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TITLE:
Isolation, Detection, and Analysis of Circulating Tumor Cells in Microfluidic Devices for Monitoring Pancreatic Cancer Treatment Response

HYPOTHESIS:
Several challenges exist in acquiring pancreatic tissue for histological diagnosis and tumor biopsies to monitor pharmacodynamic responses during clinical trials of novel treatments. Circulating tumor cells (CTCs) can act as a minimally invasive liquid biopsy for cancer, providing clinicians and researchers with a tool to learn about the metastatic process and assess the risks of metastasis in localized disease. Longitudinal enumeration of CTCs performed by isolating and detecting these malignant cells from the peripheral blood of pancreatic cancer patients may provide us with predictive and prognostic information related to clinical outcomes of patients undergoing therapy. Such information could prove to be invaluable in guiding cancer therapy by revealing treatment resistance earlier than normal, as well as potentially revealing overexpressed antigens for targeted therapies.

BACKGROUND/AIMS:
Pancreatic cancer is one of the most aggressive solid tumors clinically characterized by local invasion, early metastasis, and resistance to standard chemotherapy. Consequently, pancreatic cancer has one of the lowest overall survivals among all cancers. Up to date, the best strategies to monitor pancreatic cancer patients during treatment are to perform routine radiographic imaging to track changes in the size of the primary and/or metastatic tumor masses and track CA19-9 (carbohydrate antigen 19-9) levels; however, both strategies have severe downfalls and thus better biomarkers are needed for pancreatic cancer diagnosis and prediction of therapeutic efficacy.

CTCs are cancer cells that originate from a primary or metastatic tumor that have made their way into blood vessels, and are ultimately responsible for the formation of new metastases. Studies have shown that determinants of long-term survival in pancreatic cancer patients appear to be dictated majorly by the biology of the tumor masses. CTCs are a potential biomarker for pancreatic cancer not only because of their specificity, but also because CTCs allow repeated study of tumor genetics, proteomics, and molecular biology of the cancer cells. CTCs have shown to provide predictive/prognostic information related to disease relapse, overall survival, and tumor response to therapy in a variety of cancers. In this work, we explore the potential role of CTCs in the prognosis and therapy of pancreatic cancer via a microfluidic platform that has been optimized for high capture efficiency and purity of target cancer cells.

METHODS:
CTC analysis systems are hindered by sub-optimal sensitivity and specificity, as well as lack of capability of characterizing CTCs with clinically-relevant biomarkers. For this work, we previously developed and optimized polydimethylsiloxane (PDMS) microfluidic devices, fabricated via soft lithography, that are functionalized with antibodies specific for the epithelial cell-adhesion molecule (EpCAM). EpCAM has been shown to be overexpressed in many carcinomas relative to normal epithelium, and appears to be involved in regulating gene transcription and cell proliferation associated with anchorage-dependent growth and invasiveness.

Peripheral blood (4 mL per test) drawn from patients undergoing palliative chemotherapy treatment is treated and processed through EpCAM-functionalized devices to capture tumor cells. Captured cells are then stained with anticytokeratin-FITC, anti-CD45-PE, and DAPI to differentiate tumor cells from normal blood cells. To define CTCs, the FDA-approved definition is used: a nucleated cell expressing EpCAM, cytokeratin, and negative for the pan-leukocyte marker, CD45 (EpCAM+/CK+/DAPI+/CD45-); biomechanical characteristics are also considered during detection.

RESULTS & CONCLUSIONS:
CTCs were detected in the majority of peripheral blood samples (96.2%) from 38 metastatic pancreatic cancer patients undergoing chemotherapy. An average of 4.22 CTCs per mL were detected in 234 blood samples processed. Blood was typically processed within 4 hours after drawing. CTC analysis exhibited a large amount of patient heterogeneity in terms of CTCs detected at baseline (mean = 6.96 CTCs/mL, S.D. = 2.4), as well as CTC fluctuations due to therapy. Furthermore, we found that CTC numbers correlated and, in some cases, predicted disease progression, usually marked by changes in the sizes of the primary and/or metastatic tumors (confirmed by CT scans). Herein, we have established the capability of our antibody-based microfluidic platform to detect low number pancreatic CTCs and the potential for this type of liquid biopsy technology to make an impact in the future of personalized medicine.