Polarization gating spectroscopy of normal-appearing duodenal mucosa to detect pancreatic cancer

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Background: According to the field effect theory, by detecting microvasculature changes such as early increase in blood supply (EIBS) in the surrounding tissue, neoplastic lesions can be identified from a distance.

Objective: To determine the feasibility and efficacy of a fiberoptic probe containing novel polarization gating spectroscopy technology to identify patients with pancreatic adenocarcinoma (PAC) by the field effect theory.

Design: Prospective cohort (pilot) study.

Setting: Outpatient tertiary care center.

Patients: Adult ($\geq$18 years) patients undergoing EGD-EUS were screened. Patients with PAC were included in the "cancer" group and patients without PAC were included in the "control" group. We excluded patients with other known malignancies and gastroduodenal premalignant lesions.

Interventions and Main Outcome Measures: Spectroscopic measurements of EIBS variables, such as deoxyhemoglobin concentration (DHb) and mean blood vessel radius (BVR), were obtained from 5 periampullary locations. The Mann-Whitney rank sum test was used for the statistical analysis ($P \leq .05$).

Results: Fourteen patients (mean age 72 years, 79% male) in the cancer group and 15 patients (mean age 63 years, 60% male) in the control group were included in the final analysis. At the ampullary site, both DHb ($P = .001$) and BVR ($P = .03$) were higher in PAC patients than in the control subjects. The DHb alone (92% sensitivity, 86% specificity) or in combination with BVR (92% sensitivity, 79% specificity) can differentiate PAC from control subjects with high accuracy.

Limitations: Small sample size, unmatched control subjects.

Conclusions: Spectroscopic measurements of EIBS by fiberoptic probes are feasible. Preliminary evidence suggests that in vivo measurement of normal-appearing duodenal tissue can differentiate PAC patients from a distance with high accuracy. (Gastrointest Endosc 2014;80:786-93.)

(footnotes appear on last page of article)

INTRODUCTION

Pancreatic cancer is the fourth leading cause of cancer death in the United States and is associated with a poor prognosis. The mortality rate is approximately 74% at 1 year and 94% at 5 years.[1] The average life expectancy after the diagnosis is approximately 5 to 8 months.[2] At present, successful surgical resection is the only curative therapy that can improve long-term survival. However, long-term survival can be achieved only when a tumor is detected at an early stage.[3] Unfortunately, because of nonspecific symptoms associated with pancreatic cancer, it is commonly detected in the later stages of the disease.[4]

In the past decade, there has been significant improvement in the quality of imaging studies (eg, CT, MRI, and EUS) and development of disease-specific molecular markers (eg, CA 19-9). However, their use has failed to significantly improve the mortality rate of the pancreatic

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cancer.[5] Unfortunately, fewer than 20% of patients are considered eligible candidates for curative surgical resection at the time of diagnosis.[6] This suggests that although modern imaging methods may define the diagnosis of pancreatic cancer with more precision, they have failed to improve the prognosis associated with it. Therefore, identifying patients with early pancreatic cancer and developing screening strategies for high-risk patients are of immense importance.[7,8] To date, no definite biomarkers or imaging techniques have proven to be safe, sensitive, and cost-effective strategies for pancreatic cancer screening in the general population. However, technologic advances have been able to detect early neoplastic changes in the field of tissue surrounding the pancreas and other solid tumors.[9-12]

In this study, we hypothesized that pancreatic adenocarcinoma (PAC) could be detected by measuring the changes in the early increase in blood supply (EIBS)[13] found in the surrounding normal-appearing duodenal tissue with Polarization Gating Spectroscopy (PGS) technology. Our goal was to evaluate the feasibility and efficacy of PGS measurements in the duodenum during endoscopy procedures and to evaluate EIBS markers, deoxyhemoglobin concentration (DHb), and average blood vessel radius (BVR) in patients with PAC versus control subjects.

Take-home Message

- Detection of the field effect changes such as early increase in blood supply surrounding the malignant lesion can potentially be useful to detect a malignant lesion from a distance. We can detect variables of early increase in the blood supply with a simple through-the-endoscope fiberoptic probe and can predict the presence of pancreatic adenocarcinoma with high accuracy.
- Such technology may bring additional tools to stratify the malignant potential of the pancreatic lesion.

METHODS

Study design and sample size

This pilot study was a single-center, open-label, prospective cohort study performed at the Mayo Clinic in Jacksonville, Florida, in partnership with the Biomedical Engineering Department of Northwestern University in Evanston, Illinois. We planned to recruit a total of 15 patients with pathologically confirmed PAC (cancer group) and 15 patients without PAC (control group) to compare spectroscopic measurements. The sample size was based on both feasibility and the primary aim to gain sufficient pilot data to provide reasonable point estimates for each measurement to plan future studies. The Institutional Review Board of the Mayo Clinic approved the study, and all patients signed the informed consent documentation. (Clinical trial registration number: NCT01015820.)

Study patients

We screened all patients prescheduled to receive EGD with upper EUS. Patients with a known, recent history of PAC (untreated) were included in the cancer group, and patients without a known history of PAC were included in the control group. Patient eligibility in the cancer group or the control group was determined based on the inclusion and exclusion criteria of our study (Table 1). All patients in the cancer group were diagnosed with PAC. Patients with pancreatic neuroendocrine tumors were excluded from the study. As described in the exclusion criteria, patients in the control group had no malignant or premalignant lesions in the pancreas or gastroduodenal area. Patients with chronic pancreatitis were excluded from the PAC group but not from the control group. All patients with visible inflammatory conditions in the upper GI tract were excluded from both the PAC group and the control group. Patients in the control group received an upper EUS for the indication of abdominal pain. Patients with EGD/upper EUS findings who did not meet the exclusion criteria were considered a "screen failure" and were excluded from the final study analysis.

Polarization gating spectroscopy

PGS is an optical method that measures the intensity of light scattering with the help of both polarization and wavelength (λ) (Fig. 1). The polarization dependence of the scattered light allows depth-selective interrogation of tissue. The collection of polarized light parallel ($I_{\|}$) and perpendicular ($I_{\perp}$) to the incident beam allows the analysis of the $I_{\|}$, $I_{\perp}$ and difference between $I_{\|}$ and $I_{\perp}$ signals.[14] PGS light signals interrogate progressively deeper into the tissue at estimated maximum penetration depths of 200, 270, and 400 μm. Analysis of the wavelength dependence of these signals allows measurements of oxyhemoglobin concentration, DHb, and the mean BVR via a modified Beer-Lambert algorithm (see Supplement 1 for details, available online at www.giejournal.org).[14-16]

The measurement method with a PGS fiberoptic probe was described previously in detail.[16,17] The PGS measurement unit consists of the following components: (1) a mobile cart with a light source, spectrometer, computer processor, monitor, keyboard, and calibration equipment (Fig. 2A) and (2) a fiberoptic probe (Fig. 2B). The reusable fiberoptic probe was sterilized and reprocessed with Cidex solution (Ethicon Endo-surgery Inc., Cincinnati, Ohio) in a similar standardized fashion as with other endoscopes.

Procedural details

Each patient received EGD with upper EUS as scheduled by an experienced endoscopist participating in the study (M.W., M.R., or T.W.). All patients were sedated with monitored anesthesia under the guidance of an anesthesiology provider.

TABLE 1. Inclusion and exclusion criteria of the study

	Criteria
Inclusion	Age 18 years or older
	Able to provide informed written consent
	Patient scheduled for previously planned EGD with upper EUS
	Patients with known adenocarcinoma of the pancreas were included in the cancer group
	Patients with abdominal imaging studies (eg, CT abdomen or MRI abdomen) negative for malignancy in past 5 years were included in the control group
Exclusion	Unable to obtain biopsy specimen or FNA results of the pancreas lesion (eg, coagulation disorder, inadequate sample)
	Presence of malignant lesion* in the pancreas or the duodenum (eg, neuroendocrine tumor, GI stromal tumor)
	Known familial disorder with high risk of pancreatic cancer development (eg, familial adenomatous polyposis syndrome, hereditary nonpolyposis colorectal cancer syndrome, juvenile polyposis syndrome)
	Significant family history of pancreatic cancer (at least 1 first-degree relative with pancreatic cancer)
	Presence of premalignant lesions (eg, duodenal adenoma, pancreas intraductal papillary mucinous neoplasm)
	Active visible inflammation/ulcer in the stomach or the duodenum
	Patients with known chronic pancreatitis were excluded from the cancer group†
	Known pregnancy or sexually active women of childbearing age not receiving an accepted form of birth control method

*Other than pancreatic adenocarcinoma.
†Chronic pancreatitis patients were allowed to be included in the control group only.

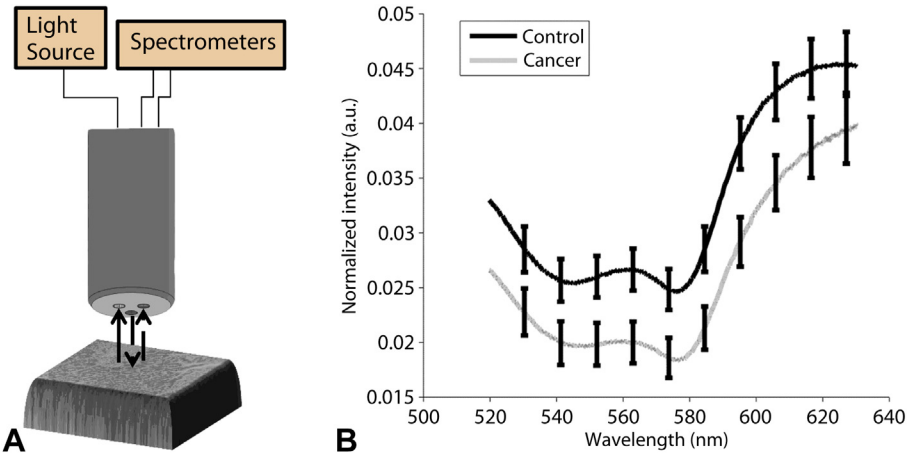

Figure 1. Schematic of the polarization-gated probe **(A)** and representation of data acquisition graph **(B)**.

Before obtaining each spectroscopic measurement, calibration of the fiberoptic system was performed using air, water, and 99% diffuse reflectance standards. The air and water standards accounted for stray reflections in the probe, whereas the reflectance standard was used to account for the intensity spectrum of the light source and system. During EGD, the optic probe was inserted inside the accessory channel of the upper endoscope and advanced to the tip of the endoscope (Fig. 2C). The optic probe was gently brought in contact with the periampullary duodenal mucosa at 5 different desired locations: (1) directly on the ampulla, (2) approximately 5 mm proximal from the ampulla, (3) approximately 5 mm distal from the ampulla, (4) 1 cm proximal from the ampulla, and (5) 1 cm

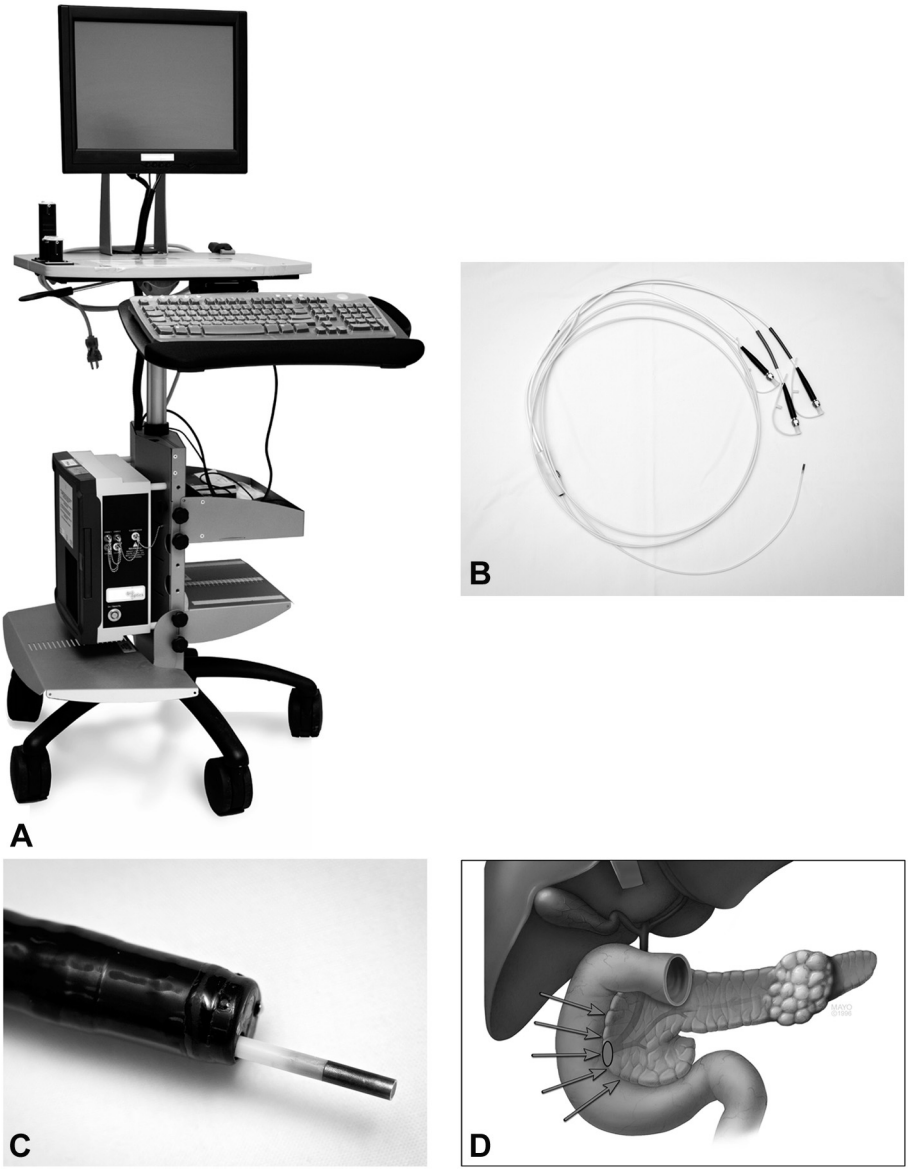

Figure 2. A, Spectroscopy processor, monitor, and keyboard of PGS measurement unit. **B,** Fiberoptic probe of polarization gating spectroscopic measurement unit. **C,** Polarization gating fiberoptic probe fed through the accessory channel of the standard upper endoscope. **D,** Periampullary locations where spectroscopy measurements were obtained.

distal from the ampulla. We obtained spectroscopy measurements 4 times in each of these 5 periampullary locations (Fig. 2D). The rest of the EGD and upper EUS–FNA endoscopy procedures were then completed as clinically indicated. All results, including FNA results (if obtained), were recorded. During the procedure, all visualized mucosal abnormalities were recorded and photographed.

Patients were monitored and then discharged after the endoscopy procedure per standard protocol. Upon discharge, participants were provided information to contact the study investigator if they developed any new or worsening symptoms. Any reported postprocedural adverse events were recorded.

Data analysis

After obtaining spectroscopy measurements, data were stored in the spectroscopy processor and transferred to Northwestern University biomedical engineering laboratory for final analysis. Spectra measurements at each desired location with an $\frac{I_{\|}}{I_{\perp}}$ ratio between 1 and 5, $I_{\|}$ signal intensity $>$ 1% of the reflectance standard intensity, and oxygenation saturation $>$ 2% were considered optimal quality. Spectra not meeting the above criteria were excluded from the final analysis. These criteria avoided inadequate intensity signals in the analysis. EIBS variables, DHb, and BVR were calculated from each spectra, and their average value at each desired periampullary location was calculated. A given

TABLE 2. Demographics of patients in cancer and control groups

Demographic variables	Cancer group (n = 14)	Control group (n = 15)
Mean age, y (± standard deviation)	72 ± 10	63 ± 11
Male gender	11 (79%)	9 (60%)
White ethnicity	13 (93%)	13 (87%)
History of smoking tobacco	7 (50%)	9 (60%)
History of alcohol use	6 (43%)	13 (87%)

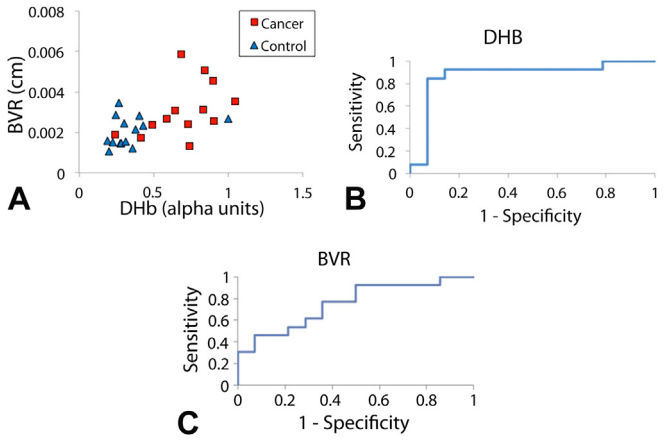

Figure 3. A, Scatterplot of ampullary measurements among patients in the cancer and control groups. **B,** Receiving operating characteristic curve using DHb. **C,** Receiver operating characteristic curve using BVR to differentiate presence of pancreatic cancer compared with control.

periampullary location had to possess at least 2 satisfactory spectra for the average values to be included in the final analysis. To minimize the bias, the engineer performing the data analysis was blinded to the patient status (cancer vs control).

Demographic information was described using frequency statistics. The DHb and BVR measurements were compared between patients in the cancer and control groups by using the Mann-Whitney rank sum test using Minitab version 16.1.1 (Minitab Inc., State College, Pa). At various cut-off values on the receiver operating characteristic curve, the sensitivity and specificity of DHb and BVR were determined to differentiate patients with PAC from control subjects. The leave-one-out cross-validation procedure was performed using Stata version 8 (StataCorp, College Station, TX). The results were assessed for any effect of potential confounding factors, such as age, gender, ethnicity, history of smoking or alcohol use, tumor size, or tumor location, using the analysis of covariance test. The p value of less than 0.05 was used to determine statistical significance of the test results. This article was prepared and reviewed by the contributing authors of this study. Additionally, all authors had access to the study data and reviewed and approved the final manuscript.

RESULTS

We enrolled 37 patients in the study. Of these, 5 patients with neuroendocrine tumors and 2 patients with duodenal anatomy limiting the use of the probe were excluded because of screen failure. We recruited 15 patients with PACs to the cancer group and 15 patients without PACs to the control group. These individuals met all inclusion and exclusion criteria of the study. Among the 15 patients in the cancer group, 1 patient was excluded from the final analysis because of suboptimal measurements. In the final analysis, 14 patients in the cancer group were compared with 15 patients in the control group. The demographics of the recruited patients in the cancer and control groups are shown in Table 2. Among the 15 patients in the control group, 2 patients were found to have chronic pancreatitis based on 5 or more EUS features. The rest of the patients had normal pancreatic architecture and received the diagnosis of functional abdominal pain. The preparation of the cart and the spectroscopic measurements took on average approximately 10 minutes.

Ampulla: optimal location for spectroscopy measurements

Among all 5 periampullary locations, the EIBS variables, DHb ($P = .001$), and BVR ($P = .03$) calculated from the cross-polarized signal and measured directly from the ampulla had the least variability and showed maximal ability to differentiate the PAC patients from control subjects (Supplement 2, available online at www.giejournal.org). Below, we discuss the details of the measurements obtained directly from the ampulla.

Accuracy to differentiate patients with PAC

The scatterplot of DHb and BVR measurements from the ampulla between patients in the cancer and control groups is shown in Figure 3A. The scatterplot demonstrates the higher levels of both DHb and BVR among patients in the cancer group compared with patients in the control group. After plotting DHb and BVR measurements on receiver operating characteristic curves (Fig. 3B and C), their sensitivity and specificity were determined using variable cut-off values (Table 3). In receiver operating characteristic curve analysis, DHb measurements alone achieved a sensitivity of 92% and specificity of 86% to differentiate PAC from control subjects. DHb in combination with BVR measurements achieved a sensitivity of 92% and specificity of 79% to differentiate PAC from control subjects. Upon performing leave-one-out cross-validation analysis, the DHb retained a sensitivity of 92% and

TABLE 3. Accuracy of BVR and DHb measurements

Variables	Sensitivity	Specificity	Area under the curve in receiver operating characteristic
DHb	92%	86%	.87
BVR	77%	64%	.75
Combined DHb and BVR	92%	79%	.87

DHb, Deoxyhemoglobin concentration; *BVR*, blood vessel radius.

TABLE 4. Effect of various confounding variables on measurements obtained in patients with pancreatic cancer compared with control subjects

Confounding variables	DHb (*P* value*)	BVR (*P* value*)
Age	.37	.58
Gender	.02	.46
White ethnicity	.28	.45
History of active smoking	.76	.43
History of active alcohol use	.70	.65

DHb, Deoxyhemoglobin concentration; *BVR*, blood vessel radius.
*Statistical analysis was performed using analysis of covariance test.

specificity of 71%. After excluding patients with chronic pancreatitis (n = 2) from the control group, DHb measurements alone retained a sensitivity of 92% and specificity of 67% to differentiate PAC from control subjects, and DHb in combination with BVR measurements retained a sensitivity of 92% and specificity of 50% using leave-one-out cross-validation.

Effects of confounding factors

We observed no statistically significant effect of age, race, and history of smoking tobacco or alcohol use on the EIBS measurements between the 2 groups (Table 4). DHb measurements were found to be higher in men than in women ($P = .02$), whereas gender did not show a statistically significant effect on BVR measurements ($P = .46$).

In terms of tumor characteristics, the average size of the PAC tumor was 3.3 cm (±1.0 cm standard deviation). The distribution of the PAC tumors was 43% (n = 6) in the head/neck region and 57% (n = 8) in the body/tail region of the pancreas. Tumor size and tumor location in the pancreas did not show any statistically significant effect on the EIBS measurements.

Adverse events

None of the patients who participated in our study reported any adverse events during or after the endoscopy procedure with this spectroscopic measurement.

DISCUSSION

Our study results suggest that patients with PAC have field effect changes in the normal-appearing duodenal mucosa that is distant from the site of the tumor. PGS, a novel optical biomarker, is able to distinguish PAC from control subjects without cancer by detection of EIBS changes in the microvasculature of the adjacent normal-appearing duodenum. We found that the measurement of EIBS markers, DHb, and BVR in the duodenal mucosa by using a PGS fiberoptic probe was simple, feasible, and safe during the endoscopy. In this early study, DHb alone or in combination with BVR can differentiate patients with PAC from control subjects with high sensitivity and specificity.

Development and progression of neoplastic lesions are known to be associated with EIBS (primarily through angiogenesis) and high oxygen demand.[16,18] Field effect theory proposes that neoplastic changes, such as epigenetic alteration and angiogenesis, can be present in contiguous normal tissues. Detecting such changes can reveal the presence of a neoplastic lesion from a distance.[16,18] The use of field effect biomarkers has already been reported in colon cancer,[12] stomach cancer,[19] lung cancer,[20] and breast cancer.[21]

The development of modern molecular technology such as 4-dimensional elastic light-scattering spectroscopy has been replicated in a miniature form, PGS, to explore the molecular abnormalities in tissue microarchitecture that appears to be histologically normal.[11] PGS allows quantitative assessment of the absorption spectra and light scattering in real time based on how the light interacts with the tissue.[14,22-25]

According to theory, field effect changes may be present at several locations surrounding the pancreas, but the measurement of optical and biochemical biomarkers from the duodenum is appealing because of its accessibility and anatomic continuation with the pancreatic tissue and the potential for fewer adverse events related to pancreatic duct manipulation. PGS via a fiberoptic probe is a relatively convenient device applied via the accessory channel of the standard upper endoscope. The preparation and measurement processes add only a few minutes to endoscopy procedure time. PGS technology uses the subset of normal white light (specific wavelength between 450 and 650 nm), which does not impose any risk of radiation injury. The spectroscopic measurement involves bringing the fiberoptic probe in contact with the tissue

surface and therefore adds very limited risks to the planned endoscopy procedure. Contrary to other advanced imaging techniques (eg, confocal microscopy, magnification narrow-band imaging), PGS technology can objectively quantify field effect changes without the need of complex training in image interpretation or, in the case of confocal endomicroscopy, a contrast agent.[26]

A previous study of spectroscopy measurements on an ex vivo duodenal biopsy sample showed a 95% sensitivity and 91% specificity in detecting the presence of pancreatic cancer,[27] which is comparable with our study results of spectroscopic measurements obtained in vivo. Common confounding factors, such as age, race, history of smoking tobacco, history of alcohol use, tumor size, and tumor location, did not show significant impact on spectroscopic measurements in either group.

Limitations

The number of the patients in the cancer and control groups is relatively small to derive any strong conclusion. However, having approximately 15 patients in each group is a reasonable number for a pilot study designed to understand the feasibility, safety, and rough estimation of the efficacy of this technology to determine the presence of PAC. We performed a leave-one-out cross-validation statistical analysis that showed comparable accuracy of the results.

The patient demographics of a higher number of older male patients in the cancer (PAC) group are suggestive of a potential referral bias and a possible representation of similar demographics of PAC patients in the general population. However, other confounding variables did not show statistically significant effects on the spectroscopic measurements. Similar to prior reports, DHb measurements were also found to be higher in male patients compared with female patients.[28] The cause of gender differences in DHb measurement is currently unknown.

Spectroscopy technology has limitations in differentiating EIBS changes from the neoplastic lesion versus tissue inflammation. After excluding patients with visible inflammation in the gastroduodenal region, the results achieved high accuracy to differentiate the presence of PAC from control subjects. So far, the effect of chronic pancreatitis on the spectroscopic measurements is not well understood. To increase the validity of the study results, a small number of patients with chronic pancreatitis were included in the control group. However, the study sample size is not large enough to accurately determine the true effect of chronic pancreatitis on the spectroscopic measurements. Some theoretical limitation of the front-viewing endoscope within the duodenum necessitated a tangential view to obtain spectral measurement of the periampullary tissues. However, the standard upper endoscope, instead of the side-viewing ERCP scope, was chosen to increase its potential applicability and reduce the risk of damaging the delicate fiberoptic probe by the elevator. In our experience, we have found that the fiberoptic probe is quite sturdy with no significant issue maneuvering it through the endoscope. Although the intensity of pressure applied to the tissue with the probe is subjective, the equipment provides immediate feedback if there is poor contact, prompting a repeat measurement. It does not, however, compensate for excessive contact or artifacts from luminal contents (bile) or mucosal abrasion. Fortunately, these were rare and adjusted for by moving the probe to a nearby location.

Future implications

This PGS technology was shown to be a safe, feasible, and accurate option to differentiate the presence of PAC during endoscopy. However, its validity and the effect of potential confounding variables have yet to be tested in a large number of patients with PAC.

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Abbreviations: BVR, blood vessel radius; DHb, deoxyhemoglobin concentration; EIBS, early increase in blood supply; PAC, pancreatic adenocarcinoma; PGS, polarization gating spectroscopy.

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APPENDIX

Supplement 1

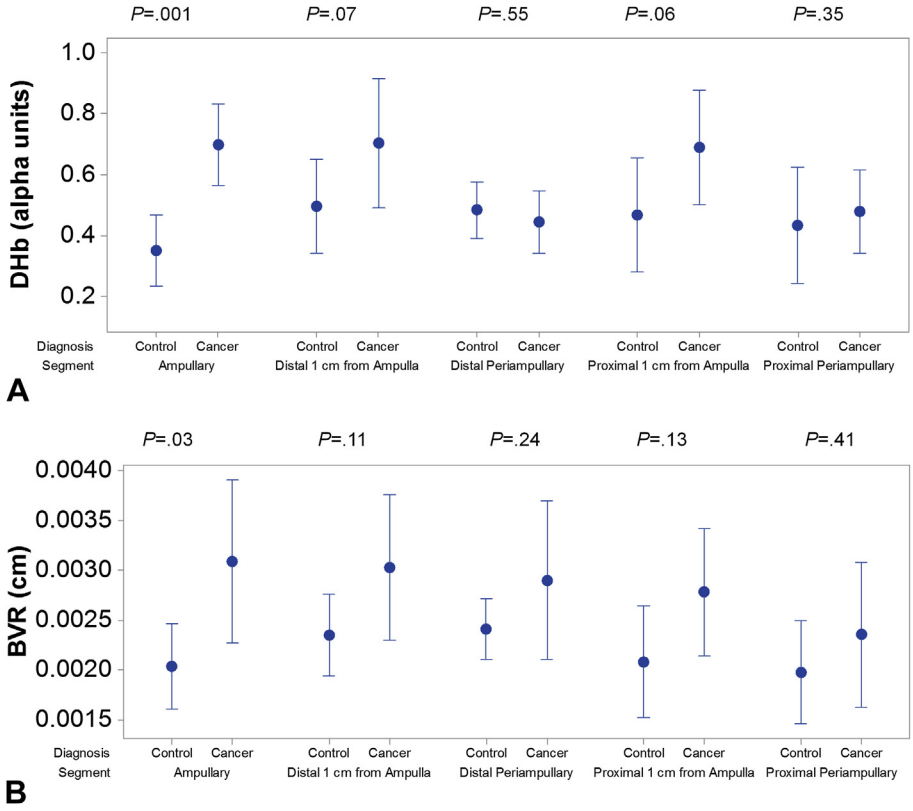
The basis for extraction of hemoglobin content from the collected signals from tissue is the following modified Beer-Lambert equation:

$$I(\lambda) = d\lambda^{2m-4} \exp(-C(\lambda)\alpha_{Hb}[S\varepsilon_{OHb}(\lambda) + (1-S)\varepsilon_{DHb}(\lambda)]), \quad \text{(Eq 1)}$$

where d is the proportionality constant, m is the shape parameter of the refractive index correlation function,[29] α_{Hb} is the product of effective path length and total hemoglobin concentration under Beer's law, S is the oxyhemoglobin saturation, and ε is the molar extinction spectrum for oxyhemoglobin and DHb. The variable C is a correction factor to account for hemoglobin packing in the blood vessels and is given by the following formula[30,31]:

$$C(\lambda) = \frac{1 - \exp\left(-2R_{vessel}\mu_{a,bl}(\lambda)\right)}{2R_{vessel}\mu_{a,bl}(\lambda)}, \quad \text{(Eq 2)}$$

where R_{vessel} is the mean BVR and $\mu_{a,bl}$ is the absorption coefficient of whole blood. We extracted quantitative information on oxyhemoglobin, DHb, and BVR from our readings by minimizing the sum of the square error between the measured and calculated signals over a wavelength range of 520 to 630 nm using α, S, and R_{vessel} as fitting parameters.[16] It should be noted that the accuracy of Eq 1 for hemoglobin concentration and BVR measurement is dependent on several assumptions[32]: (1) hemoglobin concentration within whole blood is 150 g/L; (2) the Fahraeus effect, by which hematocrit is lower in the capillaries than whole blood, can be neglected; and (3) Eq 2, which is derived from modeling vessels as infinite cylinders of uniform size, can be accurately applied to realistic tissue geometries.



Supplement 2. A, DHb and, B, BVR in the PAC and control group at all 5 periampullary locations.