**Chapter 27**

***Molecular Testing in Pancreatic Cancer***

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**CASE 1**

**Introduction:** Pancreatic ductal adenocarcinoma (PDAC) has an estimated prevalence of 40 per 100,000 people and a 5-year surviving rate of 7.2%. As of now, the only curative treatment is based on a surgical resection, which implies early cancer diagnosis. In this context, diagnostic tests are needed which detect pancreatic cancer reliably at an early and potentially curable disease state.

**Primary Question:** ***An optimal screening test to diagnose PDAC in its earliest stage needs to fulfill which of the following criteria?***

1. Only sensitivity of 90% is sufficient.
2. Only specificity of 90% is sufficient.
3. Both sensitivity and specificity of 90% are sufficient.
4. Both sensitivity and specificity need to be higher than 99.99%.

**Correct Response:** D

**Explanation of Correct/Incorrect Response** - Screening tests for rare diseases like PDAC require highest sensitivity and specificity. A screening test with a specificity of 90% would result in nearly 10 million false positive test results in the US. A specificity of 99.9% would still have approximately 100,000 false positive test results. In order to avoid unnecessary invasive testing or surgery, highest sensitivity and specificity, preferable above 99.99% are paramount.

**First Reply Question** - Current diagnostic markers for PDAC are inadequate in order to be used in the context of a screening test. ***Which of the following statements about an optimal screening test for PDAC is true?***

1. High sensitivity and specificity are paramount.
2. The positive predictive value is not affected by the disease prevalence.
3. The negative predictive value is not affected by the disease prevalence.
4. Test accuracy cannot be calculated from sensitivity and specificity.

**Correct Response:** A

**Explanation of Correct/Incorrect Response** - Screening tests for rare diseases like PDAC require highest sensitivity and specificity in order to limit false positive and false negative results. Both positive and negative predictive values are affected by disease prevalence. With increasing disease prevalence, the positive predictive value rises and negative predictive value decreases. The test accuracy can by calculated as the following:

Accuracy = (Sensitivity) x (Prevalence) + (Specificity) x (1-Prevalence)

**Second Reply Question** - ***Besides highest sensitivity and specificity, which of the following test characteristics are important for an optimal screening test for PDAC?***

1. High test accuracy is important.
2. High test precision is important.
3. It is preferable that the tested specimen is easily and safely accessible.
4. The optimal screening test is cost effective.
5. All above are correct.

**Correct Response:** E

**Explanation of Correct/Incorrect Response** - Optimal screening tests require highest sensitivity and specificity along with high accuracy and precision. The optimal specimen for testing needs to be easily and safely accessible like urine, saliva or serum to avoid unnecessary iatrogenic injury in light of screening for diseases. Finally, cost effectiveness is an important aspect of any screening test given its expected frequent usage.

**CASE 2**

**Introduction:** Diagnostic markers play an important role in identifying diseases in patients and hereby distinguishing from healthy or normal subjects. Moreover, their role is also to distinguish diseases from other potentially resembling pathologic conditions. In order to do so, diagnostic markers need to fulfill a magnitude of criteria. The search for an optimal diagnostic marker for pancreatic ductal adenocarcinoma (PDAC) is particularly challenging. This is also due to the fact that PDAC arises from different biologic pathways. Additionally, the biologic pathways of benign and malignant pancreatic conditions overlap.

**Primary Question -** ***Which one of the following statements is correct regarding diagnostic markers for PDAC?***

1. A single diagnostic marker is as sensitive and specific as a panel of diagnostic markers.
2. All pancreatic ductal adenocarcinoma have similar biologic background.
3. Diagnostic markers for PDAC need to distinguish PDAC only from normal pancreas but not from mucinous cystic neoplasms (MCN) or chronic pancreatitis.
4. Diagnostic markers are required to diagnose PDAC at a disease stage 3 and 4.
5. None of the statements is correct.

**Correct Response:** E

**Explanation of Correct/Incorrect Response** - Despite similar anatomic location, PDAC can have different biologic background reflecting a significant heterogeneity of this cancer, which consequently requires different diagnostic markers. In this context, multiple studies proved that a panel of markers yielded better test performance than a single diagnostic marker. Non-invasive pre-malignant conditions which are chronic pancreatitis, MCN and intrapapillary mucinous neoplasms (IPMN) can be misinterpreted as PDAC and vice versa. In order to avoid unnecessary invasive testing or surgery, diagnostic markers need to distinguish reliably all pre-malignant conditions from PDAC. The goal of a screening test is to identify a medical condition at a treatable and preferably curable stage, as the chances of cure fall when PDAC invades adjacent blood vessel and lymph nodes or when distant metastasis are present. Therefore, the goal is to detect PDAC in its earliest invasive stage (stage 1).

**First Reply Question** - Diagnostic markers for PDAC need to fulfill a magnitude of criteria. ***Which one of the following statements is correct regarding diagnostic markers for PDAC?***

1. Optimal diagnostic markers for PDAC need to distinguish it from pancreatic cystic neoplasms (PCN).
2. Diagnostic markers of PDAC need to diagnose PDAC at its earliest stages.
3. A panel of diagnostic markers is likely to be more sensitive and specific compared to a single diagnostic marker.
4. All statements are correct.

**Correct Response:** D

**Explanation of Correct/Incorrect Response** - Pancreatic cystic neoplasms (PCN) encompass mucinous cystic neoplasms (MCN) and intrapapillary mucinous neoplasms (IPMN) and both are pre-malignant conditions. Moreover, PCN include also serous cystic neoplasms (SCN) which have a much lower malignant potential. An optimal diagnostic marker needs to distinguish all PCN from PDAC. A diagnostic marker utilized for a screening test needs to identify a medical condition at a treatable and preferably curable state, which is in PDAC preferable disease stage 1. PDAC has significant biologic heterogeneity for which a panel of markers is likely to yield a better test performance than a single diagnostic marker.

**Second Reply Question** - Diagnostic markers for pancreatic ductal adenocarcinoma (PDAC) need to distinguish PDAC from chronic pancreatitis and pancreatic cystic neoplasms (PCN). ***Which one of the following statements is correct regarding the current research for diagnostic markers for PDAC?***

1. Diagnostic markers of PDAC are frequently false positive when pancreatic cystic neoplasms (PCN) are present.
2. Diagnostic markers of PDAC are frequently false positive when mucinous cystic neoplasms (MCN) are present.
3. Diagnostic markers of PDAC are frequently false positive when intrapapillary mucinous neoplasms (IPMN) are present
4. Diagnostic markers of PDAC are frequently false positive in chronic pancreatitis without evidence of malignancy.
5. All statements above are correct.

**Correct Response:** E

**Explanation of Correct/Incorrect Response** - A challenge to identify an optimal diagnostic marker or a panel of markers for PDAC is the significant biologic overlap between PDAC and pancreatic cystic neoplasms (PCN) which encompass mucinous cystic neoplasms (MCN) and intrapapillary mucinous neoplasms (IPMN). Additionally, PDAC and chronic pancreatitis have significant biologic overlap which results in frequent overlapping of potential diagnostic markers.

**CASE 3**

**Introduction:** The pathogenesis of PDAC encompasses both inherited and acquired mutations in cancer-related genes. The mutations lead to pancreatic intraepithelial neoplasia (PanIN) which progresses from low grade dysplasia to high grade dysplasia depending on the type and quantity of mutated genes. Comparable cancer pathways have been also reported for pancreatic cystic neoplasms (PCN).

Primary Question - ***Which of the following genes were shown to be mutated in pancreatic ductal adenocarcinoma PDAC?***

1. K-ras
2. p16
3. p53
4. c-Kit
5. BRCA2
6. All above genes were shown to be mutated in PDAC

**Correct Response:** F

**Explanation of Correct/Incorrect Response** - K-ras is the most commonly found mutation in PDAC (90%), followed by p16, and p53. BRCA-2 mutation is detected in up to 17% of PDAC, especially familial pancreatic cancer. C-Kit mutations are not commonly reported. However they are found in 6% to 77% of PDAC cases.

**First Reply Question** - A variety of genetic mutations have been reported in the pathogenesis of PDAC. ***Which of the following mutated genes is commonly reported to be an early event in the pathogenesis of pancreatic ductal adenocarcinoma (PDAC)?***

1. K-ras
2. p53
3. BRCA2
4. All above genes were shown to be mutated early in PDAC.

**Correct Response:** A

**Explanation of Correct/Incorrect Response** - K-ras is commonly found to be an initial mutation in the pathogenesis of PDAC given its high frequency in pancreatic intraepithelial neoplasia 1 (PanIN1). However, p53 and BRCA-2 mutation are detected more frequently in PanIN3 lesions arguing for a later event in the pathogenesis of PDAC.

**Second Reply Question** - A variety of genetic mutations have been reported in the pathogenesis of PDAC. The mutations include the activation of an oncogene or the deactivation of a tumor suppressor gene. ***Which of the following gene defects encompasses the activation of an oncogene which is commonly reported to be involved in the pathogenesis of pancreatic ductal adenocarcinoma (PDAC)?***

1. K-ras
2. p53
3. BRCA2
4. All above genes are oncogenes.

**Correct Response:** A

**Explanation of Correct/Incorrect Response** - K-ras is an oncogene which is activated in the pathogenesis of PDAC. Contrary, both p53 and BRCA2 are tumor suppressor genes which are inactivated in the pathogenesis of PDAC.

**CASE 4**

**Introduction**: Virtually all diagnostic markers for diseases derive from the human body. Especially when implementing diagnostic markers for an early disease diagnosis, easily and safely accessible specimens are paramount in order to avoid any iatrogenic complications.

**Primary Question** - ***Which of the following specimens have not been evaluated for their diagnostic utility as screening biomarker carrier for pancreatic ductal adenocarcinoma (PDAC)?***

1. Serum
2. Bile
3. Pancreatic juice
4. Feces
5. Endoscopic ultrasound with fine needle aspiration (EUS-FNA)
6. Urine

**Correct Response:** E

**Explanation of Correct/Incorrect Response** - All of the above but EUS-FNA have been evaluated for their utility as a carrier for a screening marker for PDAC, with serum and pancreatic juice being the most extensively studied specimens. EUS-FNA is an invasive technique which is used for diagnostic purposes in the setting of PDAC evaluation. However given its invasiveness, EUS-FNA plays no role as screening tool for PDAC.

**First Reply Question** - The medical literature encompasses multiple specimens which were used as carrier of diagnostic markers for PDAC. ***Which of the following specimens have been most frequently studied in the search for a diagnostic marker for PDAC?***

1. Serum
2. Bile
3. Pancreatic juice
4. Feces
5. Urine

**Correct Response:** A

**Explanation of Correct/Incorrect Response** - Serum is the best studied specimen as carrier for diagnostic markers of PDAC. In this context, less data is available for feces, followed by bile, pancreatic juice and urine.

**Second Reply Question** - The collection of bile has been described in the context of diagnostic markers for PDAC. ***Which of the following statements is correct when comparing bile collection with serum, urine or feces collection to detect a diagnostic marker for PDAC?***

1. Bile has a higher quantity of diagnostic markers for PDAC in comparison with serum or urine given its proximity to the pancreas.
2. The collection of bile is as safe as the collection of serum.
3. Typically, patients do not require sedation in order to collect bile.
4. In comparison to bile collection, pancreatic juice collection requires cannulation of the pancreatic duct.
5. All statements above are incorrect.

**Correct Response:** E

**Explanation of Correct/Incorrect Response** - As of today, no study demonstrated in a head-to-head comparison that bile has a higher quantity of diagnostic markers for PDAC in comparison to serum or urine. However, bile has the theoretical advantage given its proximity to the pancreas for which bile undergoes dedicated evaluation as a carrier of diagnostic markers for PDAC. Given the need for endoscopy or placement of a naso-duodenal tube, bile collection is more invasive in comparison with the acquisition of serum. In most studies, especially when endoscopy was performed, patients were sedated for bile collection. Collection of pancreatic juice has been also reported without cannulation of the pancreatic duct. In this context, pancreatic juice was collected in the duodenum following secretin stimulation during upper endoscopy utilizing a 7 French catheter.

**CASE 5**

**Introduction**: A multitude of genetic and epigenetic mutations and alterations has been described in the pathogenesis of pancreatic ductal adenocarcinoma (PDAC). Consequently, the detection of these mutations and alterations was studied for its yield of detecting PDAC and more importantly distinguishing PDAC from benign as well as pre-malignant conditions.

**Primary Question -** ***Which of the following alterations have been evaluated for their utility as a potential diagnostic marker for pancreatic ductal adenocarcinoma (PDAC)?***

1. Accumulation of DNA mutations
2. Micro-RNA level alterations
3. Increased amount of methylated DNA
4. Changes in mRNA levels
5. All above

**Correct Response:** E

**Explanation of Correct/Incorrect Response** - All above mentioned alternations have been evaluated for their utility in the early diagnosis of PDAC. The best studied DNA mutation in PDAC is K-ras mutation which has been also evaluated in setting of PDAC screening. However, this showed to be insufficient when used as a single marker. Micro-RNAs (miRNA) were recently evaluated for their potential use as diagnostic marker in PDAC with miR-21, miR-155, miR-205, miR-210, miR-492, and miR-1427 being found to be altered in PDAC. Large cohort studies are pending to document their value. Multiple recent studies found aberrantly methylated DNA in PDAC including in p16, SARP2 and mucin genes. Changes in mRNA expression level belong to the best studied potential diagnostic markers for PDAC, although none of them proved to have sufficient sensitivity and specificity to be utilized as an early diagnostic marker for PDAC.

**First Reply Question** - Alterations of the levels of micro-RNA or methylated DNA are very promising diagnostic markers of pancreatic ductal adenocarcinoma (PDAC). ***In this context, which of the following specimens have been studied to contain alterations in the levels of either micro-RNA or methylated DNA in order to be used a diagnostic markers for PDAC?***

1. Serum
2. Bile
3. Pancreatic juice
4. Feces
5. All of the specimens above

**Correct Response:** E

**Explanation of Correct/Incorrect Response** - Alterations in the levels of micro-RNA or methylated DNA for the purpose of PDAC diagnosis have been studied in serum, bile, pancreatic juice as well as feces.

**Second Reply Question** - Epigenetics is an umbrella term which has been implemented in the pathogenesis of PDAC. ***Which of the following alterations are implied in the term epigenetics?***

1. Accumulation of DNA mutations
2. Micro-RNA level alterations
3. Increased amount of methylated DNA
4. Changes in mRNA levels
5. Answers B and D are correct.

**Correct Response:** E

**Explanation of Correct/Incorrect Response** - Epigenetics encompasses the study of changes in organisms caused by modification of gene expression but not by changes in the [DNA](http://en.wikipedia.org/wiki/DNA) sequence. Micro-RNAs are non-coding RNAs which regulate gene expression. Methylation of DNA does not change the DNA sequence however it is implicated in the regulation of gene expression. DNA mutations imply changes in the DNA sequence which per definition is not epigenetics. Changes in the mRNA expression can be a result of epigenetic regulation, but per se do not fall under the term epigenetics.

**CASE 6**

**Introduction**: Carbohydrate antigen 19-9 (CA19-9) is a tumor marker which is frequently used as a serum marker in the context of pancreatic ductal adenocarcinoma (PDAC). In addition CA 19-9 can be elevated in other gastrointestinal malignancies. CA19-9 is an antibody against Sialyl-LewisA which is a tetrasaccharide [carbohydrate](http://en.wikipedia.org/wiki/Carbohydrate) on the surface of cells.

**Primary Question** - ***Which of the following statements about CA19-9 in the context of PDAC diagnosis is correct?***

1. Serum CA19-9 yields a sensitivity of 97% and specificity of 99% for PDAC in symptomatic individuals.
2. Obstructive jaundice decreases the level of CA19-9.
3. Combination of CA19-9, alpha-1 chymotrypsin (AACT), thrombospondin-1 (THBS1) and haptoglobin (HPT) have a better ability to distinguish PDAC from healthy controls than CA19-9 alone.
4. Combination of CA19-9, CA125 and LAMC2 did not show to improve the ability to distinguish PDAC from healthy controls compared with CA19-9 alone.

**Correct Response:** C

**Explanation of Correct/Incorrect Response** - A systematic review of literature concluded that CA19-9 has a median sensitivity of 79% and specificity of 82% for the diagnosis of PDAC in symptomatic individuals. Obstructive jaundice (due to any cause) is commonly associated with an increased serum level of CA19-9. A combination of alpha-1 chymotrypsin (AACT), thrombospondin-1 (THBS1) and haptoglobin (HPT) with CA19-9 had a better ability to distinguish PDAC from healthy controls (AUC 0.99 vs. 0.89 for CA19-9 alone). Combining CA19-9, CA125 and LAMC2 distinguished PDAC from benign conditions with an AUC of 0.87 vs AUC 0.8 for CA19-9 alone.

**First Reply Question** - CA19-9 has been reported as a marker for PDAC. ***Which of the following malignancies have been found to be associated with elevated serum CA19-9 levels as well?***

1. Colon cancer
2. Hepatocellular cancer
3. Esophageal adenocarcinoma
4. Cholangiocarcinoma
5. All malignancies above

**Correct Response:** E

**Explanation of Correct/Incorrect Response** - Elevated serum levels of CA19-9 have been reported in colon cancer, hepatocellular cancer, esophageal adenocarcinoma and cholangiocarcinoma however the sensitivity and specificity of elevated CA19-9 levels are low for these conditions which limit its use as a diagnostic marker.

**Second Reply Question** - CA19-9 is an antibody against Sialyl-LewisA which is a tetra-saccharide [carbohydrate](http://en.wikipedia.org/wiki/Carbohydrate) on the surface of cells. Approximately 7-10% of Caucasian population lacks the Lewis antigen. ***Which of the following statements of CA19-9 levels are true for patients who lack the Lewis antigen?***

1. CA19-9 cannot be detected or is detected only in very low levels, independent on PDAC stage.
2. CA19-9 can be detected in high levels already early in the disease course of PDAC.
3. CA19-9 can be detected in high levels only in the late course of PDAC.
4. CA19-9 is always detected in high levels independent of the presence or absence of PDAC.

**Correct Response:** A

**Explanation of Correct/Incorrect Response** - In patients who lack the Lewis antigen, CA19-9 can generally not been detected or is detected in only very low levels, independently on the stage of PDAC or any other gastrointestinal malignancy. This is due to the absence of fucosyltransferase in patients who lack the Lewis antigen which is needed for the CA19-9 production.

**CASE 7**

Introduction: The optimal diagnostic marker for malignant diseases is the one with highest sensitivity and specificity, which is especially true for pancreatic ductal adenocarcinoma (PDAC). Multiple markers for PDAC has been described including elevated or decreased protein or mRNA levels, as well as alterations in mi-RNA levels or methylated DNA in pancreatic tissue, serum, bile etc.

**Primary Question** - ***Which of the following markers reached most consistently highest sensitivity to diagnose pancreatic ductal adenocarcinoma (PDAC)?***

1. Pancreatic juice derived K-ras
2. Bile derived carcinoembryonic cell adhesion molecule 6 (CEAM6)
3. Bile derived neutrophil gelatinase-associated lipocalin (NGAL)
4. Combining biliary NGAL level and serum CA 19-9

**Correct Response:** C

**Explanation of Correct/Incorrect Response** - Pancreatic juice derived K-ras detected PDAC in only 61%. CEAM6 and NGAL levels in bile level yielded a sensitivity of 93% and 94%, respectively. Interestingly, the combination of bile derived NGAL and serum CA 19-9 yielded a sensitivity of only 85%.

**First Reply Question** - A diagnostic marker for PDAC needs to be preferably accessed easily and safely which is not optimal when using bile as the carrier of diagnostic markers. Multiple pilot studies analyzed urine, feces, and saliva as potential sources for diagnostic markers of PDAC. ***Which of the following statements about diagnostic markers of PDAC is correct?***

1. A panel of urine derived matrix metalloproteases (uMMPs) uMMP-2 and uTIMP-1A showed sensitivity below 50% to diagnose PDAC.
2. A panel of saliva derived K-ras, MBD3L2, ACRV1, DPM detected PDAC with a sensitivity and specificity of 90% and 95%, respectively.
3. Methylated BMP3 in feces reached a sensitivity of nearly 100% for PDAC.
4. A panel of perspiration derived p53, K-ras and uMMP-2 reached a sensitivity of 97-99% to diagnose PDAC.

**Correct Response:** B

**Explanation of Correct/Incorrect Response** - A panel of urine derived matrix metalloproteases (uMMPs) uMMP-2 and uTIMP-1A yielded a sensitivity and specificity of 91% and 75%, respectively. A panel of saliva derived K-ras, MBD3L2, ACRV1, DPM detected PDAC with a sensitivity and specificity of 90% and 95%, respectively, distinguishing patients with PDAC from patients with chronic pancreatitis and healthy controls. Methylated BMP3 in feces reached a sensitivity and specificity of 51% and 90%, respectively. As of now, perspiration was not evaluated as diagnostic marker for PDAC.

Second Reply Question - Assuming that a panel of diagnostic markers for PDAC yields higher sensitivity and specificity compared to a single diagnostic marker, one can also assume that a combination of specimens yields higher diagnostic quality for PDAC than one single specimen type alone. ***Which of the following combinations of specimens have been evaluated for their diagnostic yield of PDAC?***

1. Bile and saliva
2. Pancreatic juice and saliva
3. Feces and bile
4. Urine and saliva
5. None of the combinations above

**Correct Response:** E

**Explanation of Correct/Incorrect Response** - Despite the importance to identify a reliable diagnostic marker for PDAC, no authors analyzed a combination of diagnostic maker carriers for their combined yield to diagnose PDAC.

**CASE 8**

**Introduction**: Cytology is frequently used to detect neoplastic cells, especially from fine needle aspirates (FNA). Significant attention has been drawn to the detection of neoplastic cells in bile and pancreatic juice, which are potential carriers of shed pancreatic cells. This is especially important as pancreatic ductal adenocarcinoma (PDAC) is believed to derive from pancreatic ductal cells, which are in direct contact with pancreatic juice.

**Primary Question** - ***Which of the following statements about cytology of shed pancreatic ductal cells in pancreatic juice (PJ) is correct?***

1. Most studies analyzed the diagnostic yield of cytology from PJ which was collected in the duodenum.
2. Increasing the number of PJ collections does not increase the diagnostic yield of cytology.
3. Brush cytology increased the diagnostic yield of cytology.
4. Combined cytology and human telomerase reverse transcriptase (hTERT) immunohistochemistry meets criteria as a screening test for PDAC.

**Correct Response:** C

**Explanation of Correct/Incorrect Response** - Historically, cytology of shed pancreatic ductal cells in PJ was mainly obtained by endoscopic retrograde cholangio-pancreatography (ERCP). The sensitivity and specificity to detect PDAC increased to 80-100% and 83.3-100%, respectively, when PJ was collected 5 times (mean). The addition of brush cytology increased the sensitivity for PDAC diagnosis compared with PJ alone from 21.3% to 62.2%. Combined cytology and human telomerase reverse transcriptase hTERT immunohistochemistry yielded a sensitivity and specificity of 92% and 75%, respectively, which is inadequate as a screening test for PDAC.

**First Reply Question** - Shed pancreatic ductal cells can be detected in both PJ and bile. ***Which of the following statements about PJ is correct?***

1. The collection of PJ is more frequently associated with acute pancreatitis in comparison to the collection of bile.
2. The collection of bile is cheaper in comparison to the collection of PJ.
3. Medication used for sedation can affect the protein composition of PJ and bile.
4. None of the statements above is correct.

**Correct Response:** B

**Explanation of Correct/Incorrect Response** - In order to obtain PJ most authors utilize Secretin stimulation. Secretin is injected IV and stimulates the exocrine pancreas to secrete fluid with high concentration of bicarbonate. However, Secretin is expensive and increases the procedure cost. On the other hand, the collection of PJ is not associated with a higher risk of acute pancreatitis in comparison to the collection of bile which does not require Secretin stimulation. Medications for sedation have not been reported to be associated with alterations of protein in PJ or bile.

Second Reply Question - Cytological analysis of shed pancreatic ductal cells encompasses different cytological staining techniques. In addition polymerase chain reaction (PCR) and immunohistochemistry (IHC) allow to measure gene and protein expression on a cellular basis. ***Which of the following statements about staining techniques of shed pancreatic cells in PJ is correct?***

1. IHC cannot be used for cytological analysis.
2. PCR cannot be used for cytological analysis.
3. Papanicolaou staining is a common cytology staining method including for shed pancreatic cells in PJ.
4. Giemsa staining cannot be used for shed pancreatic cells in PJ.

**Correct Response:** C

**Explanation of Correct/Incorrect Response** - Both IHC and PCR can be used to detect alterations in mRNA and protein expression in cytological specimens. Papanicolaou as well as Giemsa staining have been reported most commonly as staining methods of choice for cytological specimens derived from PJ.

**CASE 9**

**Introduction**: Multiple carriers of diagnostic markers have been evaluated for their utility to diagnose pancreatic ductal adenocarcinoma (PDAC). An optimal carrier of a diagnostic marker is easily and safely accessible. In this context, fecal material has been evaluated which has already been shown to yield a sensitivity of 92% to detect early-stage colorectal cancer. Approximately 1000-1500 ml pancreatic juice is secreted daily into the duodenum, of which a part is excreted as fecal material.

**Primary Question** - Feces have been evaluated as a carrier for pancreatic biomarkers. ***Which of the following statements is correct?***

1. K-ras mutation was detected in all patients with pancreatic ductal adenocarcinoma (PDAC).
2. p53 mutation was detected in all patients with PDAC.
3. Adnab-9 monoclonal antibody yielded a higher sensitivity for PDAC than K-ras.
4. Micro-RNAs (mi-RNA) could not be measured in feces.

**Correct Response:** C

**Explanation of Correct/Incorrect Response** - K-ras and p53 mutation were detected in 66 of 75 (88%) and 23 of 62 (37.1%) patients with PDAC, respectively. Adnab-9 monoclonal antibody yielded a sensitivity and specificity of 67-80% and 87-91%, respectively. Elevated and lower level of multiple mi-RNA (miR-21, miR-143, miR-155, miR-196a, miR-210, miR-216a, miR-375) were detected in feces from patients with PDAC. Sensitivity and specificity calculations are currently pending.

First Reply Question - DNA mutations, methylated DNA markers and micro-RNA levels have been studied in feces for their utility as diagnostic markers for pancreatic ductal adenocarcinoma (PDAC). ***Which of the following statements of diagnostic markers in feces is correct?***

1. miR-143, miR-155, miR-196a, and miR-216a were detected in higher concentrations in patients with PDAC, in comparison with patients with chronic pancreatitis.
2. K-ras mutation was detected in 88% in feces of patients with PDAC.
3. Methylated BMP3 yielded a sensitivity of 90% for PDAC.
4. A panel of K-ras mutations, age and methylated BMP3 reached a sensitivity of up to 99% for PDAC.

**Correct Response:** B

**Explanation of Correct/Incorrect Response** - miR-143, miR-155, miR-196a, and miR-216a have been detected in feces from patients with PDAC in lower concentration in comparison with patients with chronic pancreatitis and healthy controls. K-ras mutation has been detected in up to 88% in feces of patients with PDAC. Methylated levels of EYA4, MDFI, UCHL1, and BMP3 were analyzed in feces of patients with PDAC. In this context, BMP3 yielded a sensitivity of 51% and specificity of 90% to diagnose PDAC. Moreover, a panel consisting of two K-ras mutations, age and methylated BMP3 level yielded sensitivities of 52-79%.

**Second Reply Question -** Feces consist of a significant amount of bacteria. ***Which of the following statements about the bacterial fecal flora is correct?***

1. Bacterial DNA can be used as diagnostic marker for PDAC.
2. The bacterial flora is inactive and does not alter human derived proteins in the feces.
3. Usage of antibiotics alters the bacterial flora which in response affects the yield of diagnostic fecal tests for PDAC.
4. In order to limit any molecular biologic alterations of feces, most authors immediately froze the feces following collection.

**Correct Response:** D

**Explanation of Correct/Incorrect Response** - Human DNA but not bacterial DNA has been assessed for its diagnostic yield to detect PDAC in feces. The bacterial flora is active in feces and can result in alterations of protein and DNA, for which feces is frozen immediately following its collection. Although it is known that antibiotics alter bacterial gut flora, the impact of antibiotics on the yield of feces as carrier for diagnostic marker of PDAC is unknown.

**CASE 10**

**Introduction**: New-onset of diabetes mellitus at age 50 years or older has been reported to be associated with a new diagnosis of pancreatic ductal adenocarcinoma (PDAC) within 3 years.

**Primary Question** - ***Which of the following statements about new-onset diabetes mellitus and PDAC is correct?***

1. The ratio of observed-to-expected PDAC incidence is 7.9 in patients with new-onset diabetes mellitus at age 50 years or older.
2. Diabetes mellitus has a low prevalence in PDAC.
3. Only 20% of patients with stage I or II PDAC have diabetes mellitus.
4. An abnormal glucose tolerance was detected in less than 50% of patients with early stage PDAC.

**Correct Response:** A

**Explanation of Correct/Incorrect Response** - A ratio of observed-to-expected PDAC incidence of 7.9 (95% CI: 4.7-12.5) has been reported for patients with new-onset diabetes mellitus at age 50 years or older. Diabetes mellitus is prevalent in patients with PDAC. In fact, up to 50% of patients with stage I or II PDAC were reported to have diabetes mellitus. Moreover, an abnormal glucose tolerance was found in up to 61% of patients with early stage PDAC.

**First Reply Question** - In the absence of a highly reliable diagnostic marker for pancreatic ductal adenocarcinoma (PDAC), endoscopic ultrasound (EUS) remains a highly sensitive tool to diagnose PDAC. ***Which of the following statements about EUS is correct?***

1. EUS-guided fine needle aspiration (FNA) allows tissue sampling of suspected pancreatic lesions with a high diagnostic accuracy.
2. EUS has a sensitivity of up to 100% and specific of above 95% for pancreatic lesions under 2cm.
3. EUS is operator dependent and highest sensitivity and specificity are mainly reached in tertiary referral centers.
4. All statements above are correct.

**Correct Response:** D

**Explanation of Correct/Incorrect Response** - EUS-guided FNA of pancreatic lesions yields a diagnostic accuracy of up to 92%. Pancreatic lesions of less than 2 cm can be diagnosed with EUS yielding a sensitivity and specificity of up to 100% and 95% respectively. A disadvantage of EUS is its significant operator dependence.

**Second Reply Question** - Computer tomography (CT) and magnetic resonance imaging (MRI) are frequently used cross-sectional imaging studies in the work up on pancreatic tumors. ***Which of the following statements about CT and MRI is correct?***

1. In order to obtain optimal CT imaging quality non-contrast, arterial, pancreatic parenchymal and portal venous phases are recorded.
2. The sensitivity of CT imaging ranges from 75%-100% for the diagnosis of PDAC.
3. MRI was shown to yield a sensitivity and specificity for PDAC of 100% and 88% respectively.
4. Combined MRI and CT offer highest sensitivity and specificity to diagnose PDAC and can guide resectability of PDAC in most cases.
5. All statements above are correct

**Correct Response:** E

**Explanation of Correct/Incorrect Response** - CT reaches highest sensitivity and specificity for PDAC when non-contrast, arterial, pancreatic parenchymal, and portal venous phase contrast are used. In this context, sensitivities of 75%-100% have been reported. However, for pancreatic lesions less than 2 cm, the sensitivity drops to 68%-77%. MRI has been reported to achieve sensitivity, specificity and accuracy of 100%, 88%, and 98% respectively to diagnose PDAC. A combination of CT with MRI achieves highest sensitivity and specificity and guides resectability of PDAC in most of the cases.