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Light scattering spectroscopy identifies the malignant potential of pancreatic cysts during endoscopy

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Pancreatic cancers are usually detected at an advanced stage and have poor prognosis. About one-fifth of these arise from pancreatic cystic lesions. Yet not all lesions are precancerous, and imaging tools lack adequate accuracy for distinguishing precancerous from benign cysts. Therefore, decisions on surgical resection usually rely on endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA). Unfortunately, cyst fluid often contains few cells, and fluid chemical analysis lacks accuracy—which has dire consequences, including unnecessary pancreatic surgery for benign cysts and the development of cancer. Here, we report an optical spectroscopic technique, based on a spatial gating fibre-optic probe, that predicts the malignant potential of pancreatic cystic lesions during routine diagnostic EUS-FNA procedures. In a double-blind prospective study in 25 patients, with 14 cysts measured *in vivo* and 13 postoperatively, the technique achieved an overall accuracy of 95%, with a 95% confidence interval of 78–99%, in cysts with definitive diagnosis.

Pancreatic cancer has the lowest survival rate among all major cancers, typically six months from diagnosis[1]. This is due to an inability to detect it early, while still treatable, largely because of the inaccessible location of the pancreas deep in the abdomen. Also, the disease often metastasizes while it is still asymptomatic[2]. About one-fifth of pancreatic cancers arise from cystic lesions that can potentially be identified in early, treatable stages with non-invasive imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI). Cystic lesions are relatively common, occurring in at least 2% of adults[3], with some studies describing the incidental finding of pancreatic cysts in more than 10% of abdominal MRIs obtained for non-pancreatic indications[4]. It should therefore come as no surprise that cystic lesions account for one-third of all pancreatic surgeries[5]. However, while CT and MRI could be used to screen for cystic lesions, they have limited accuracy with regard to identifying the type of pancreatic cyst. Currently, there is no sufficiently accurate diagnostic technique that can reliably distinguish cancerous and precancerous cysts from benign cysts. The resulting uncertainty in diagnosis of pancreatic cystic lesions can lead to a delay in surgical resection of precancerous lesions, as well as unnecessary surveillance and even surgery for benign cysts. Considering the high mortality and morbidity of pancreatic surgeries and the even higher mortality from untreated pancreatic cancers, there is an obvious need for the development of new diagnostic methods to accurately identify pancreatic cysts that need surgical intervention.

The best currently available diagnostic method for identifying malignancy in pancreatic cyst lesions is based on the minimally invasive EUS-FNA procedure, which is performed in at least 90% of cases when the decision to undergo surgery is required. This procedure has an overall sensitivity of less than 50% for detecting malignancy, with the majority of results being non-diagnostic[6,7]. During the EUS-FNA procedure, the cyst fluid is collected and then analysed both for tissue evaluation (cytopathology), and also for the presence of certain molecular markers or glycoproteins, such as carcinoembryonic antigen (CEA).

There are two primary types of precancerous pancreatic cystic lesions, intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms (MCNs)[8,9], that could be treated surgically, achieving a high cure rate. However, the majority of cystic pancreatic lesions have no malignant potential and do not require surgery. Certain types of precancerous cysts can be safely monitored over years, and may not require surgical resection. Higher-risk precancerous cysts should be removed surgically before cancer development. Pancreatic surgery is complex and is associated with significant morbidity and mortality. For instance, pancreatoduodenectomy, also known as the Whipple procedure, involves removing the head of the pancreas, two-thirds of the duodenum and one-third of the stomach, and has a mortality rate of more than 11% when averaged over all US hospitals[10]. Therefore, the decision to consider surgery for a pancreatic cyst requires the treating physician to weigh data from potentially inaccurate EUS-FNA results with several even less conclusive imaging tests, as well as with the patient's ability to tolerate the surgery. As a result, of the pancreatoduodenectomies that are performed on cystic lesions, only about 42% are later confirmed as featuring precursor lesions with malignant potential[11]. On the other hand, precancerous and small resectable cancerous cysts, when left untreated, have the risk of progressing to incurable cancer.

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There is a critical need for the development of a diagnostic method that improves the accuracy of cyst evaluation and can be employed during the EUS-FNA procedure. The basic physical principle that elastic light scattering can distinguish precancerous and early cancerous lesions has already been demonstrated[12]. There are three main components of tissue light scattering spectra. The largest is a diffuse background signal from submucosal tissue; next is scattering by small organelles; and, lastly, a relatively small backscattered component from epithelial cell nuclei. The submucosal background can be excluded by one of several gating techniques[12–18] and the smaller organelles have a very different scattering spectral dependence than that of the nuclei. Elastic light scattering can also be used to measure other cellular compartments, such as mitochondria[19], whose spectra[20] are sufficiently different from that of nuclei to be distinguished[21]. The combination of gating and difference in spectral behaviour allows the epithelial nuclear scattering spectrum to be isolated in the processed light scattering spectroscopy (LSS) signal. A significant contribution from nuclear backscattering and clear correlation of dysplasia with nuclear size has been demonstrated in earlier studies[12–16,22–24]. Direct comparison of the nuclear size distribution extracted from the backscattering signal to that of histological examination of the corresponding haematoxylin and eosin (H&E)-stained sections has also been demonstrated[17,18].

Now we have developed a new instrument that uses this principle to solve the difficult problem of identifying precancerous and early cancerous lesions in the pancreas. The instrument can probe the internal surface of pancreatic cysts, obtaining multiple noninvasive optical 'biopsies' from each cyst in a matter of seconds, performing significantly better than existing cytology and cyst fluid CEA markers. Our results indicate that this technology has significant potential to aid in identifying both precursor lesions and early-stage pancreatic cancers.

Results

To develop an *in vivo* LSS system and diagnostic algorithm, we first performed an *ex vivo* pilot study to evaluate the ability of LSS to differentiate cystic neoplasms with varying grades of malignancy from benign cysts. We then designed a needle-based LSS instrument for *in vivo* use during EUS-FNA procedures and have collected spectra from the pancreatic cysts of 14 consecutive subjects who satisfied the study enrollment criteria. The diagnostic cut-offs were determined prospectively and the experimenters performing the data collection and analysis were blinded to the patient diagnosis, while the gastroenterologists making the patient diagnosis were blinded to the LSS results.

Studies in freshly resected pancreatic samples. Measurements on freshly resected pancreatoduodenectomy and distal pancreatectomy samples from 11 subjects including a total of 13 pancreatic cystic lesions were obtained. The LSS spectra from pancreatic resection samples were measured with the clinical LSS system and polarization gated probe, developed previously for Barrett's oesophagus (BE) studies[13]. The spectra were collected from multiple locations with the measured sites marked with India ink, and photographed to ensure proper co-registration with the subsequent histopathology examination. To differentiate various cystic neoplasm histopathologies, we employed the diagnostic parameter Δ introduced in our BE studies[13], with the only difference in that the root mean square normalized spectrum, employed in the diagnostic algorithm, was now calculated using all cystic lesion measurements. In our BE studies, if this diagnostic parameter was greater than 0.1 (10% of the mean squared spectrum summed over all spectral points), the site was considered to be dysplastic. Following the same logic, our diagnostic criteria for cystic lesions classified $\Delta < 0.1$ as benign, $0.1 < \Delta < 0.2$ as low-grade dysplasia (LGD), and $\Delta > 0.2$ as high-grade dysplasia (HGD). The cut-offs $\Delta = 0.1$ and $\Delta = 0.2$ correspond to 25% and 50% enlarged nuclei according to our earlier work[13]. The use of the same diagnostic criteria as in the BE studies is rationalized by the fact that the two most common types of precancerous pancreatic cystic lesions, IPMNs and MCNs, are characterized by a similar type of lining as in the BE — columnar epithelium. The data analysis was performed in a double-blind manner, before postoperative histopathology results were available. The preoperative cytology results and CEA levels were not taken into account.

To check if LSS would improve the diagnosis of cystic neoplasms, we compared our findings with preoperative imaging results, cytology results and cyst fluid CEA levels as well as postoperative histopathology, which is considered the gold standard. These results are summarized in Table 1 and discussed below.

In all cases, LSS diagnosis of benign and dysplastic cysts agreed with histopathology. When dysplasia grades were taken into account, two benign cases, four LGD cases and six HGD cases were correctly identified, while one HGD case was identified as LGD. Figure 1e

Table 1 | *Ex vivo* differentiation of cystic neoplasms.

Cyst	MRI/CT	CEA (ng ml^{-1})	Cytology	Histopathology cyst type	Histopathology diagnosis	LSS (Δ)	LSS diagnosis
1*	CNET	686	-	IPMN	LGD	0.11	LGD
2*	-	-	-	Serous	Benign	0.07	Benign
3	Serous	67	Scant benign cells	IPMN	LGD	0.12	LGD
4	IPMN	142	-	IPMN	HGD	0.74	HGD
5	IPMN	430	LGD IPMN	IPMN	LGD	0.19	LGD
6	-	-	-	Pseudocyst	Benign	0.08	Benign
7	-	-	HGD IPMN	IPMN	HGD	0.76	HGD
8	IPMN	1.8	HGD IPMN	IPMN	HGD	0.19	LGD
9	IPMN	151	HGD	IPMN	HGD	0.23	HGD
10†	IPMN	-	-	IPMN	LGD	0.17	LGD
11†	IPMN	-	Adenocarcinoma	IPMN	HGD	0.22	HGD
12	IPMN	-	Carcinoma	ITPN	HGD	0.29	HGD
13	IPMN	122	HGD IPMN	IPMN	HGD	0.26	HGD

Polarization gated LSS optical spectroscopic technique versus MRI/CT, CEA level, preoperative cytology and postoperative histopathology. The two last columns present Δ parameter and the LSS diagnosis. MRI includes both abdominal MRI and MRCP. CNET, cystic neuroendocrine tumour; ITPN, intraductal tubulopapillary neoplasm. Dashes indicate no information due to lack of imaging classification, cellular material or absence of data on CEA level. *Cysts 1 and 2 are from the same subject. †Cysts 10 and 11 are from the same subject.