Pancreatic cyst fluid VEGF-A and CEA: a highly accurate test for the diagnosis of serous cystic neoplasm

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ABSTRACT

BACKGROUND: Accurate differentiation of pancreatic cystic lesions is important for pancreatic cancer early detection and prevention as well as avoidance of unnecessary surgical intervention. Serous cystic neoplasms (SCN) have no malignant potential, but may mimic premalignant mucinous cystic lesions: mucinous cystic neoplasm (MCN) and intraductal papillary mucinous neoplasm (IPMN). We recently identified vascular endothelial growth factor (VEGF)-A as a novel pancreatic fluid biomarker for SCN. We hypothesize that combining cyst fluid carcinoembryonic antigen (CEA) with VEGF-A will improve the diagnostic accuracy of VEGF-A.

METHODS: Pancreatic cyst/duct fluid was collected from consenting patients undergoing surgical cyst resection with corresponding pathologic diagnoses. Pancreatic fluid VEGF-A and CEA levels were detected by ELISA.

RESULTS: One hundred forty-nine patients with pancreatic cystic lesions met inclusion criteria. Pathologic diagnoses included pseudocyst (n=14), SCN (n=26), MCN (n=40), low/moderate grade IPMN (n=34), high grade IPMN (n=20), invasive IPMN (n=10) and solid pseudopapillary neoplasm (n=5). VEGF-A was significantly elevated in SCN cyst fluid compared to all other diagnoses ($p<0.001$). With a threshold of >5,000 pg/ml, VEGF-A alone has 100% sensitivity and 83.7% specificity to distinguish SCN from other cystic lesions. With a threshold of ≤10ng/ml, CEA alone identifies SCN with 95.5% sensitivity and 81.5% specificity. Sensitivity and specificity of the VEGF-A/CEA combination are 95.5% and 100% respectively. The c-statistic increased from 0.98 to 0.99 when CEA was added to VEGF-A alone in the ROC analysis.

CONCLUSIONS: Although VEGF-A alone is a highly accurate test for SCN, the combination of VEGF-A with CEA approaches the gold-standard of pathologic diagnosis, thus importantly avoiding false positives. Patients with a positive test indicating benign SCN can be spared a high risk surgical pancreatic resection.

Key Words: pancreatic cyst fluid, pancreatic cancer prevention, VEGF, CEA, biomarker

Abbreviations

IPMN: Intraductal papillary mucinous neoplasm

MCN: Mucinous cystic neoplasm

SCN: Serous cystic neoplasm

VEGF-A: Vascular endothelial growth factor A

CEA: Carcinoembryonic antigen

SPN: Solid pseudopapillary neoplasm

ROC: Receiver operator curve

VHL: Von Hippel Lindau

PNET: Pancreatic neuroendocrine tumor

Introduction

Pancreatic cysts are increasingly diagnosed due to advancements in radiographic imaging allowing for higher diagnostic sensitivity and higher test volume. 2.6% of patients undergoing abdominal imaging will have incidentally diagnosed cystic lesions of the pancreas.[1] Pancreatic cysts can be differentiated based upon malignant potential. While mucinous cysts such as intraductal papillary mucinous neoplasm (IPMN) and mucinous cystic neoplasm (MCN) harbor malignant potential, serous cystic neoplasm (SCN) and pseudocysts virtually never progress to invasive carcinoma.[2] Consequently, management ranges from observation to aggressive pancreatic resection. Pancreatic surgery is associated with significant mortality and morbidity in the contemporary literature.[3] Diagnostic accuracy is therefore of paramount importance.

SCN is a benign lesion that represents 16% of resected pancreatic cysts and less than 1% of all pancreatic lesions.[4, 5] Only three serous cystadenocarcinomas were found within the largest series of SCN patients to date (n = 2,622), demonstrating the extremely low rate of malignant progression.[6] Furthermore, SCN-specific mortality approaches nil at 0.1%.[6] For these reasons, in addition to the low rate of symptoms, the majority of SCN can be managed non-operatively.[6-8] Despite this, the retrospective multinational series by Jais *et al*. found only 39% of SCN patients avoid surgical resection.[6] Major indications for surgery included presence of symptoms, cyst size or growth rate, and lack of pre-operative diagnosis (60%).[6] While exact thresholds for cyst size or growth rate are controversial, most agree that diagnostic uncertainty is unacceptable.[2, 7, 9, 10]

Existing diagnostic modalities (CT, MRI, endoscopic ultrasound, and fine-needle aspiration with cyst fluid analysis) are imperfect and leave many cysts undiagnosed or incorrectly diagnosed.[8, 11] Biomarker research is being pursued in hopes of improving the diagnostic accuracy of pancreatic cysts. However, the majority of emerging biomarker

investigations focus on mucinous cysts with malignant potential. It is equally important to develop diagnostic tools for benign lesions in order to avoid unnecessary morbidity and mortality of surgery. We previously identified vascular endothelial growth factor (VEGF)-A as a valuable pancreatic fluid biomarker for differentiating benign SCN from all other pancreatic cysts.[12] In the present study, we aim to further validate VEGF-A in a larger cohort and establish an improved VEGF-A/CEA diagnostic test for SCN. This test will facilitate avoidance of unnecessary pancreatic resection for SCN.

Methods

Patient Samples

Samples were either obtained from the Indiana University Pancreatic Tissue-Fluid Bank following approval by Indiana University Institutional Review Board or kindly provided by Johns Hopkins University (n=12, Dr. Anne Marie Lennon). Patients signed informed consent for collection of pancreatic fluid at the time of routine endoscopy (EUS or ERCP) and/or operation (OR). We have previously confirmed that the method of fluid procurement does not affect VEGF-A measurement.[12] Fluid specimens were placed immediately on ice after procurement and aliquoted for storage at -80 degrees. In total, samples from 149 patients collected between 2003 and 2015, including serous cystic neoplasm (SCN, n=26), pseudocyst (n=14), mucinous cystic neoplasm (MCN, n=40), intraductal papillary mucinous neoplasm (IPMN) low/moderate grade (n=34) or high-grade (n=20) or invasive (n=10), and solid pseudopapillary neoplasm (SPN, n=5), were pathologically confirmed following surgical resection. IPMN dysplasia was determined according to the World Health Organization (WHO) criteria. VEGF-A levels were previously reported for 87 of these patients.

VEGF-A and CEA measurement

Pancreatic fluid samples (1-50ul) were analyzed for VEGF-A by Quantikine ELISA (R&D Systems, Minneapolis, MN) according to the manufacturer's protocol. CEA was determined by Beckman Coulter DxI 800 analyzer or in cases of low fluid volume, by ELISA (Sigma-Aldrich, St. Louis, MO). CEA values obtained by ELISA were converted to the Beckman automated analyzer scale using linear regression.

Molecular Genetic Analysis of Von Hippel Lindau (VHL)

DNA was extracted from 3 SCN samples and their matched adjacent normal tissue using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA). A custom multiplex PCR reaction capturing all coding exons and significant portions of introns and UTRs was designed using the Ion Torrent Ampliseq Designer (Life Technologies, South San Francisco, CA). Barcoded libraries were prepared using the Ion Ampliseq Kit for Chef DL8 (A29024, ThermoFisher Scientific), followed by sequencing using Ion Hi-Q Sequencing Technology. Sequencing data was mapped to the human genome (hg19) and analyzed for somatic mutations using the Ion Torrent Suite v5.0.3 and Ion Reporter v5.2.

Statistical Analysis

Descriptive statistics including mean, median, and range were calculated for each cyst type. VEGF-A and CEA were analyzed for statistical significance using Kruskal-Wallis for all groups and Wilcoxon Rank Sum test for SCN versus non-SCN comparisons as our data was not normally distributed. P-values of <0.05 were considered statistically significant. Receiver

operator characteristic (ROC) curve analysis was further used to evaluate the diagnostic accuracy of each biomarker test. Cut-points for VEGF-A and CEA were then chosen using Youden's index for the combined VEGF-A and CEA tests. In this case, the test was classified as positive for SCN if the thresholds for both VEGF-A and CEA were met, and negative if either of the thresholds were not met. We then modified the thresholds after examining the data to minimize false positives for SCN. These thresholds were then used to calculate the sensitivity, specificity, and likelihood ratios of VEGF-A and CEA alone and in combination.

Results

VEGF-A levels in pancreatic fluid

Patient pancreatic fluid samples (n=149) were collected from cystic lesions of the pancreas and correlated with surgical pathologic diagnosis. These included 26 serous cystic neoplasms (SCN), 14 pseudocysts, 40 mucinous cystic neoplasms (MCN), 5 solid pseudopapillary neoplasms (SPN), and 64 intraductal papillary mucinous neoplasms (IPMN). IPMN was further divided based on grade of dysplasia according to WHO criteria: 34 (22.8%) low to moderate grade dysplasia, 20 (13.4%) high grade dysplasia, and 10 (6.7%) invasive IPMN.

Pancreatic fluid samples were analyzed for VEGF-A by ELISA (**Figure 1**). Median VEGF-A concentrations and ranges were as follows for SCN: 35,598 (5,318 – 290,960 pg/mL), pseudocyst: 1283 (0 – 16,204 pg/mL), MCN: 2,074 (6 – 15,233 pg/mL), low/moderate grade IPMN: 2,285 (753 – 9,483 pg/mL), high grade IPMN: 2,951 (30 – 16,324 pg/mL), invasive IPMN: 4,341 (1,184 – 19,555 pg/mL), and SPN: 295 (151 – 584 pg/mL). VEGF-A level in SCN cyst fluid was significantly higher than VEGF-A in non-SCN pancreatic fluid (35,598 vs 2,149

pg/mL; $p<0.0001$), and likewise higher compared to each specific cyst group ($p<0.0001$), confirming our previously published findings. Additionally, Von Hippel-Lindau (VHL) status was determined in three SCN patients with elevated cyst fluid VEGF levels, for which cyst tissue and matching normal adjacent tissue were available. Genetic alterations that inactivate the tumor suppressor VHL may result in upregulated VEGF expression. Indeed, VHL gene alterations were detected in the three SCNs – one with a point mutation/splice site alteration (chr3:10188197G>A) and two with deletions/frameshifts (chr3:10183820 and 10183845), confirming our previous report.

VEGF-A concentration of greater than 5,000 pg/mL was chosen as the threshold for a positive diagnostic test to detect SCN. This threshold was chosen to maximize sensitivity and specificity of the VEGF-A/CEA combination test. Using this criterion for VEGF-A, all SCNs were correctly classified with a sensitivity of 100%. Twenty non-SCN pancreatic cysts (1 pseudocyst, 7 MCN, 5 low/moderate grade IPMN, 4 high grade IPMN, and 3 invasive IPMN) were incorrectly categorized as SCN by this VEGF-A threshold, demonstrating a specificity of 83.7%. The highest non-SCN VEGF-A was an invasive IPMN at 19,555 pg/mL. The positive and negative likelihood ratios were 6.2 and 0; a negative VEGF-A test was 100% accurate. ROC curve analysis revealed AUC of 0.983 (**Figure 2**). With an alternate VEGF-A threshold of 7,858 pg/mL chosen to maximize sensitivity/specificity of the single test, sensitivity and specificity were 96.2% and 91.9%. Thus, VEGF-A alone is an accurate SCN biomarker; however, a clinical diagnostic test for benign SCN must achieve 100% specificity to avoid misclassification of potentially malignant pancreatic cysts, or false positives.

CEA levels in pancreatic fluid

Of the 149 pancreatic fluids tested for VEGF-A, 130 had sufficient volume available for determination of CEA concentration (**Figure 3).** Median CEA levels and ranges were as follows for SCN: 0.5 (0.09 – 65.4 ng/mL), pseudocyst: 20.4 (0.5 – 310 ng/mL), MCN: 1,000 (0.1 – 25,194 ng/mL), low/moderate grade IPMN: 244 (0.8 – 511,473 ng/mL), high grade IPMN: 260.5 (2.9 – 7,550 ng/mL), invasive IPMN: 1,878 (3.2 – 38,730 ng/mL), and SPN: 0.1 (0.1 – 0.1 ng/mL). CEA level in SCN cyst fluid was significantly lower than CEA in non-SCN pancreatic fluid ($p<0.0001$), and likewise lower compared to each specific cyst group ($p<0.0001$). A pancreatic fluid CEA threshold of less than 10 ng/ml was considered a positive diagnostic test for SCN. Using this criterion, one SCN was misclassified with a CEA of 65.4 ng/mL and twenty non-SCN cysts were incorrectly categorized as SCNs. This demonstrates that pancreatic fluid CEA has a sensitivity and specificity of 95.5% and 81.5%, respectively, to detect SCN. Positive and negative likelihood ratios were 5.2 and 0.06. ROC curve analysis resulted in AUC of 0.945 for CEA alone (**Figure 2**).

VEGF-A/CEA combination test for SCN

We sought to determine whether combining VEGF-A with CEA could improve the diagnostic accuracy of VEGF-A as a biomarker of SCN. Both VEGF-A greater than 5,000 pg/mL and CEA less than 10 ng/mL was considered a positive combination test for SCN (**Figure 4).** Of the cysts analyzed for CEA (SCN: n=22; non-SCN: n=108), none of the other pancreatic cysts (non-SCN) cysts had a positive combination test, and only one SCN was misclassified with a negative test. Sensitivity and specificity of VEGF-A/CEA were 95.5% and 100%, respectively, with an infinite positive likelihood ratio. The single SCN outlier had VEGF-A of 104,275 pg/mL

and CEA of 65.4 ng/mL. AUC for the VEGF-A/CEA combination was 0.993 on ROC analysis, an improvement over each biomarker alone (**Figure 2**).

Diagnostic utility of VEGF-A/CEA test

To evaluate the potential diagnostic utility of the VEGF-A/CEA test for SCN, we reviewed the indications for surgery of the SCN cohort (**Figure 5**). Surgical indications included unclear diagnosis, presence of symptoms, cyst size, cyst growth, young patient age, and patient preference. All patients underwent at least two diagnostic modalities (CT and EUS-guided FNA), with twelve patients (50%) undergoing three or greater (including MRI/MRCP, ERCP, or PET). Despite the abundance of diagnostic methodologies, preoperative diagnoses in patients with SCN were often unclear or unknown (n=15, 63%). Patients with multiple diagnoses, most commonly MCN or IPMN, were also categorized as "unclear diagnoses." Unclear diagnosis was the most common indication for surgical resection. 25% (n=6) of patients underwent operative management for the sole indication of unclear or unknown diagnosis. Importantly, all of these patients would have been identified as SCN with a positive VEGF-A/CEA diagnostic test and therefore avoided major surgery.

Discussion

Optimal clinical management of pancreatic cysts with variable malignant potential relies upon accurate differential diagnosis to stratify patient risk. Currently, SCN diagnosis is most commonly made by radiographic appearance, a highly variable, imprecise modality for pancreatic cysts with overlapping features.[7] SCN are described as microcystic, macrocystic, mixed, or solid type by Kimura et al.[8] Microcystic SCN are composed of multiple, small (<1 cm)

cysts lined by glycogen rich cuboidal epithelium, similar in appearance to a honeycomb.[5, 7, 13] The classically described central scar is present in only 15-20%. Microcystic SCN account for the majority of SCN and are the most diagnostically straightforward. Alternatively, macrocystic SCN are composed of one or several larger cysts (>1 cm), a pattern shared by some IPMN and MCN.[7, 8] Multiple studies have found low sensitivity and specificity of radiographic imaging for SCN diagnosis, supporting this diagnostic challenge. In a study by Bassi *et al*., SCN was correctly diagnosed by CT and MRI 54% and 74% of the time, and incorrectly diagnosed 34% and 26% of the time.[11] Kimura *et al*. later reported 63% of resected SCN were not pre-operatively diagnosed as such.[8] Alternative diagnoses included IPMN (most commonly), cystic pancreatic neuroendocrine tumors (PNET), and MCN. In support, we report here that 63% of SCN were preoperatively misdiagnosed/not diagnosed.

Endoscopic ultrasound (EUS) with or without fine-needle aspiration (FNA) is the next step in pancreatic cyst diagnosis, but still often fails to accurately diagnose the cyst.[11] EUS alone has a sensitivity and specificity similar to CT with many of the same diagnostic difficulties and the added drawback of being an invasive procedure.[11] Although all SCN patients included in this series underwent at least CT and EUS imaging, a high rate of uncertain diagnoses were still observed. No patients with SCN had 100% certain diagnoses preoperatively, as none underwent the gold-standard core biopsy with pathologic evaluation. Core biopsy is not recommended for the routine diagnosis and management of pancreatic cysts. FNA may be added for additional diagnostic options but also has significant shortcomings. Cytologic analysis is often not possible due to pauci- or acellular aspirate accounting for the low sensitivity of FNA.[14-16] Molecular genetics may offer further diagnostic clues. Although not universal, K-RAS and GNAS mutations are common in mucinous pancreatic cysts, unlike non-mucinous SCN.[17, 18] Von Hippel

Lindau (VHL) tumor suppressor gene mutations are also associated with SCN development and may be present in hereditary forms of SCN.[19, 20] However, sporadic SCN are vastly more prevalent, and VHL mutations may be associated with SCN as well as PNET.

Cyst fluid carcinoembryonic antigen (CEA) has repeatedly proven useful in differentiating mucinous from non-mucinous pancreatic cysts. CEA level cutoffs vary widely, resulting in equally diverse reports of diagnostic accuracy. 192 ng/mL is a commonly accepted CEA threshold that was found to yield a diagnostic accuracy of 79%, better than all other tests evaluated (EUS, cytology, CA 125, and CA 19-9).[21] Similar studies report accuracy ranging from 70-86%, sensitivity of 61-89%, and specificity of 63-77%, using CEA thresholds of 5-800 ng/mL.[22-26] CEA has also been used as part of combination tests to achieve further diagnostic accuracy; however, none of these specifically identify SCN.[25, 27, 28]

A diagnostic test for SCN must be near perfect to avoid the consequences of misdiagnosis (i.e. unnecessary surgery of benign lesions or surveillance of potentially malignant lesions). We previously identified VEGF-A as a highly accurate pancreatic fluid biomarker for SCN.[12] These findings were validated in the present study with a larger cohort from two institutions. Additionally, we demonstrated that combining cyst fluid CEA with VEGF-A achieves 95.5% sensitivity and 100% specificity as a diagnostic test for SCN. To our knowledge, the VEGF-A/CEA combination is the only SCN-specific, highly accurate test reported in the literature to date. Although two small studies report alpha-inhibin as a highly sensitive immunohistochemical tissue and cyst fluid marker for SCN, control non-SCN cysts were not included in either of these studies to address specificity.[29, 30]

Interestingly, four cystic pancreatic neuroendocrine tumors (PNET) were also classified as SCN based upon a positive VEGF-A/CEA combination test (unpublished observations).

Although cystic PNET may be in the differential diagnosis for SCN, these lesions are extremely rare. PNET account for 1-2% of all pancreatic lesions with only 10-15% having cystic components.[31, 32] Furthermore, cystic PNET are adequately identified with FNA and cytologic analysis. Multiple studies evaluate the accuracy of FNA for all PNET (very few or no cystic PNET) and report sensitivities of 83-87% and accuracy of up to 93%.[33-35] Baker *et al*. however, reported 100% positive predictive value of EUS-guided FNA for cystic PNET.[36] In the present study, of those that underwent pre-operative EUS-guided FNA (n=3), all were cytologically diagnosed as cystic PNET, confirming that cystic PNET are accurately diagnosed on cytologic examination. Taken together, diagnostic cytology must be performed prior to VEGF-A/CEA combination test in order to exclude possible PNETs.

Several limitations were encountered in this study. Although 149 total pancreatic cyst fluid samples were included in this study, only 26 SCN were included. Of those, 22 were amenable to CEA measurement. Six of the 26 SCN patients had small, asymptomatic lesions of uncertain preoperative diagnoses that would have avoided surgery with the VEGF-A/CEA combination test. Despite the modest number that would definitely avoid surgery with VEGF-A/CEA testing, all would ultimately benefit from increased confidence in diagnosis. Many additional patients would avoid surgery as other surgical indications such as symptoms, patient preference, and cyst size would play less of a role in surgical decision making if SCN diagnosis was certain. Existing single institution series of surgical SCN have comparable numbers due to the relative rarity and typical non-operative management of these lesions. Larger, multi-institutional studies are needed to confirm findings published here. All patients in this series underwent surgical pancreatic resection. As many patients never receive operative care, data collected from surgical patients may not be generalizable to all SCN patients. Confounding

variables could account for the elevated VEGF-A in surgical SCN. The surgical indication might also contribute to VEGF-A levels (i.e. symptoms, size, growth). Our previous study examined cyst size as a potential confounder but confirmed no association between size and VEGF-A.[12] An additional study limitation was the two methods used to obtain cyst fluid CEA levels, necessitated by the small fluid volumes available.

Preoperative diagnosis of SCN is challenging, as supported by our findings of 63% misdiagnosed/not diagnosed, and contributes to unnecessary pancreatic surgical resection. 25% of patients undergoing operative intervention for the sole indication of lacking a clear pre-operative diagnosis would have avoided surgery with the use of VEGF-A/CEA combination test. Additional patients with unclear diagnosis as part of their surgical indication might have been spared surgery with increased confidence in the benign diagnosis.

Conclusions

Due to the increasing number of patients with incidentally detected pancreatic cysts (2.6% of those undergoing abdominal imaging)[1], surgeon awareness of available as well as promising diagnostic tests is important in order to effectively manage pancreatic cyst patients. In high risk patients with pancreatic cysts, there is an urgent need for an accurate and reliable diagnostic test to stratify and improve clinical care. Patients with SCN are commonly misdiagnosed, thus undergoing unnecessary operative intervention. Although VEGF-A alone is a highly accurate test for SCN, we have demonstrated that the combination of VEGF-A with CEA approaches the gold-standard of pathologic diagnosis (sensitivity: 95.5%, specificity: 100%). Patients with a positive test indicating benign SCN can be spared a high-risk surgical pancreatic resection.

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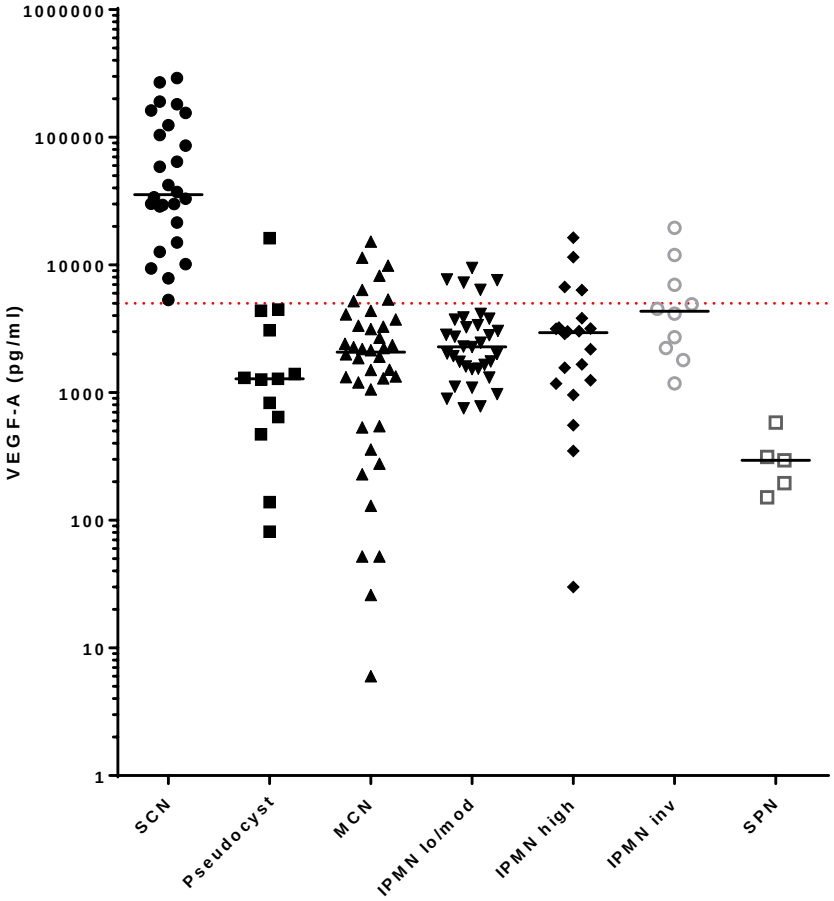
Figure 1: Pancreatic Cyst Fluid VEGF-A: VEGF-A levels in pancreatic cyst fluid as determined by ELISA. Each VEGF-A value is shown on a log linear scale within the column consistent with pathologic diagnosis: SCN, pseudocyst, MCN, IPMN, or SPN. Horizontal black lines represent median values for each cyst type. The red dotted line is the cut-off (5,000 pg/mL) giving 100% sensitivity and 83.7% specificity.

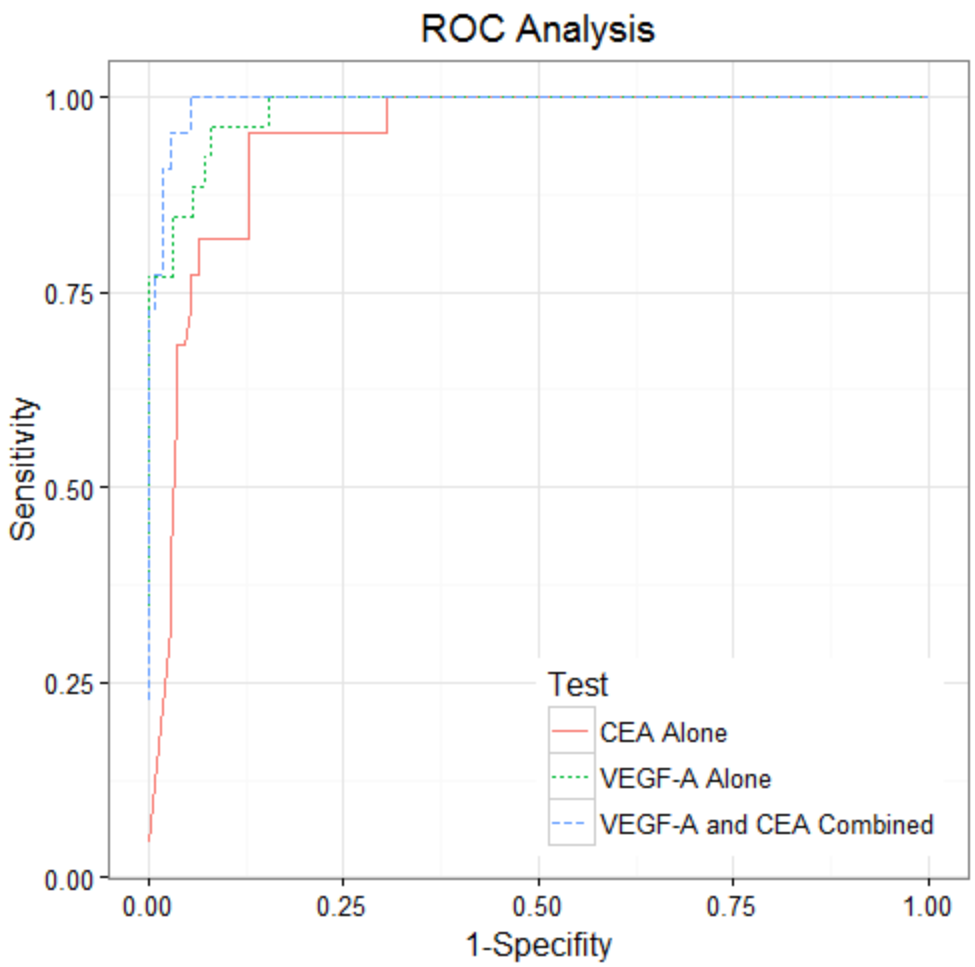
Figure 2: ROC Curves: ROC Curves for each biomarker alone and in combination. The AUC for CEA alone is 0.945, for VEGF-A alone is 0.983, and for VEGF-A/CEA Combination test is 0.993.

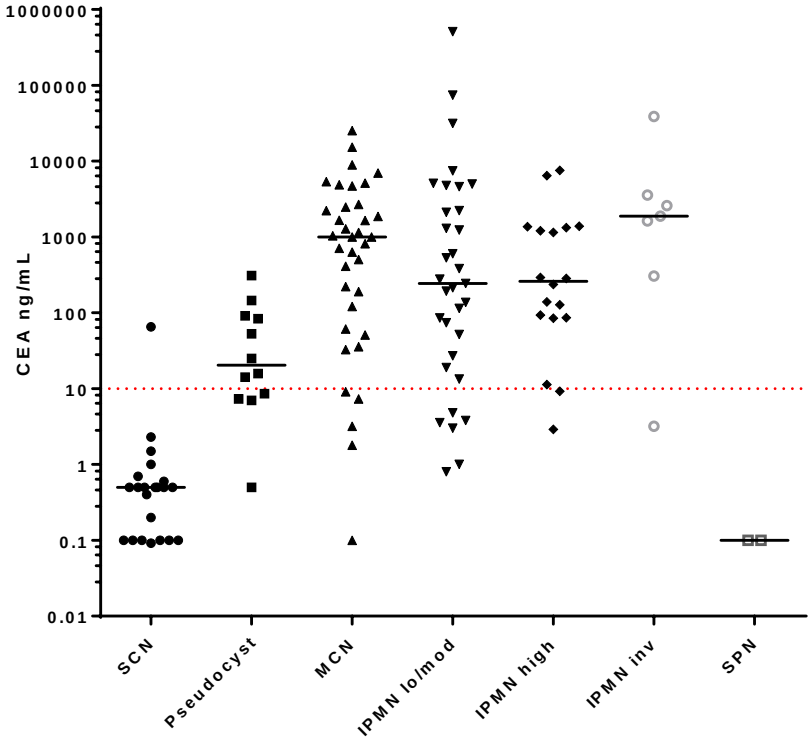
Figure 3: Cyst Fluid CEA: CEA levels in pancreatic cyst fluid. Each CEA value is shown on a log linear scale within the column consistent with pathologic diagnosis: SCN, pseudocyst, MCN, IPMN, or SPN. Horizontal black lines represent median values for each cyst type. The red dotted line is the cut-off (10 ng/mL) giving 95.5% sensitivity and 81.5% specificity.

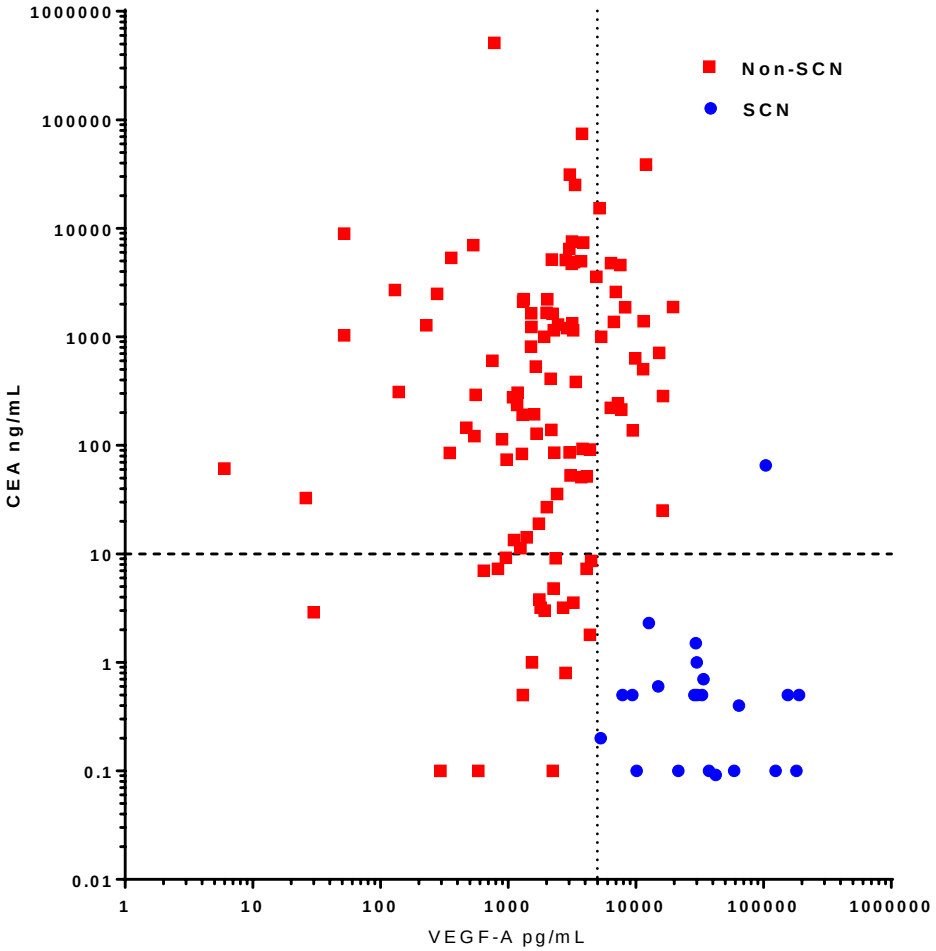
Figure 4: CEA vs VEGF-A for SCN vs non-SCN: CEA vs VEGF-A for SCN vs non-SCN. Each point displays the cyst fluid CEA and the VEGF-A level for a single pancreatic cyst on a log linear scale. Blue points are SCN, and red points are all other cysts. The horizontal dashed line is the CEA cut-off of 10 ng/mL; all cysts plotted below this line had a positive CEA test result. The vertical dotted line is the VEGF-A cut-off of 5,000 pg/mL; all cysts plotted to the right of this line had a positive VEGF-A result. The right lower quadrant formed by the two threshold lines contains cysts with a positive VEGF-A/CEA combination test. These are exclusively SCN, demonstrating 100% specificity and infinite positive likelihood ratio of the combination test.

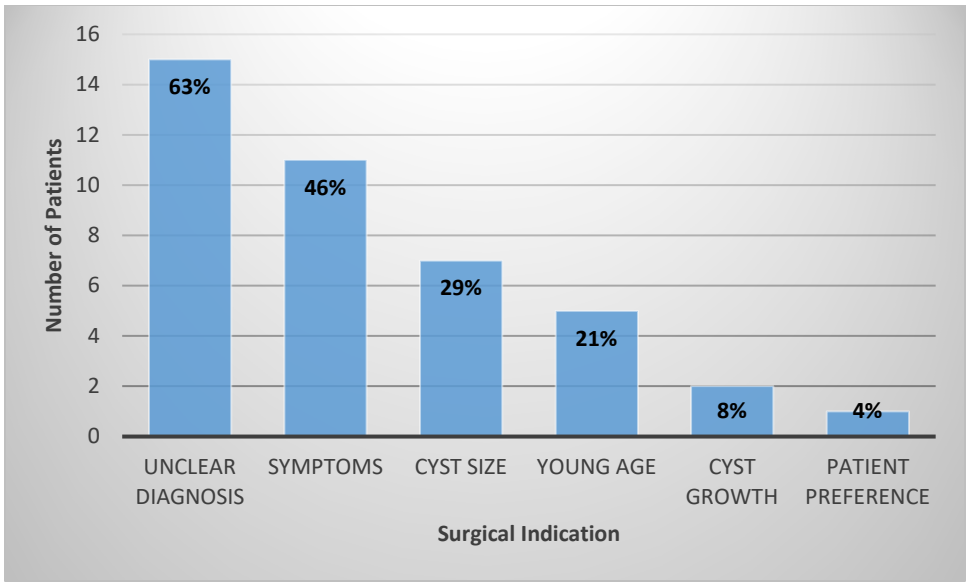
Figure 5: Surgical indication of patients with SCN: Indication for surgery in SCN patients. The Y axis shows the number of patients with each specific surgical indication. The frequency of each indication within all SCN patients is displayed at the top of each column. Many patients had more than one indication for surgery; each indication is represented in this graph.


1000000
100000
10000
1000
100
10
1
VEGF-A (pg/ml)
SCN
Pseudocyst
MCN
IPMN lo/mod
IPMN high
IPMN inv
SPN


ROC Analysis
Sensitivity
1-Specifity
0.00
0.25
0.50
0.75
1.00
Test
CEA Alone
VEGF-A Alone
VEGF-A and CEA Combined


1000000
100000
10000
1000
100
10
1
0.1
0.01
CEA ng/mL
SCN
Pseudocyst
MCN
IPMN lo/mod
IPMN high
IPMN inv
SPN


Non-SCN
SCN
CEA ng/mL
VEGF-A pg/mL
1000000
100000
10000
1000
100
10
1
0.1
0.01
1
10
100
1000
10000
100000
1000000


16
14
12
10
8
6
4
2
0
Number of Patients
63%
46%
29%
21%
8%
4%
UNCLEAR DIAGNOSIS
SYMPTOMS
CYST SIZE
YOUNG AGE
CYST GROWTH
PATIENT PREFERENCE
Surgical Indication