Vascular Endothelial Growth Factor, a Novel and Highly Accurate Pancreatic Fluid Biomarker for Serous Pancreatic Cysts

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BACKGROUND: Mucinous pancreatic cysts (intraductal papillary mucinous neoplasm and mucinous cystic neoplasm) have the potential to progress to invasive pancreatic adenocarcinoma, presenting an opportunity for early detection, prevention, and cure. Serous cystic neoplasms (SCN) have no malignant potential, but can mimic mucinous pancreatic cysts on imaging. Therefore, identification of biomarkers that can distinguish between cystic lesions is critically important. We hypothesize that vascular endothelial growth factor (VEGF)-A levels in pancreatic fluid correlate with pathologic diagnosis.

STUDY DESIGN: Pancreatic cyst/duct fluid samples were prospectively collected from patients undergoing pancreatic resection and correlated with surgical pathology. VEGF levels were detected by ELISA. VEGF-A and VEGF receptor 2 expression in pancreatic tissue was localized by immunohistochemistry. Genetic alterations of the von Hippel-Lindau gene were determined by targeted next-generation sequencing.

RESULTS: Eighty-seven patients met inclusion criteria for enrollment. Final pathologic diagnoses included pseudocyst (n = 9), SCN (n = 17), mucinous cystic neoplasm (n = 24), low/moderate grade intraductal papillary mucinous neoplasm (n = 16), high-grade/invasive intraductal papillary mucinous neoplasm (n = 10), and pancreatic ductal adenocarcinoma (n = 11). VEGF-A was significantly upregulated in SCN cyst fluid compared with all other diagnoses ($p < 0.0001$). With a cut-off of 8,500 pg/mL, VEGF-A has 100% sensitivity and 97% specificity as an SCN biomarker. VEGF-A and VEGF receptor 2 are overexpressed in SCN cyst tissue. VEGF-C was also significantly elevated in SCN cyst fluid ($p < 0.0001$). With a cut-off set at 200 pg/mL, VEGF-C identifies SCN with 100% sensitivity and 90% specificity. The presence of a von Hippel-Lindau mutation in SCN cyst tissue correlates with elevated cyst fluid VEGF levels.

CONCLUSIONS: This is the first report of a cyst fluid protein biomarker that can positively identify SCN. The ability to distinguish SCN from premalignant/malignant pancreatic cysts can spare the cost and risk of surveillance and surgical intervention in select patients. (J Am Coll Surg 2014; 218:608–619. © 2014 by the American College of Surgeons)

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Pancreatic adenocarcinoma (pancreatic cancer) is the fourth leading cause of cancer-related deaths, with mortality nearly equal to incidence. Approximately 5% of patients survive 5 years from the time of diagnosis, and pancreatic cancer is anticipated to account for nearly 40,000 deaths in the United States this year alone.[1] Surgery performed early in the course of disease can rarely cure patients with pancreatic cancer; other treatment modalities have been largely ineffective due to innate or acquired cancer cell resistance. Pancreatic cancer represents one of the deadliest cancers. Despite these dismal statistics, a substantial improvement in clinical outcomes is possible by identification and screening of patients at risk for pancreatic cancer developing.

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Abbreviations and Acronyms

GNAS = guanine nucleotide binding protein α stimulating activity polypeptide 1
IPMN = intraductal papillary mucinous neoplasm
MCN = mucinous cystic neoplasm
SCN = serous cystic neoplasm
VEGF = vascular endothelial growth factor
VEGFR-2 = vascular endothelial growth factor receptor 2
VHL = von Hippel-Lindau

Although many cases of pancreatic cancer occur sporadically, 2 groups of patients have been identified that are at risk of pancreatic cancer. One group is patients with familial or hereditary pancreatic cancer,[2] and the other is patients with cystic lesions of the pancreas.[3] Cystic lesions of the pancreas are diagnosed in increasing numbers due to the use of high-resolution imaging and increased awareness of pancreatic cyst symptoms[4]; in fact, >2% of American adults have pancreatic cysts. Cystic lesions include mucinous (intraductal papillary mucinous neoplasm [IPMN] and mucinous cystic neoplasm [MCN]) and serous (serous cystic neoplasm [SCN]) types.[3]

Cystic lesions of the pancreas exhibit variable malignant potential. Mucinous cystic lesions, IPMN and MCN, have considerable potential to progress to invasive pancreatic adenocarcinoma. Because these patients are at increased risk of pancreatic cancer developing, screening, risk stratification, and pancreatic cyst removal in select patients at highest risk (ie, where risk of cancer significantly outweighs the risk of surgery) will promote early detection and prevention of pancreatic cancer. Serous cystic neoplasm, unlike IPMN and MCN, is a nonmucinous, benign cyst of the pancreas that has little or no risk of malignant transformation; however, SCN can mimic IPMN and MCN on imaging and symptom presentation. Radiologists, when blinded to pathologic diagnosis, are at best approximately 50% accurate in differentiation. In addition, the overall accuracy of cytology in identifying mucinous lesions is only approximately 50%.[5,6] Cyst fluid cytology analysis can often be difficult to perform due to insufficient cells or contamination. In addition, there are no known protein biomarkers to date that can accurately distinguish SCN from mucinous cystic lesions[7]; patients with SCNs that are not accurately distinguished from mucinous cystic lesions might undergo surgical intervention and its attendant risks unnecessarily. Pancreatic surgery has high morbidity (40% to 50%) and potential mortality (1% to 5%), even in high-volume pancreatic surgery centers. To guide the management of mucinous cystic lesions, the International Consensus Guidelines were established in 2006 and included parameters such as cyst size, intramural nodules, cyst fluid cytology, and symptoms as indications for surgical resection. In a retrospective study of 147 patients, these guidelines were reported to have a sensitivity of 100%, but a specificity of only 23%—all patients with malignancy were identified as surgical candidates, but 85% of the patients who underwent surgical resection had benign disease; other studies have confirmed these findings.[8-12]

To accurately identify groups of patients at low or high risk of pancreatic cancer developing, improvements must be made in the ability to distinguish between serous and mucinous cysts.[13] Commercial biomarkers, such as pancreatic cyst fluid CEA level and K-Ras mutation status, exist that can help diagnose mucinous cysts. Mucinous cysts typically show elevated CEA levels (>192 ng/mL) with a sensitivity and specificity of 75% and 84%, respectively.[5] K-Ras mutations are common, although not universal, in mucinous cysts, so molecular analysis for K-Ras mutations can also identify some mucinous cysts.[14] Although these tests can identify a portion of precancerous mucinous cysts, none can accurately identify serous cysts or distinguish benign serous cysts from all other cysts. We report here that vascular endothelial growth factor (VEGF)-A, a critical regulator of vascular growth and function, is considerably elevated in the pancreatic cyst fluid from patients with SCN and can differentiate SCN, a completely benign pancreas cyst, from other pancreas cysts with 100% sensitivity and 97% specificity.

METHODS

Patient samples

Patients signed informed consent for collection of pancreatic fluid at the time of routine endoscopy (endoscopic ultrasonography or ERCP) and/or operation for the Indiana University Pancreatic Tissue-Fluid Bank. Fluid specimens were placed immediately on ice after procurement and aliquoted for storage at −80°C. For this study, samples from 87 patients collected between 2003 and 2012, including pseudocyst (n = 9), SCN (n = 17), MCN (n = 24), IPMN (low/moderate grade, n = 16 or high-grade/invasive, n = 10), and pancreatic ductal adenocarcinoma (n = 11), were pathologically confirmed. Intraductal papillary mucinous neoplasm dysplasia was determined according to the WHO criteria.

Enzyme-linked immunosorbent assay

Pancreatic fluid samples (1−50 uL) were analyzed for VEGF-A or VEGF-C by Quantikine ELISA (R&D Systems) according to manufacturer's protocol. Fluid