes (AUC = 0.89), cyst (AUC = 0.82), and chronic pancreatitis (CP) (AUC = 0.90). Adding CA 19-9 to the panel increased the performance (AUC = 0.99) suggested a high diagnostic potential in distinguishing pancreatic cancer from the other benign pancreatic conditions. However, this panel showed limited performance in distinguishing pancreatic cancer from obstructive jaundice. In addition, this case-control study used mostly late-stage cancer, generating some concern that the panel may not detect early stage cancer. The performance in an independent validation sample with pre-diagnostic cases is not known.

Antibody Arrays

Antibody arrays are another discovery approach used to identify potential cancer biomarkers as they allow for the detection of multiple proteins in low sample volumes. Using a cross-species antibody microarray containing over 130 relevant antibodies, Mirus et al.²³ identified a 3-protein panel of plasma biomarkers for early diagnosis of pancreatic cancer. Samples used were collected from 14 KPC (LSL-Kras^{G12D/+}; LSL-Trp53^{R172H/+}; Pdx-1-Cre) transgenic mice as well as 223 human plasma samples consisting of 87 prediagnostic and 87 matched controls, and 25 diagnostic cases and 24 unmatched controls. The panel of 3 markers, ERBB2, TNC and TCM, were tested further. The AUC for this panel was 0.68 and 0.86 for the prediagnostic and diagnostic specimens respectively. When CA 19-9 was added to the panel the AUC was 0.71 and 0.97 for prediagnostic and diagnostic specimens, respectively, suggesting the possibility for use as a diagnostic biomarker panel.

Using a combination of antibody-based and LC-MS/MS proteomics, Yoneyama et al.²⁴ identified IGFBP2 and IGFBP3 as compensatory biomarkers for CA 19-9 for detection of early stage pancreatic cancer. This study focused on 130 proteins known to be increased in pancreatic cancer and for which antibodies were available. After reverse-phase protein array and LC-MS/MS quantification they identified 2 markers, IGFBP2 and IGFBP3 which had the ability to discriminate patients with invasive ductal adenocarcinoma of pancreas (N = 101) at an early stage from healthy controls (N = 38). When combined with CA 19-9 the AUC was 0.94, indicating this combination of biomarkers can distinguish diagnostic cases from healthy controls and could be a promising diagnostic biomarker.

Other investigators are exploring the value of using large panels of protein markers as a diagnostic test. Gerdts et al evaluated an antibody array containing 350 human recombinant antibodies targeting cytokines and a variety of other molecules to detect circulating biomarkers of pancreatic cancer. They found signatures of 17–29 antibodies which when assayed in a small test set gave AUC values of 0.71 and 0.87 for early stage and all PDAC (respectively) versus controls,²⁵ although the number of early stage tumors was small. It remains to be seen if such approaches can improve the diagnosis of early stage pancreatic cancer.

Mass Spectrometry

Mass spectrometry profiling is another high throughput method used to discover cancer biomarkers. Jenkinson et al. used iTRAQ (isobaric tags for relative and absolute quantitation) to profile serum proteins from UKCTOCS prediagnostic samples. They found reduced levels of TSP-1 up to 2 years prior to a diagnosis of pancreatic cancer.²⁶ The MS results were verified using ELISA. The combination of TSP-1 and CA 19-9 performed better (AUC = 0.85) than either alone (AUC = 0.69 for TSP-1 and AUC = 0.77 for CA 19-9) in these prediagnostic specimens and suggests that the combination would be promising for early detection.

Metabolites

Mayers et al.²⁷ performed plasma metabolite profiling from four cohorts including individuals who later developed pancreatic cancer and matched controls. They found that

elevated branch chain amino acid profiles were weakly associated with an increased likelihood of being diagnosed with pancreatic cancer in the subsequent two to five years. There was considerable overlap between cases and controls. This association was independent of other co-morbidities associated with pancreatic cancer risk. In a genetically engineered mouse model of pancreatic cancer they found that elevated branch chain amino acids arose from muscle catabolism.

Urine

Although most investigators use sera or plasma, some investigators have used urine as a noninvasive source of potential genomic and proteomic biomarkers. Radon et al²⁸ used a discovery/validation design of diagnostic specimens to identify and validate a 3-protein biomarker panel in urine for early detection of PDAC. In the discovery phase, they used mass spectroscopy to perform comprehensive proteomic analysis on samples from 18 patients (healthy controls N = 6, chronic pancreatitis N = 6, and patients with PDAC N = 6). The three most commonly deregulated proteins (LYVE1, REG1A and TFF1) were validated by ELISA in an independent cohort (49 PDAC and 28 healthy) and this panel when used with age and creatine gave an AUC of 0.92. A major unanswered question is the specificity of the biomarker panel. The performance of the test was best in the healthy vs PDAC comparison and was less robust when trying to distinguish chronic pancreatitis from PDAC. The panel was not specific for PDAC when including patients with other pancreatobiliary neoplasms, and the specificity with respect to other cancer types is not known. Another concern is the need for assay standardization, as one protein was left out of later experiments due to a change in the commercial ELISA assay.

Exosomes

In the past, several years, exosomes and their molecular contents have been investigated as potential cancer biomarkers. Exosomes are small endocytic vesicles, which can contain DNA fragments, mRNAs, microRNAs and proteins. The meeting reviewed the results of a study by Melo et al who reported that glypican-1 (GPC1) positive exosome levels were much higher in patients with pancreatic cancer than in controls and could distinguish patients with PDAC from healthy controls with nearly 100% accuracy (FPR = 0 and TPR = 1). They also found patients with precancerous lesions (IPMNs) had elevated GPC1 positive exosomes, however the sample size was small. There is a need to evaluate this biomarker in a blinded fashion using an independent sample set that contains more early stage PDACs.²⁹ It remains to be determined if this or a similar test can detect pancreatic cancer specific exosomes that could be used to detect prediagnostic cancer.

SESSIONS 4: IMAGING BIOMARKERS

Endoplasmic ultrasound (EUS), CT and Magnetic resonance imaging (MRI) are the most commonly used methods to diagnose PDAC. EUS is the most commonly used method and may be able to find small masses that are not detected by current MRI and CT methods. However, none of these imaging methods have the sensitivity to reliably detect early stage PDAC or PanIN-3 lesions. Consequently, there is considerable interest in developing

improved imaging methods which could be used as stand alone or in combination with biomarkers.

Radiography

Canto et al³⁰ screened 225 high-risk individuals using CT scan, EUS and MRI and compared the results in a blinded fashion. EUS was most sensitive in detecting abnormalities, magnetic resonance cholangiopancreatography (MRCP) and CT missed 19/92 lesions detected by EUS. There was better concordance with EU and MRI (91%) vs EUS and CT (73%). This was a well-designed study and a large cohort and has a potential for high sensitivity, but it is difficult to determine the utility without information on specificity.

In a retrospective study Kamisawa et al³¹ used diffusion-weighted MRI (DWI) to differentiate patients with autoimmune pancreatitis (AIP) (N = 13) from those with PDAC (N = 40). They showed a AUC of 0.89 for PDAC vs AIP suggesting diffusion-weighted MRI might be useful diagnostically to distinguish AIP from PDAC, but the results need independent validation. While DWI has clinical relevance, the practical use has challenges.

Imaging Probe

Kelly et al³² proposed to develop a nanoparticle imaging probe to detect pancreatic cancer and/or high grade lesions. This study used genetically engineered mouse models (GEMM) of pancreatic cancer, normal duct cells from wild type mice, human pancreatic cancer cell lines and human pancreatic tumor samples to identify peptides that specifically bind to the cell surface of pancreas cancer cells. They developed a magnetofluorescent nanoparticle fused to a Plectin-1 binding peptide for targeting pancreatic cancer cells. Using confocal microscopy, they showed focal uptake the targeting peptide in KPC mice that corresponded to the presence of tumor nodules. Some concerns with this study were the lack of benign controls (pancreatitis) and the high uptake in the liver and kidney.

Radiomics

Permeth et al³³ hypothesized that combining miRNA classifier with radiographic features, including radiomics, would be more predictive than either alone. They studied 38 patients (28 benign and 18 malignant) with pre-op CTs and miRNA data by radiogenomics. The miRNA classifier, which included miR-200a-3p, 1185-5p, 33a-5p, 574-4p, 664b, was identified in a previous study.³⁴ Combining the high-risk stigmata (main duct (MD) involvement/ dilatation > 10 mm, obstructive jaundice with a cystic lesion in the pancreatic head, or an enhanced solid component within the cyst) with the miRNA classifier produced an AUC of 0.95, while combining worrisome features (MD dilation 5–9 mm, cyst size > 3 cm, thickened enhanced cyst walls, non-enhanced mural nodules, or acute pancreatitis) with radiomics and the miRNA classifier produce an AUC of 0.93. This radiogenomics study could be useful in distinguishing benign from malignant IPMN but the sample size was small and independent validation is needed.

Metabolites

Penet et al³⁵ used metabolic imaging to detected altered choline metabolism in PDAC. This was a pre-clinical study using a human pancreatic cancer cell line, tumors and the

appropriated controls. They used ^1H magnetic resonance spectroscopic imaging to identify noninvasive biomarkers and uncover potential metabolic targets. They showed that choline levels were higher in all cancers cell lines compared to the non-neoplastic cells. These results could potentially be useful for early detection of pancreatic cancer; however, the sample size is small and needs to be validated.

CONCLUDING REMARKS

A careful evaluation of recent publications on biomarkers for the early detection of pancreatic cancer indicates that there have been significant improvements in both the reported performance of the biomarkers (sensitivity and specificity) and in the design of the studies (use of appropriate samples and populations and use of independent training and test samples). However, to date, none of the reported biomarkers appear ready for use in the clinic. In general, biomarkers measured in cystic fluid or pancreatic juice appear closer to being ready for largescale biomarker validation trials than those measured in blood. This most likely reflects the proximity of the pancreatic juice to the PDAC and the direct sampling of the cyst fluid which harbors the neoplasm. It will be interesting to determine the performance of a panel of biomarkers collected from cyst fluid or from pancreatic juice. A panel might include several classes of biomarkers, such as mutant DNA, epigenetic alterations, elevations in protein expression, changes in enzymatic activity or changes in metabolites that can be easily assayed using minimal amounts of aspirated fluid. A panel of biomarkers measured in pancreatic juice will most likely be useful for high-risk patients (e.g., patients with a family history of PDAC) who are undergoing surveillance by endoscopic ultrasound (EUS).

Several papers reported promising results on biomarkers measured in cyst fluids to help surgeons determine which cysts are likely to be cancerous and need to be removed. The workshop participants suggested that a collaborative study be performed to compare the performance of these different biomarkers in a common set of cyst fluids both to directly compare their performances and to determine if they can be combined into a panel to increase accuracy. Recently, several participants of the workshop formed the Pancreatic Cyst Biomarker Alliance (PCBA). The PCBA will use pancreatic cyst fluids collected from multiple centers to validate multiple biomarkers to help surgeons determine which cysts are likely to be cancerous and need to be removed. Many of the markers to be tested were discussed at the Data Jamboree.

For screening either a high-risk population or an average-risk population for PDAC, blood-based biomarkers need to have very high specificity to avoid a very high number of false positives. Several of the protein biomarkers discussed have sensitivities in the range of 50–60% at 95% specificity. At 99% specificity, which may be required for screening, the reported sensitivities are considerably reduced. Either biomarkers or panels of biomarkers with improved specificity need to be developed or a two-stage screening strategy needs to be developed. The first stage will be a biomarker or panel of biomarkers with very good sensitivity and specificity followed by an imaging modality that can detect early stage (small) PDAC. Current imaging methods cannot accurately detect small PDACs and

improved screening methods will need to be developed. Several potential improved imaging methods were discussed.

Since the Data Jamboree workshop in December 2016, there have been several reports of new pancreatic cancer biomarkers, many by members of the Alliance. These biomarkers include thrombospondin-2 (THBS-2)³⁶, plasma tissue factor pathway inhibitor (TFPI),³⁷ tenascin C (TNC-FN III-C), tissue inhibitor of metalloproteinase 1 (TIMP1), and Leucine-rich repeat-containing G protein-coupled receptor 1 (LGR1),³⁸ mucin-5AC (MUC5AC)³⁹ and a panel of methylated gene markers to predict the grade of dysplasia in pancreatic cysts.⁴⁰ Others have evaluated exosome-derived DNA and RNA^{41,42} and cell free DNA and miRNA markers^{43–46} as potential biomarkers for early detection. This is not a complete list of published manuscripts nor were these manuscripts evaluated by the criteria described in this manuscript.

Participants of the workshop have formed The Alliance of Pancreatic Cancer Consortia for Biomarkers for Early Detection to continue the discussion on biomarkers for pancreatic cancer. The Alliance will assist in developing methodologies and accruing prediagnostic specimens to validate current and future biomarkers for early detection of pancreatic cancer and for evaluating potential markers for recommendation of surgical removal of cancerous cysts. The Alliance agreed to meet in 2 years to discuss new papers and to determine if there are any potential biomarkers that need further validation.

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