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Near-infrared fluorescence imaging of a solitary fibrous tumor of the pancreas using methylene blue

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ABSTRACT

A 67-year-old female presented with unexplained abdominal pain. A contrast-enhanced CT scan of the abdomen incidentally revealed a mass in the uncinate process of the pancreas. This mass was resected and based on histopathological findings, diagnosed as a solitary fibrous tumor of the pancreas. A solitary fibrous tumor is an extremely rare benign mesenchymal tumor that in 65% of cases affects the visceral pleura but can also affect extra-pleural sites. The intraoperative demarcation of pancreatic tumors, such as a solitary fibrous tumor, can be challenging. In this report, the first clear intraoperative identification of a solitary fibrous tumor of the pancreas using near-infrared fluorescence and methylene blue in a human was shown.
INTRODUCTION

Pancreatic tumors are most often adenocarcinomas, but can also be rare neuroendocrine tumors and even rarer benign mesenchymal tumors. Diagnostic workup of pancreatic tumors is typically performed using contrast-enhanced Doppler ultrasound (US), helical computed tomography (CT), enhanced magnetic resonance imaging (MRI), and endoscopic US (EUS). However, during pancreatic surgery, tumor localization and assessment of the extent of disease is presently made using visual inspection and palpation, and in some cases, intraoperative US. Inadequate intraoperative tumor identification can lead to recurrent disease and/or the need for re-exploration.

Near-infrared (NIR) fluorescence imaging is a promising technique to facilitate intraoperative, real time, visual identification of tumors, which uses tumor-specific fluorescent contrast agents. Although several targeted NIR fluorophores have shown promise in preclinical model systems, there are no tumor-specific contrast agents presently available for clinical use. In fact, the only 2 NIR fluorescent contrast agents available clinically, and approved for other indications, are the 700 nm fluorophore methylene blue (MB) and the 800 nm fluorophore indocyanine green (ICG); both agents are classified as non-specific with respect to targeting. MB and ICG have been clinically used for decades. MB is used in parathyroid surgery and for the treatment of septic shock. ICG is used to assess the clearance capacity of the liver and for angiographies and has been used off label for NIR fluorescence sentinel lymph node mapping.

Although the enhanced permeability and retention (EPR) effect can potentially be used to accumulate non-targeted contrast agents in tumors, a recent clinical study by our group suggested that intravenously injected ICG did not result in improved identification of adenocarcinoma of the pancreas from this effect. In a different preclinical study by our group, it appeared that intravenous injection of MB into transgenic mice resulted in high sensitivity detection of insulinomas and a pancreatic neuroendocrine tumor. The optimal dose of MB in that study was 1 to 2 mg/kg. Based on these results, a clinical study was initiated in which patients with a suspected neuroendocrine tumor of the pancreas were administered 1.0 mg/kg MB intraoperatively and the pancreas was imaged using an optimized NIR fluorescence imaging system.
CASE REPORT

A 67-year-old female presented with unexplained abdominal pain. The patient did not have jaundice or other symptoms of obstruction. A contrast-enhanced CT scan of the abdomen incidentally revealed a mass of 1.6 x 2.8 cm in the uncinate process of the pancreas (Fig. 1; dashed outline). The mass showed hyperattenuation during the arterial phase of contrast administration. Static total body and SPECT / low-dose CT scans using In-111-Octreotide showed a normal distribution in the body and no uptake in the pancreatic mass (data not shown). Moreover, no hormonal hypersecretion was observed. Family history of multiple endocrine neoplasia syndrome was negative. A non-functioning neuroendocrine tumor was suspected and the patient was planned for resection. During surgery, directly after exposure of the uncinate process, NIR fluorescence imaging of the pancreatic mass using MB was performed. Afterwards, the lesion was enucleated (Fig. 1) while sparing the pancreatic duct and processed for histology. No surgical complications or adverse events were reported and the patient was released from the hospital 7 days postoperatively.

Intraoperative NIR Fluorescence Imaging System

NIR fluorescence imaging was performed using the Mini-FLARE™ image-guided surgery system as described in detail previously (Fig 2) \(^ {16} \). Briefly, the system consists of 2 wavelength separated light sources: a “white” LED light source, generating 26,600 lx of 400 to 650 nm light to illuminate the surgical field and an NIR LED light source, generating 7.7 mW / cm\(^2\) of 670 nm fluorescence excitation light. White light and NIR fluorescence images are acquired simultaneously and displayed in real time, using custom designed optics and software. A pseudo-colored (neon red) image of NIR fluorescence superimposed over the white light image is also displayed, to provide the NIR fluorescence signal in the proper anatomical context.

Intraoperative NIR Fluorescence Imaging and Fluorescence Microscopy

The current study was approved by the Medical Ethics Committee of the Leiden University Medical Center and was performed in accordance with the ethical standards of the Helsinki Declaration of 1975. One patient with a suspected pancreatic neuroendocrine tumor was included and gave written informed consent. In this patient, a dose of 1.0 mg/kg MB (64 mg in 6.4 ml of water; 10 mg/ml final stock solution concentration) was infused intravenously over 5 min directly
after exposure of the uncinate process. After the start of infusion, NIR fluorescence imaging of the pancreatic mass and the pancreas was performed approximately every 30 seconds for a period of 20 min. *Ex vivo* NIR fluorescence imaging of the sliced resection specimen was performed at the pathology department. NIR fluorescence imaging using the Mini-FLARE™ system enabled clear visualization of the pancreatic lesion (Fig. 3A). After the infusion time of 5 min, a tumor-to-background ratio of ≈ 3 was reached and was stable for the next 15 min (Fig. 3B). No adverse effects occurred.

**Histopathology**
Part of the excised tissue was fixed in formalin and embedded in paraffin for hematoxylin and eosin (H&E) and immunohistopathological staining, and part of the excised tissue was snap frozen for fluorescence microscopy. Macroscopically, a clearly defined well-circumscribed pale tan firm nodule was seen. Sections showed an unencapsulated, circumscribed tumor, consisting of a spindle cell proliferation with hypercellular areas alternating with hypocellular foci (Fig. 4A). The hypercellular areas are made up of spindle shaped cells without cytological atypia. There is no cellular pleomorphism, mitoses or necrosis. Furthermore, deposition of abundant hyalinized collagen was identified (sometimes keloid-like) and the stroma contained several vessels, a few of which were dilated and hemangiopericytic. Several normal pancreatic acinar elements are embedded within the mass. No islets of Langerhans were identified within the lesion. Additional, immunohistochemical staining shows that the tumor cells were positive for CD34, CD99, and Bcl2 (Fig. 4A). CD117 and b-catenin were negative (data not shown). Based on these histopathological findings the tumor was diagnosed as a solitary fibrous tumor (SFT) of the pancreas.

**Fluorescence Microscopy**
Snap frozen tissue was sectioned at 6 µm for fluorescence microscopy and additionally stained with a blue 4',6-diamidino-2-phenylindole (DAPI) nuclear staining. Fluorescence microscopy was performed on a Zeiss LSM 700 Confocal Laser Scanning Microscope and a Zeiss HBO 100 Microscope Illuminating System (Jena, Germany) using a 633 nm laser for excitation and a 650 nm high pass emission filter. Subsequently, tissue sections were stained with H&E and overlay images of NIR fluorescence were created. Fluorescence signal was located in remnants of the exocrine pancreatic tissue, encapsulated by the tumor tissue (Fig 4B).
**Figure 1:** Presurgical and intraoperative visualization of the pancreatic mass (dashed outline)

**Figure 2:** The Mini-FLARE imaging system
Figure 3A: Intraoperative near-infrared (NIR) fluorescence imaging

Figure 3B: Signal-to-background ratio of the solitary fibrous tumor

Figure 4A: Histopathological evaluation
Figure 4B: Fluorescence microscopy
DISCUSSION

Despite the availability of many preoperative imaging modalities, intraoperative identification and demarcation of pancreatic tumors remains challenging. In this report, the first clear identification of a SFT of the pancreas using NIR fluorescence and MB in a human was shown. A SFT is an extremely rare benign mesenchymal tumor that in 65% of cases affects the visceral pleura but can also affect extra-pleural sites. Until now, 9 cases of a SFT affecting the pancreas have been reported.17-25 In these cases, patients were asymptomatic or complained of abdominal pain.

Although the current study was initiated to assess the ability to intraoperatively detect neuroendocrine tumors using NIR fluorescence and MB, the unexpected findings in this patient with a SFT in the pancreas are encouraging. Although SFTs mimic neuroendocrine tumor radiologically, it remains unknown whether MB will provide strong contrast in other non-adenocarcinoma pancreatic tumors. SFTs, like neuroendocrine tumors, typically show a hypervascular, hyperenhancing mass using contrast-enhanced CT, and because MB is a phenothiazine derivative that acts as a perfusion tracer, it remains likely that future clinical testing will confirm what has been seen preclinically in neuroendocrine tumors.15 Our clinical study enrolling patients with a suspected neuroendocrine tumor remains open to accrual. Moreover, NIR fluorescence imaging using methylene blue could potentially be translated to patients with other diseases giving hypervascular lesions, such as hyperparathyroidism and angiodysplasia of the gut. A clinical trial in hyperparathyroidism patients using methylene blue and NIR fluorescence is currently ongoing in our center.

In conclusion, the current study demonstrated the first in human NIR fluorescence imaging of a SFT in the pancreas using MB, a registered and approved pharmaceutical. Larger series will be needed to confirm this result.
REFERENCES
