Cytologic Characteristics of Circulating Epithelioid Cells in Pancreatic Disease

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BACKGROUND: Circulating epithelioid cells (CECs), also known as circulating tumor, circulating cancer, circulating epithelial, or circulating nonhematologic cells, are a prognostic factor in various malignancies that can be isolated via various protocols. In the current study, the authors analyzed the cytomorphologic characteristics of CECs isolated by size in a cohort of patients with benign and malignant pancreatic diseases to determine whether cytomorphological features could predict CEC origin. **METHODS:** Blood samples were collected from 9 healthy controls and 171 patients with pancreatic disease who were presenting for surgical evaluation before treatment. Blood was processed with the ScreenCell size-based filtration device. Evaluable CECs were analyzed in a blinded fashion for cytomorphologic characteristics, including cellularity; nucleoli; nuclear size, irregularity, variability, and hyperchromasia; and nuclear-to-cytoplasmic ratio. Statistical differences between variables were analyzed via the Fisher exact test. **RESULTS:** No CECs were identified among the 9 normal healthy controls. Of the 115 patients with CECs (positive or suspicious for), 25 had nonmalignant disease and 90 had malignancy. There were no significant differences in any of the cytologic criteria noted between groups divided by benign versus malignant, neoplastic versus nonneoplastic, or pancreatic ductal adenocarcinoma versus neuroendocrine tumor. **CONCLUSIONS:** CECs were observed in patients with malignant and nonmalignant pancreatic disease, but not in healthy controls. There were no morphologic differences observed between cells from different pancreatic diseases, suggesting that numerous conditions may be associated with CECs in the circulation and that care must be taken not to overinterpret cells identified by cytomorphology as indicative of circulating tumor cells of pancreatic cancer. Additional studies are required to determine the origin and clinical significance of these cells. ***Cancer Cytopathol* 2017;125:332-40**. © *2017 American Cancer Society*.

KEY WORDS: circulating epithelial cells (CEC); circulating tumor cells (CTC); cytomorphological features; pancreas; isolation by size of epithelial tumor cells (ISET).

INTRODUCTION

Circulating epithelioid cells (CECs) are defined as cells with epithelioid cytological characteristics found in the peripheral blood, often at a very low frequency. In different contexts, these are known alternatively as circulating tumor cells (CTCs), circulating cancer cells, circulating epithelial cells, or circulating nonhematologic cells. We have chosen to term these cells as CECs instead of CTCs to refer to cells present in the circulation that appear epithelial-like on microscopy (ie, epithelioid) but lack additional studies to confirm the cells as epithelial or to determine a tumor as the site of origin. As would be expected from the numerous ways to refer to these cells, there are several methods of enriching, isolating, and studying them.

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The widely cited and, to our knowledge, first published observation of CECs occurred in 1869, when, at autopsy, an Australian physician noticed atypical cells in the blood of a patient that closely mimicked the individual's widely metastatic carcinoma.[1] CellSearch (Janssen Diagnostics, LLC, Raritan, NJ), the first clinical assay for CECs approved by the US Food and Drug Administration, only received this approval in 2004.[2] This assay uses antibodies for epithelial markers (normally epithelial cell adhesion molecule [EpCAM]) attached to magnetic beads to isolate cells of epithelial origin. This isolation step is followed by labeling with fluorophore-conjugated antibodies to other epithelial markers (typically cytokeratins) for visualization, enumeration, and characterization.[3] CellSearch positivity has been correlated with worse prognosis in numerous carcinomas, including breast cancer,[4,5] non-small cell lung carcinoma,[6] cholangiocarcinoma,[7] and colorectal cancer,[8] among others.

In recent years, there has been a significant increase in research regarding CEC analysis. Similar to its application in hematopathology but using antibodies for keratins and EpCAM, flow cytometry has been used to identify CECs. In addition, some newer flow cytometry machines allow for a degree of cell imaging and localization of markers to the individual cell locations (nuclear, cytoplasmic, membranous, etc), possibly providing a more comprehensive picture of their phenotype.[9]

Extremely sophisticated microfluidic chips have been developed to both characterize and isolate CECs. These chips use the flow properties of the larger cells, along with antibody-coated surfaces to isolate cells with particular characteristics.[10] Chips also have been designed that rely on intracellular cohesion to sort out cells in clusters.[11] In addition to allowing for very high-purity isolation and characterization of CECs, these chip systems allow for the collection of unmodified cells, which can be studied further by genetic or immunologic analysis. Through the isolation and sequencing of single cells, researchers have been able to demonstrate clonality between CTCs and both primary and metastatic foci in a case of widely metastatic prostate cancer.[12]

Another method for isolating CECs is the isolation by size of epithelial tumor cells (ISET) method, which uses size exclusion filters to collect cells.[13] One platform for this method is ScreenCell (Westford, Mass), which consists of single-use filters with 8-μm pores that allow erythrocytes and leukocytes to pass through but trap cells larger than normal hematologic elements.[14] These filters have the advantage that they are relatively easy and inexpensive to use and have the ability to trap unmodified cells, which can be characterized with conventional stains or immunohistochemistry or sent for molecular analysis.[15] ISET positivity has been described in several gastrointestinal tumors,[16,17] and is associated with worse prognosis in numerous malignancies, including colorectal cancer,[18] uveal melanomas,[19] non-small cell lung carcinoma,[20] and pancreatic cancer.[21,22]

Although these different isolation methods theoretically are searching for the same CECs, they have somewhat different operating characteristics. For example, in patients with lung cancer, ISET yields CTCs in a larger percentage of cases compared with CellSearch,[23] and another study found only a weak correlation in the number of CECs isolated by the 2 methods among patients with metastatic lung, prostate, and breast cancer.[24] This difference in performance may indicate that different cells are being isolated by the disparate methods and calls into question what actually is being isolated by these techniques.

We previously published a study of a series of surgical patients with pancreatic pathology who were found to have suspicious or malignant-appearing CECs when characterized by cytology alone.[25] In this group of patients, cells were detected in patients with malignant and nonmalignant pancreatic pathology.[25] In this article, we will discuss the cytomorphologic characteristics of the CECs isolated by ScreenCell in a variety of pathological conditions of the pancreas, with the objective of determining features that can distinguish benign from malignant conditions.

MATERIALS AND METHODS

The current study was performed with Institutional Review Board approval from Massachusetts General Hospital. A total of 171 adult patients with pancreatic disease who presented to the Massachusetts General Hospital pancreatic surgical clinic between October 2011 and October 2013 were recruited before any surgical or medical therapy was administered. In addition, 9 healthy adults lacking any known pancreatic pathology were recruited as controls. All patients consented to a blood draw as per Institutional Review Board protocol and had their samples processed by the ScreenCell technique within 3 hours as described elsewhere.[14] In brief, 1 mL of peripheral blood was added to a lysis buffer and passed through a filter with a low-pressure

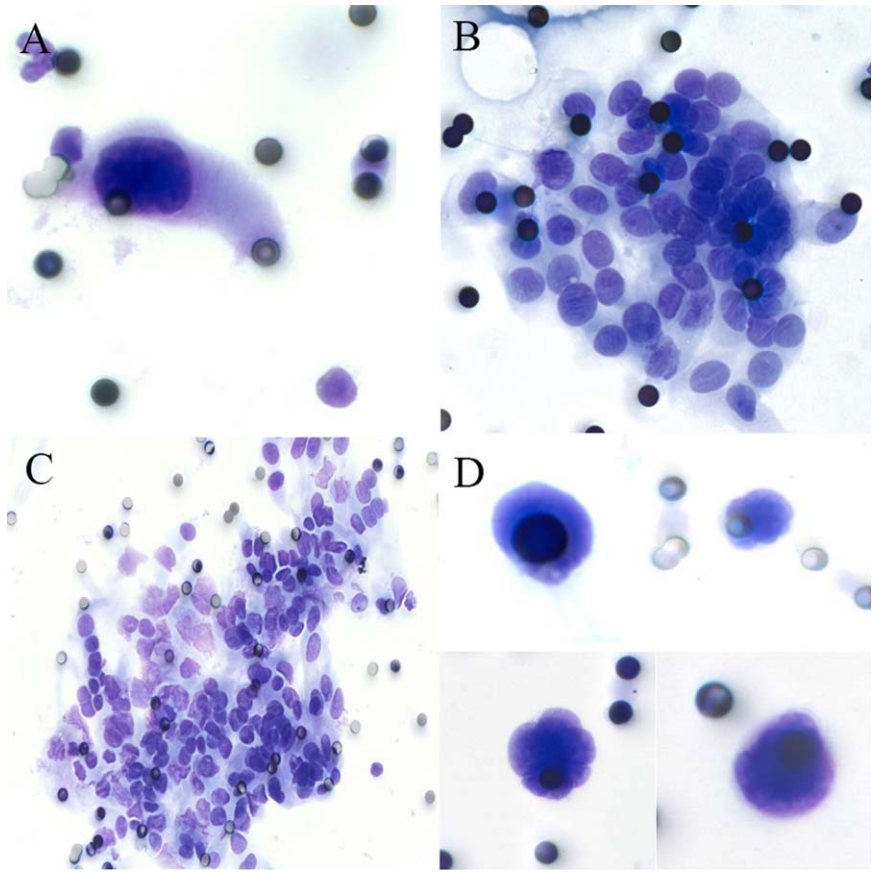

Figure 1. Cytologic characteristics of circulating epithelial cells. (A) A paucicellular (<10 cells) specimen consisting of a single markedly enlarged cell with nuclear enlargement (>3 times the pore size), nuclear hyperchromasia, and nuclear membrane irregularity. (B) A moderately cellular specimen (10-100 cells) consisting of clusters of epithelioid cells with oval nuclei and occasional nuclear grooves. (C) A markedly cellular specimen (>100 cells) with clusters of epithelioid cells. (D) Suspicious specimens consisting of markedly enlarged, irregular nuclei but no visible cytoplasm.

vacuum. These filters were stained as per the manufacturer's instructions with Giemsa stain (Haem 3; Thermo Fisher Scientific, Waltham, Mass). Filters were evaluated by a cytopathologist experienced in pancreatic cytopathology (M.B.P.) who was blinded to patient diagnosis. Slides were interpreted as negative (no CECs present), suspicious (atypical epithelioid cells without definitive malignant features), and positive (CECs with malignant features). CECs also were analyzed for established characteristics of pancreatic malignancy: high nuclear-to-cytoplasmic (N/C) ratio, enlarged nuclei, irregular nuclear borders, nuclear hyperchromasia, and anisonucleosis.[26,27] Similar criteria have been used to describe malignant appearance in ISET-isolated CECs in patients with lung cancer.[28]

Samples with well-visualized CECs were analyzed for cellularity (low [<10 cells] [Fig. 1A], moderate [10-100 cells] [Fig. 1B], high [>100 cells] [Fig. 1C], and naked nuclei only [Fig. 1D]). Cells were characterized by large nuclei (≥3 times the pore size [8 μm]) (Fig. 1A), cell clustering (single cells, clusters ≥5 cells [Fig. 1B], or both), irregular nuclear borders (Fig. 2A), nuclear hyperchromasia (Fig. 2A), a high N/C ratio (>0.75) (Fig. 2A), anisonucleosis (>2-fold variability) (Fig. 2B), and nucleoli (not seen [Fig. 2B], visible [Fig. 2C], and prominent [Fig. 2D]). CECs also were classified by overall impression (negative, suspicious, or positive).

Positive CECs were enlarged (>2 times the pore size), with either irregular hyperchromatic nuclei and scant cytoplasm (Fig. 1A) or clusters of cells with round-to-oval nuclei with occasional grooves and visible cytoplasm (Fig. 1B). Suspicious CECs were epithelioid but fell short of the criteria listed above or lacked clear cytoplasm (Fig. 1D).

Differences were analyzed based on the final histopathologic diagnosis of the patient at the time of surgical

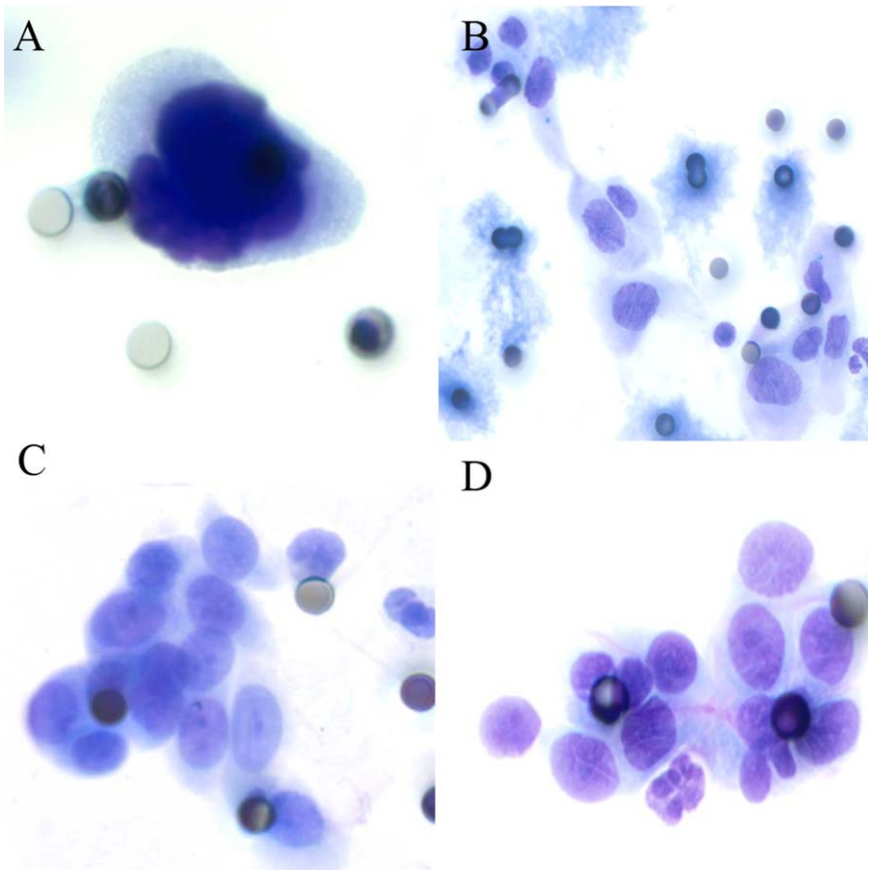

Figure 2. Nuclear and cytoplasmic characteristics of circulating epithelial cells. (A) Malignant-appearing cell with nucleomegaly (>3 times the pore size), an increased nuclear-to-cytoplasmic (N/C) ratio (>0.75), marked nuclear irregularity, and hyperchromasia. (B) Cells demonstrating nucleomegaly, irregular nuclear borders, and marked anisonucleosis (>2 times difference in nuclear size), but no visible nucleoli. (C) Cluster of cells with an increased N/C ratio and visible but nonprominent nucleoli. (D) Groups of cells with an increased N/C ratio and prominent nucleoli.

resection. Analysis compared patients with benign versus malignant lesions, neoplastic versus nonneoplastic etiologies, and patients with pancreatic ductal adenocarcinoma (PDAC) versus neuroendocrine tumors (NET) using the Fisher exact test (α set at $P<.05$).

RESULTS

There were 171 patients in the current study cohort, 115 of whom had positive or suspicious CECs (67.3%). All 9 healthy controls were found to be negative for CECs. Malignancies in the study cohort included PDAC (108 patients), cholangiocarcinoma (8 patients), ampullary adenocarcinoma (7 patients), NET (9 patients), solid pseudopapillary neoplasm (SPN) (3 patients), and acinar cell carcinoma (1 patient). Benign neoplastic lesions included intraductal papillary mucinous neoplasm (IPMN) (16 patients), serous cystadenoma (SCA) (2 patients), ampullary adenoma (3 patients), and mucinous cystic neoplasm (MCN) (1 patient). Nonneoplastic lesions included pancreatitis (12 patients) and splenic epidermoid cyst (1 patient) (Fig. 3) (Table 1). Benign lesions (IPMN, MCN, ampullary adenoma, SCA, splenic epidermoid cyst, and chronic pancreatitis) were found to be 63% CEC positive, 9% suspicious, and 29% negative compared with malignant lesions (PDAC, cholangiocarcinoma, ampullary adenocarcinoma, NET, SPN, and acinar cell carcinoma), which were 51% CEC positive, 15% suspicious, and 34% negative (P = not significant [NS]) (Table 2). There were no statistically significant differences noted between benign and malignant diagnoses with regard to cellularity, nuclear enlargement, nuclear border irregularity, cell clustering, anisonucleosis, increased N/C ratio, nuclear hyperchromasia, and nucleoli (P = NS) (Tables (3–6)).

Neoplastic diagnoses included PDAC, NET, cholangiocarcinoma, ampullary carcinoma, SPN, acinar cell

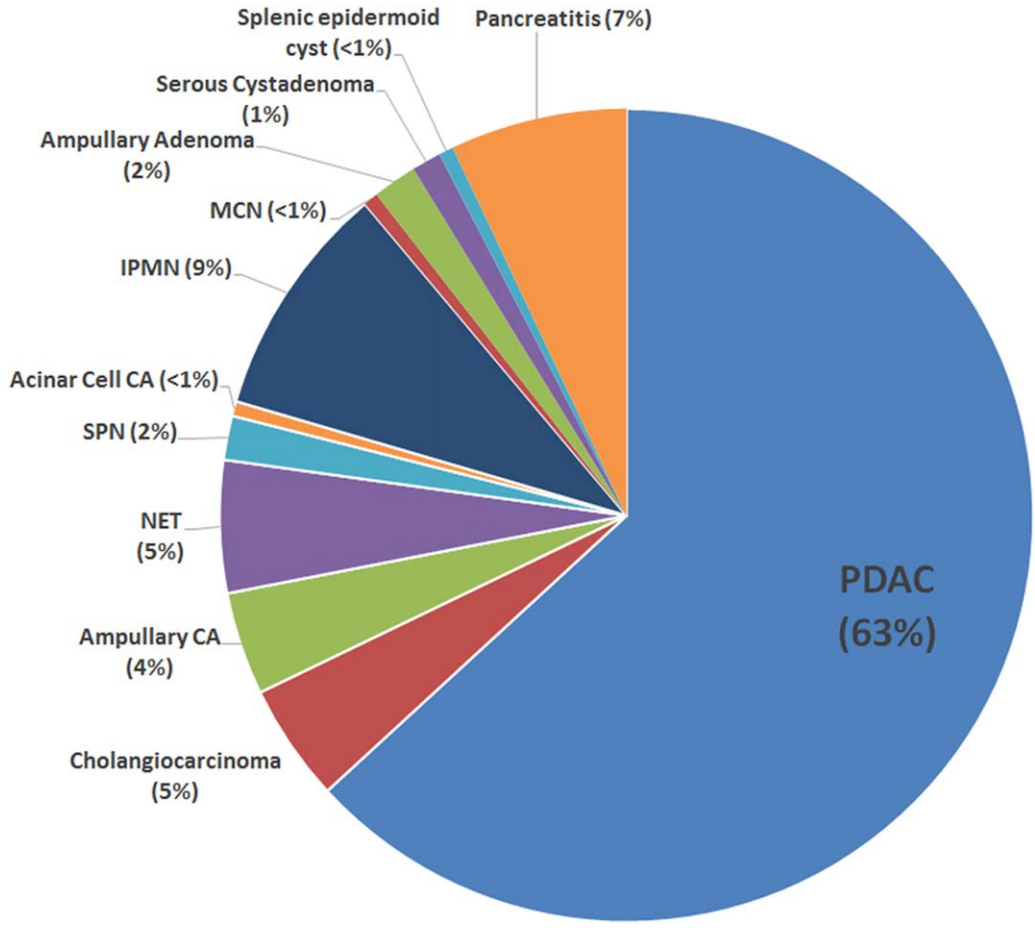

Figure 3. Percentages of different diagnoses present among 171 patients in the current study. CA indicates carcinoma; IPMN, intraductal papillary mucinous neoplasm; MCN, mucinous cystic neoplasm; NET, neuroendocrine tumor; PDAC, pancreatic ductal adenocarcinoma; SPN, solid pseudopapillary neoplasm.

TABLE 1. CEC Positivity by Diagnosis

Diagnosis	No.	CEC+ No. (%)	CEC Susp No. (%)	CEC- No. (%)
All	171	91 (53.2%)	24 (14.0%)	56 (32.7%)
PDAC	108	53 (49.1%)	19 (17.6%)	36 (33.3%)
Cholangiocarcinoma	8	4 (50.0%)	0 (0%)	4 (50.0%)
Ampullary CA	7	4 (57.1%)	0 (0%)	3 (42.9%)
NET	9	6 (66.7%)	2 (22.2%)	1 (11.1%)
SPN	3	2 (66.7%)	0 (0%)	1 (33.3%)
Acinar cell CA	1	0 (0%)	0 (0%)	1 (100.0%)
IPMN	16	12 (75.0%)	0 (0%)	4 (25.0%)
MCN	1	1 (100.0%)	0 (0%)	0 (0%)
Ampullary adenoma	3	1 (33.3%)	0 (0%)	2 (66.7%)
Serous cystadenoma	2	2 (100.0%)	0 (0%)	0 (0%)
Splenic epidermoid cyst	1	0 (0%)	0 (0%)	1 (100.0%)
Pancreatitis	12	6 (50.0%)	3 (25.0%)	3 (25.0%)

Abbreviations: +, positive; -, negative; CA, carcinoma; CEC, circulating epithelioid cells; IPMN, intraductal papillary mucinous neoplasm; MCN, mucinous cystic neoplasm; NET, neuroendocrine tumor; PDAC, pancreatic ductal adenocarcinoma; SPN, solid pseudopapillary neoplasm; Susp, suspicious.

TABLE 2. CEC Positivity by Diagnostic Grouping

Diagnosis	No.	CEC+ No. (%)	CEC Susp No. (%)	CEC- No. (%)	*P*
Malignant	136	69 (50.7%)	21 (15.4%)	46 (33.8%)	.44
Benign	35	22 (62.9%)	3 (8.6%)	10 (28.6%)	
Neoplastic	158	85 (53.8%)	21 (13.3%)	52 (32.9%)	.56
Nonneoplastic	13	6 (46.2%)	3 (23.1%)	4 (30.8%)	
PDAC	108	53 (49.1%)	19 (17.6%)	36 (33.3%)	.47
NET	9	6 (66.7%)	2 (22.2%)	1 (11.1%)	

Abbreviations: +, positive; -, negative; CEC, circulating epithelioid cells; NET, neuroendocrine tumor; PDAC, pancreatic ductal adenocarcinoma; Susp, suspicious.

carcinoma, IPMN, MCN, ampullary adenoma, and SCA. Nonneoplastic diseases included a splenic epidermoid cyst and pancreatitis. Neoplastic lesions were 54% CEC positive, 13% suspicious, and 33% negative compared with nonneoplastic lesions, which were 46% CEC positive, 23% suspicious, and 31% negative (P = NS). Similar to

TABLE 3. Specimen CEC Cellularity by Diagnostic Grouping

Diagnosis	No. (%)
Malignant: <10 cells	24 (26.7%)
Malignant: 10-100 cells	40 (44.4%)
Malignant: >100 cells	17 (18.9%)
Malignant: naked nuclei only	9 (10.0%)
Benign: <10 cells	7 (28.0%)
Benign: 10-100 cells	11 (44.0%)
Benign: >100 cells	6 (24.0%)
Benign: naked nuclei only	1 (4.0%)
P = .85	
Neoplastic: <10 cells	28 (26.4%)
Neoplastic: 10-100 cells	48 (45.3%)
Neoplastic: >100 cells	21 (19.8%)
Neoplastic: naked nuclei only	9 (8.5%)
Nonneoplastic: <10 cells	3 (33.3%)
Nonneoplastic: 10-100 cells	3 (33.3%)
Nonneoplastic: >100 cells	2 (22.2%)
Nonneoplastic: naked nuclei only	1 (11.1%)
P = .77	
PDAC: <10 cells	22 (30.6%)
PDAC: 10-100 cells	30 (41.7%)
PDAC: >100 cells	13 (18.1%)
PDAC: naked nuclei only	7 (9.7%)
NET: <10 cells	1 (12.5%)
NET: 10-100 cells	2 (25.0%)
NET: >100 cells	3 (37.5%)
NET: naked nuclei only	2 (25.0%)
P = .22	

Abbreviations: CEC, circulating epithelioid cells; NET, neuroendocrine tumor; PDAC, pancreatic ductal adenocarcinoma.

TABLE 4. CEC Clustering by Diagnostic Grouping

Diagnosis	No. (%)
Malignant: single cells	35 (38.9%)
Malignant: clusters	21 (23.3%)
Malignant: single cells and clusters	34 (37.8%)
Benign: single cells	8 (32.0%)
Benign: clusters	11 (44.0%)
Benign: single cells and clusters	6 (24.0%)
P = .14	
Neoplastic: single cells	38 (35.8%)
Neoplastic: clusters	29 (27.4%)
Neoplastic: single cells and clusters	39 (36.8%)
Nonneoplastic: single cells	5 (55.6%)
Nonneoplastic: clusters	3 (33.3%)
Nonneoplastic: single cells and clusters	1 (11.1%)
P = .30	
PDAC: single cells	29 (40.3%)
PDAC: clusters	15 (20.8%)
PDAC: single cells and clusters	28 (38.9%)
NET: single cells	3 (37.5%)
NET: clusters	3 (37.5%)
NET: single cells and clusters	2 (25.0%)
P = .56	

Abbreviations: CEC, circulating epithelioid cells; NET, neuroendocrine tumor; PDAC, pancreatic ductal adenocarcinoma.

the comparison between malignant and benign lesions, there was no difference noted between the neoplastic and nonneoplastic lesions in terms of cellularity, nuclear enlargement, nuclear border irregularity, cell clustering, anisonucleosis, increased N/C ratio, nuclear hyperchromasia, and nucleoli (Tables (3–6)).

TABLE 5. CEC Nucleolar Characteristics by Diagnostic Grouping

Diagnosis	No. (%)
Malignant: nucleoli not seen	66 (73.3%)
Malignant: nucleoli present	17 (18.9%)
Malignant: prominent nucleoli present	7 (7.8%)
Benign: nucleoli not seen	19 (76.0%)
Benign: nucleoli present	5 (20.0%)
Benign: prominent nucleoli present	1 (4.0%)
P = 1.00	
Neoplastic: nucleoli not seen	77 (72.6%)
Neoplastic: nucleoli present	21 (19.8%)
Neoplastic: prominent nucleoli present	8 (7.5%)
Nonneoplastic: nucleoli not seen	8 (88.9%)
Nonneoplastic: nucleoli present	1 (11.1%)
Nonneoplastic: prominent nucleoli present	0 (0%)
P = .84	
PDAC: nucleoli not seen	54 (75.0%)
PDAC: nucleoli present	13 (18.1%)
PDAC: prominent nucleoli present	5 (6.9%)
NET: nucleoli not seen	5 (62.5%)
NET: nucleoli present	2 (25.0%)
NET: prominent nucleoli present	1 (12.5%)
P = .52	

Abbreviations: CEC, circulating epithelioid cells; NET, neuroendocrine tumor; PDAC, pancreatic ductal adenocarcinoma.

There also was no significant difference noted between PDACs, which were 49% CEC positive, 18% suspicious, and 33% negative, and NETs, which were 67% CEC positive, 22% suspicious, and 11% negative (*P* = NS). Similar to malignant versus benign and neoplastic versus nonneoplastic lesions, there were no significant differences observed between PDAC versus NET with regard to cellularity, nuclear enlargement, nuclear border irregularity, cell clustering, anisonucleosis, increased N/C ratio, nuclear hyperchromasia, and nucleoli (Tables (3–6)).

DISCUSSION

The results of the current study demonstrate 2 important points related to CECs. First, although CECs were not present in healthy volunteers, they were present in a wide variety of patients with pancreatic diseases ranging from malignant tumors to benign, nonneoplastic pancreatitis.[25] Second, the CECs from these different diseases had identical cytologic characteristics, with similar percentages of all the features examined (cellularity, nuclear enlargement, nuclear border irregularity, cell clustering, anisonucleosis, increased N/C ratio, nuclear hyperchromasia, and nucleoli). These findings have significant implications for continuing research on CECs.

TABLE 6. Other CEC Characteristics by Diagnostic Grouping

Diagnosis, No. (%)	Large Nuclei	Irregular Nuclear Borders	Anisonucleosis	High N/C Ratio	Nuclear Hyperchromasia
Malignant: present	42 (46.7%)	52 (57.8%)	41 (45.6%)	23 (28.4%)	32 (35.6%)
Malignant: absent	48 (53.3%)	38 (42.2%)	49 (54.4%)	58 (71.6%)	58 (64.4%)
Benign: present	15 (60.0%)	18 (72.0%)	13 (52.0%)	6 (25.0%)	11 (44.0%)
Benign: absent	10 (40.0%)	7 (28.0%)	12 (48.0%)	18 (75.0%)	14 (56.0%)
P	.27	.25	.17	.80	.49
Neoplastic: present	52 (49.1%)	63 (59.4%)	52 (49.1%)	28 (28.9%)	39 (36.8%)
Neoplastic: absent	54 (50.9%)	43 (40.6%)	54 (50.9%)	69 (71.1%)	67 (63.2%)
Nonneoplastic: present	5 (55.6%)	7 (77.8%)	2 (22.2%)	1 (12.5%)	4 (44.4%)
Nonneoplastic: absent	4 (44.4%)	2 (22.2%)	7 (77.8%)	7 (87.5%)	5 (55.6%)
P	.74	.32	.72	.44	.73
PDAC: present	34 (47.2%)	42 (58.3%)	33 (45.8%)	20 (30.8%)	26 (36.1%)
PDAC: absent	38 (52.8%)	30 (41.7%)	39 (54.2%)	45 (69.2%)	46 (63.9%)
NET: present	3 (37.5%)	3 (37.5%)	3 (37.5%)	0 (0%)	2 (25.0%)
NET: absent	5 (62.5%)	5 (62.5%)	5 (62.5%)	6 (100.0%)	6 (75.0%)
P	.72	.46	.72	.17	.71

Abbreviations: CEC, circulating epithelioid cells; N/C, nuclear-to-cytoplasmic; NET, neuroendocrine tumor; PDAC, pancreatic ductal adenocarcinoma.

In the current study, we were unable to detect any significant differences in cytologic morphology between cells from these different diseases with regard to any of the cytologic characteristics investigated. In a study of patients with lung cancer using very similar cytologic criteria for malignancy, Hofman et al determined that malignant cytomorphologic CEC characteristics were common, but that they did not appear to represent the final histopathology of their tumors (ie, those from squamous carcinomas did not appear to have any evidence of squamous differentiation).[28] Although these authors did not issue descriptive statistics of their cytomorphology, the photomicrographs of the CECs isolated from these patients with lung cancer appear more or less indistinguishable from the CECs in the current study cohort, regardless of underlying disease.[28] It appears that CECs in ISET have a range of morphologic appearances, ranging from innocuous to frankly malignant; however, these appearances did not reliably distinguish benign versus malignant etiology in the cohort of patients with pancreatic disease in the current study. To demonstrate this point, the malignant-appearing cells shown in Figure 2B are actually from a patient with a benign IPMN, whereas the more banal-appearing cells shown in Figure 2C are from a patient with PDAC.

Although the vast majority of studies (including the current report) do not detect CECs in their control population, these patients typically are healthy adults lacking any known pathologic process.[5,20,28,29] In one study, the blood of 54 patients with benign colonic disease (diverticulosis, benign polyps, inflammatory bowel disease, etc) was examined using 2 different antibody-based epithelial cell isolation assays.[30] The authors found CECs in 11.3% and 18.9%, respectively, of the patients with benign colonic diseases and in none of their healthy controls. The positive rate partially depended on the assay used: 11.3% used the previously described CellSearch using anti-EpCAM and cytokeratin antibodies, and 18.9% used the EPISPOT (EPithelial ImmunoSPOT) assay, which isolated cytokeratin 19-positive viable cells after leukocyte depletion.[30] Another study examined CECs in a population of men at risk of developing prostate cancer. Using a differential centrifugation method followed by stains for prostate-specific antigen and racemase (which is commonly positive in patients with prostate cancer and negative in those with benign prostatic disease), they detected prostate-specific antigen-positive, racemase-negative CECs in 21 of 245 patients, none of whom were found to have developed carcinoma on follow-up.[31]

A large study using CellSearch found 1 CTC per 7.5 mL of blood (which is not technically positive) in 5.5% of healthy patients and 7.5% of patients with benign disease (which included benign breast disease, hypertension, diabetes, arthritis, thyroid disorders, hyperlipidemia, and asthma).[3] Although this also speaks to the importance of cutoff values on CEC enumeration for positive versus negative, it also suggests that underlying pathology may be an important factor for CEC positivity in patients with benign diseases. In the current study, and the previously mentioned prostate and colon CEC studies, the nonneoplastic pathology underlying CEC positivity often was inflammatory (pancreatitis, chronic prostatitis, and benign colonic disease) as opposed to the benign, predominantly

noninflammatory disorders in the large CellSearch study.[3,30,31] This supports that epithelial cells can be found in the circulation of patients with benign as well as malignant diseases, and that this rate of positivity may depend partially on the underlying disease process as well as on the assay used. However, the difficulty in parsing out these issues should not undermine the clinical importance of these CECs, which are a significant prognosticator in numerous cancers.

In many studies in which sequencing is performed on CECs found in patients with cancer, CECs often are clonally related to their original tumor and metastatic foci, suggesting that these cells are indeed tumor cells in transit.[12,32–35] Because these may be the cells that give rise to metastatic foci, it makes their molecular characterization useful, potentially predicting the molecular features of distant disease before it becomes clinically evident. The potential of CECs to shine light on the mutational profile of these critically important cells has been shown in a study in which whole-exome sequencing was performed on CECs, metastatic foci, and the primary tumor in a patient with metastatic prostate carcinoma.[12] The profile of CECs closely matched metastatic foci, suggesting that analysis of these cells provides insight into the true molecular profile of the most pathologic tumor cells, the ones that give rise to metastases.[12]

CECs have been described in many different carcinomas, and can be measured using various methods, including CellSearch, flow cytometry, microfluidic chips, and ISET. All these methods have certain strengths, and many have studies supporting their prognostic value in different carcinomas. The current study was limited in that additional studies were not performed to confirm the identity of the CECs isolated. Another limitation is that, although the characteristics we analyzed were defined objectively, morphologic analysis is an intrinsically subjective process. We attempted to minimize the inherent subjectivity by having all images reviewed by a pathologist who specializes in pancreatic cytopathology (M.B.P.), and chose characteristics such as the N/C ratio that have good reproducibility among pathologlists.[36]

ISET is an extremely promising modality in which low cost and ease of operation allow for scalability and the ability to perform ancillary studies (including immunohistochemistry, in situ hybridization, and gene sequencing) on isolated CECs. However, what the results of the current study demonstrate is that care is essential in all these methods, which may isolate morphologically similar nontumor cells in addition to tumor cells, especially within the context of other lesions or inflammatory conditions. The clinical context is important and ancillary testing of isolated CECs is critical for ensuring that the cells of interest are truly tumor derived and pathologically significant.

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CONFLICT OF INTEREST DISCLOSURES

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AUTHOR CONTRIBUTIONS

Matthew W. Rosenbaum: Conceptualization, methodology, validation, formal analysis, investigation, writing–original draft, writing–review and editing, and visualization. **Christy E. Cauley:** Methodology, investigation, resources, data curation, writing–review and editing, supervision, project administration, and funding acquisition. **Birte Kulemann:** Methodology, investigation, data curation, and writing–review and editing. **Andrew S. Liss:** Methodology, investigation, resources, data curation, writing–review and editing, and supervision. **Carlos Fernandez-del Castillo:** Resources and writing–review and editing. **Andrew L. Warshaw:** Investigation, resources, data curation, writing–review and editing, supervision, and project administration. **Keith D. Lillemoe:** Resources, writing–review and editing, and supervision. **Sarah P. Thayer:** Methodology, investigation, resources, writing–review and editing, supervision, and funding acquisition. **Martha B. Pitman:** Conceptualization, methodology, validation, formal analysis, investigation, resources, writing–original draft, writing–review and editing, and supervision.

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