



MICROBIOME IN PANCREATIC FLUID – PRELIMINARY ANALYSIS OF PANCREATIC CYSTIC LESIONS



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Abstract:

Introduction and objective

One of the main precursory lesions for pancreatic carcinoma is pancreatic cystic neoplasm. Differentiation between the various types of cysts is a clinical challenge. The microbiome colonizing the pancreatic cyst fluid is mostly unknown. The aim of the study was microbiological assessment of pancreatic cysts compared with biochemical parameters and histopathological results.

Material and methods

30 patients with pancreatic cysts undergoing surgery in 2022-2023 at the Department of General and Transplant Surgery, Medical University of Lodz, were enrolled in the study. Preoperative biochemical levels of blood parameters were analysed. Bacterial culture results were taken from the nasal vestibule, the skin of the groin, as well as from cyst fluid and bile (in case of cholecystectomy) and histopathological reports were analysed.

Results

Mean age was 58.77±13.56 years. 10 patients (33.3%) had malignant lesions. Nine patients (30%) had positive cultures from cyst fluid. 6 of them had malignant conditions. *Enterobacter cloacae*, *Enterococcus faecium* *Staphylococcus spp.* were found. In the malignant group, patients were statistically significantly older (68.40±5.70 y vs. 53.95±13.86 y, p=0.004), tumour diameters were smaller (4.00±2.00 cm vs. 8.50±5.77 cm, p=0.003) and CA 19-9 level were higher (100.22±186.46 ng/ml vs. 12.35±16.08 ng/ml, p=0.045) than in a benign group.

Conclusions

The occurrence of specific types of bacteria in patients with malignant pancreatic cysts appears to be of significant clinical importance. Further studies are needed.

Keywords: pancreatic cystic neoplasm, microbiome, pancreatic cancer, bacterial culture.

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive neoplasms. One of the main precursory lesions for PDAC are pancreatic cystic neoplasms (PCNs), including mucinous cystic neoplasm (MCN), intraductal papillary mucinous neoplasm (IPMN) and serous cystic neoplasm (SCN) [1, 2].

Due to the development of imaging techniques, the frequency of detection of pancreatic cystic tumors is increasing. It is estimated that pancreatic cystic tumors occur in 2% to 45% of the population [3]. Cystic lesions in the pancreas occur far more often than has been previously estimated [4].

Despite the novel diagnostic tools, differentiation between the various types of PCN is a significant clinical challenge [5]. The accuracy of identifying a specific type of pancreatic cystic tumor is between 40% and 95% for magnetic resonance imaging (MRI/MRCP) and between 40% and 81% for computer tomography (CT) [3]. Also, endoscopic ultrasonography (EUS) is not an optimal method to identify the type of pancreatic cystic tumor. Cytological analysis of the fluid from the cyst has 42% sensitivity and 99% specificity in differentiating mucinous and nonmucinous pancreatic cystic lesions [3].

As mentioned before, pancreatic cysts harbor the potential to develop into cancer. Currently, there are no optimal tools for this risk stratification, and identifying cysts that require complicated surgical treatment remains a challenge in this field. The risk of postoperative complications following pancreatic surgery is associated with a 30-40% morbidity rate and a 3-10% mortality rate [6]. Hence, the decision to operate should be clearly justified by medical indicators, such as cancer diagnosis or high risk of this cancer.

The microbiome colonizing the pancreatic cyst fluid is mostly unknown. However, some of the microbiomes with potentially detrimental functions residing in the pancreatic cystic fluid may contribute to the neoplastic process [7]. A comprehensive understanding of this phenomenon for pancreatic cancer development in pancreatic cysts is of great clinical significance.

Aim

The aim of the study was the microbiological assessment of pancreatic cysts in comparison with biochemical parameters and histopathological results.

Material and methods

The data of 30 patients with pancreatic cysts, aged 27 to 76 years, who underwent surgery in 2022 to 2023 year at the Department of General and Transplant Surgery, Medical University of Lodz, were analysed retrospectively. Informed signed consent for the operation and use of the data was obtained from all these patients. The bioethical commission of Medical University of Lodz approved this retrospective study (Number RNN/04/23/KE dated 10th of January 2023)

Preoperatively blood levels of hemoglobin, amylase, lipase, CRP, cancer markers (CA 19-9, CA 15-3, CEA, CA 125, AFP) and leukocytosis were measured. Before surgery, cultures were also taken from the nasal vestibule and the skin of the groin.

During the surgery samples of pancreatic cyst fluid and bile (in case of cholecystectomy) were taken for microbiological analysis. These samples for testing were taken in a 5 ml syringe with a 5 mm needle (*ex vivo*). Bacterial cultures were performed in the bacteriology laboratory of Barlicki Teaching Hospital in Lodz. Histopathological reports were also included and studied.

Statistical analysis was performed with the use of commercially available statistical software package (Statistica 13.1 for Windows; StatSoft Poland Ltd.).

Continuous variables were expressed as mean \pm SD, median, and minimum–maximum values. The normal distribution was verified with the Shapiro-Wilk test. The data was compared for statistical analysis using Student's t-test to evaluate differences between quantitative variables following a Gaussian distribution. Variables following a non-parametric distribution were compared with the Mann-Whitney test. Categorical data analysis was done using the Chi 2 test. Alpha < 0.05 was set as a threshold of statistical significance.

Microbiological procedures

Samples of bile and pancreas cyst fluid (PCF) were used for microbiological examinations. The inoculations were performed according to the EUCAST (European Committee on Antimicrobial Susceptibility Testing) guidelines (eucast.org). Tested specimens were inoculated onto Columbia agar with 5% sheep blood medium (bioM4rieux, Marcy l'Etoile, France) that supports growth of a variety of bacteria, MacConkey agar with crystal violet (bioM4rieux, Marcy l'Etoile, France) which allows growth of only Gram-negative bacteria, and Sabouraud gentamicin chloramphenicol 2 agar (bioM4rieux, Marcy l'Etoile, France) which allows growth of fungi. The inoculations on Columbia agar and MacConkey agar were incubated at 35°C for up to 2 days, whereas the inoculations on Sabouraud agar were incubated at 35°C for up to 7 days. Bacterial and fungal species identification and susceptibility testing were performed using a VITEC®2 Compact (bioM4rieux, Inc. Hazelwood, MO, USA).

Results

A total of 30 adult patients (18 female and 12 male) were included in the study. Baseline variables are shown in Table 1.

Among patients who had surgery, 11 (36.6%) underwent distal pancreatectomy with splenectomy, 3 (10%) patients underwent Whipple pancreatoduodenectomy, 5 (16.6%) patients palliative surgery, 2 (6.6%) enucleation of the tumor, 4 (13.2%) operative percutaneous drainage and 5 (16.6%) patients anastomosis of the pancreatic cyst with the small intestine. Cholecystectomy was performed in 10 (33.3%) patients.

Table 1. Baseline variables in the studied group.

Variable	mean±SD	Median (IQR)	min	max
Age	58.77±13.56	62.50(47.00-68.00)	27.00	76.00
Size of tumor (cm)	7.00±5.27	6.50(3.00-10.00)	2.00	30.00
CA 19-9 (U/ml)	41.64±112.85	7.90(5.70-18.70)	0.80	567.10
CA 15-3 (U/ml)	42.32±134.93	12.70(6.10-16.70)	3.70	599.00
CA 125 (U/ml)	13.78±14.35	8.80(6.00-14.00)	4.60	65.60
CEA (ng/ml)	3.34±7.25	1.25(0.90-2.80)	0.40	35.10
AFP (ng/ml)	3.92±2.92	2.65(2.00-4.90)	0.90	10.10
Hb (g/dl)	13.26±1.65	13.50(12.60-14.00)	9.10	16.50
WBC (x103 /μl)	7.86±3.75	6.80(5.40-9.10)	3.60	20.10
CRP (mg/l)	15.89±42.69	2.45(1.30-6.70)	0.20	204.50
Amylase (IU/l)	80.04±111.58	51.00(35.00-94.00)	19.00	593.00
Lipase (U/l)	55.10±62.52	29.00(15.00-67.00)	4.00	277.00
Days of hospitalisation	12.27±6.67	9.00(8.00-17.00)	3.00	27.00

CA - cancer antigen, CEA - carcinoembryonic antigen, AFP - alpha fetoprotein, Hb - haemoglobin, WBC - white blood cell count, CRP - C-reactive protein, SD - standard deviation, IQR - interquartile range

Mean age was 58.77 years (SD 13.56 years). The mean diameter of the tumor was 7,00±5,27 cm. In 5 patients (16.6%) the cyst was localized in the head of the pancreas, in 11 patients (36.6%) in the corpus, and in 14 patients (46.6%) in the tail of the pancreas. In the postoperative examination, 10 patients (33.3%) had malignant lesions while the rest of the 20 patients (66.7%) had benign cysts (Table 2).

Three patients (10%) had negative culture taken from the nasal vestibule. In the rest participants 24 had *Staphylococcus epidermidis*, three had *Staphylococcus aureus* MSSA and two patients had *Corynebacterium pseudodiphtheriticum*.

Table 2. Histopathological results.

Type of tumor	Number of patients	Malignant
Pseudocyst	9	No
Inflammatory tumor	1	No
Choledochal cyst	1	No
Cystic pancreatic adenocarcinoma	4	Yes
IPMN without dysplasia	4	No
IPMN with neuroendocrine tumor G1	1	Yes
IPMN with moderate grade dysplasia	1	Yes
IPMN with high grade dysplasia	2	Yes
IPMN with carcinoma	1	Yes
Cystic neuroendocrine tumor G1	1	Yes
MCN without dysplasia	3	No
MCN with low grade dysplasia	1	No
SCN	1	No

IPMN - intraductal papillary mucinous neoplasm, G1 - grade 1, MCN - mucinous cystic neoplasm, SCN - serous cystic neoplasm

In the skin of the groin three cultures were negative, in twenty-four cases *Staphylococcus epidermidis* was found, in three *Staphylococcus aureus* and in one case *Corynebacterium pseudodiphtheriticum* was detected.

Four patients out of ten undergoing cholecystectomy had positive culture: in three cases *Escherichia coli*, in one *Klebsiella pneumoniae*, in one *Enterobacter cloacae* and in one *Enterococcus faecium* VRE (Vancomycin-Resistant *Enterococcus* resistant to ampicillin, imipenem, levofloxacin).

Nine patients had positive culture from the cyst fluid: one *Corynebacterium*, one *Klebsiella pneumoniae*, one *Staphylococcus aureus*, one *Staphylococcus epidermidis*, one *Enterobacter cloacae*, one *Enterococcus faecalis*, one *Escherichia coli*, one *Enterococcus faecium* VRE, one *Staphylococcus capitis* and one *Staphylococcus haemolyticus*.

Patients were divided into the benign and malignant cyst group according to the postoperative histopathological result and into positive and negative culture group according to bile and pancreatic cyst fluid culture result. In the group of benign cyst tumor diameter was larger than in malignant group (8.50±5.77 cm vs. 4.00±2.00 cm, p=0.003). The difference was statistically significant. In malignant group patients were older than in benign group (68.40±5.70 y vs. 53.95±13.86 y, p=0.004). CA 19-9 level was higher in malignant group than in benign group (100.22±186.46 U/ml vs. 12.35±16.08 U/ml, p=0.045), which was statistically significant. In negative culture group AFP level was statistically significantly higher than in positive culture group (4.56±2.99 ng/ml vs. 1.68±0.84 ng/ml, p=0.038).

Discussion

In the current study, it was established that 9 out of 30 (30%) patients' PCF samples harbored a microbiome. It is especially worth noting that 6 of these patients had malignant conditions (Table 3).

Table 3. Microbiome and tumors.

	Microbiome found in PCF (Pancreatic Cystic Fluid)	Neoplasm (Benign/ Malignant)
1	Corynebacterium	Benign
2	Klebsiella pneumoniae	Benign
3	Enterobacter cloacae resistant to amoxicillin, clavulanic acid, cefuroxime axetil	Malignant
4	Enterococcus faecalis susceptible to ampicillin, gentamicin HC, teicoplanin, vancomycin, linezolid, Escherichia coli resistant to amoxicillin, clavulanic acid mic 16	Malignant
5	Staphylococcus epidermidis resistant to erythromycin and clindamycin	Malignant
6	Staphylococcus capitis not resistant	Malignant
7	Enterococcus faecium HLAR linezolid mic 2	Benign
8	Staphylococcus aureus	Malignant
9	Staphylococcus haemolyticus tetracycline mic 2, tigecycline mic 0.25, vancomycin mic 2	Malignant

mic - minimum inhibitory concentration

A total of 10 patients out of 30 had malignant conditions and in 4 (out of 10) no microbiota was found in their PCF.

In a previous study [7], *Bacillus*, *spp. Fusobacterium*, *Acinetobacter spp.*, *Anaerococcus spp.*, *Staphylococcus spp.*, *Escherichia spp.*, *Faecal bacterium*, and *Shigella* (among other) were confirmed in the PCF. Patients with both malignant and benign cysts (IPMN, pseudocysts, MCM, and SCA) participated in this study. Likewise, *Staphylococcus spp.*, *Escherichia spp.*, and *Faecal bacterium* were found in the group of patients in this study.

So far, the human gut microbiome was suggested to be an important environmental factor linked to the development of different intestinal and extra-intestinal malignancies [8-10]. In the stomach, *Helicobacter pylori* can initiate a cascade of molecular events finally leading to cancer. In the colon, *Fusobacteria nucleatum* has been linked to the mucosal dysplasia [10].

Furthermore, *Fusobacterium nucleatum* was found in pancreatic cyst fluid [7, 11] and was associated with the transformation of LGD (low-grade dysplasia) IPMN to HGD (high-grade dysplasia). *F. nucleatum* is a gram-negative, anaerobic oral bacteria that commonly resides in saliva. Equally, we found presence of well-known group of bacteria *Staphylococcus* in fluid of pancreatic cysts with malignant potential.

Only few studies worldwide have demonstrated that bacteria in the pancreatic fluid can render cancer chemotherapy less efficient since it can metabolize anti-cancer drugs [12, 13]. In a previous study on mice, the pancreatic cancer became resistant to gemcitabine [14], a commonly used drug in many countries for the treatment of PDAC. Various bacteria, including, *Enterobacter cloacae*, *Klebsiella pneumonia*, and *Escherichia Coli* responsible for this resistance were found in the examined pancreatic microbiome. These studies highlight the significance and importance of further analysis of the microbiome in pancreatic lesions.

Conclusion

In our one-centre retrospective study, the confirmed presence of specific types of bacteria in patients with malignant pancreatic cysts appears to be of significant

clinical importance. Further studies to assess pancreatic cysts microbiomes and its influence on cancerogenesis are needed.

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