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Dragan Nikolić[1,2,♣], Stojan Latinčić[1,3], Miloš Stojanović [1,2], Nikica Grubor[1,3], Lazar Ranin[1,4], Brian Nation[5]

Routes and types of microbial infection in the pathology of pancreatic adenocarcinoma

Путеви и типови микробиолошке инфекције у патологији аденокарцинома панкреаса

[1]University of Belgrade, Faculty of Medicine, Belgrade Serbia;
[2]University Clinical Centre of Serbia, Clinic for Endocrinology, Diabetes and Metabolic Diseases, (Laboratory for Human Pancreatic Islets Culture), Belgrade, Serbia;
[3]University Clinical Centre of Serbia, Clinic of Surgery, Institute of Digestive Diseases, Belgrade, Serbia;
[4]University of Belgrade, Faculty of Medicine, Institute of Microbiology and Immunology, Belgrade, Serbia
[5]Institute of Biomedical Science, 12 Coldbath Square, EC1R 5HL London, United Kingdom

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♣Correspondence to:
Dragan NIKOLIĆ
Department: Clinic for Endocrinology, Diabetes and Metabolic Diseases- Laboratory for Human Pancreatic Islets Culture
Dr Subotica 13, 11000 Belgrade, Serbia
E-mail: **dragannikolic8@yahoo.com**

Routes and types of microbial infection in the pathology of pancreatic adenocarcinoma

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SUMMARY

Introduction/Objective Pancreatic cancer may be accompanied by infections caused by various microorganisms. It is uncertain wheatear pancreatic infection precedes development of cancer or vice versa.

The aim of this study is to analyze routes of infections from duodenum through pancreatic duct to determine what types of microorganisms can get through this duct into pancreas and nearby tissue.

Methods In patients subjected to cephalic duodenopancreatectomy sec. Whipple due to adenocarcinoma of papilla Vateri, duodenum or head of the pancreas, swabs from duodenal mucosa, pancreatic duct and pancreatic tissue at the line of the resection, were taken. Microscopic slides were prepared directly from patients' specimens and also from colonies on culture plates and both were Gram stained.

Results *Candida* was present in all three types of swabs (duodenum, pancreatic duct and tissue), while bacteria, depending of the species (*Pseudomonas aeruginosa*. *α hemolytic Streptococcus, Coagulase Negative Staphylococcus, Enterococcus spp, Serratia spp)*, were present in pancreatic duct or tissue, but not in duodenum.

Conclusion There is connection between presence of microorganisms and pathology of the pancreatic adenocarcinoma. Results show that Candida infection originates from duodenum, while bacterial infections originate directly from blood or tissue injuries.

Keywords: Candida albicans; Pseudomonas aeruginosa; pancreatic adenocarcinoma; pancreas infections; duodenum

САЖЕТАК

Увод/циљ Рак панкреаса може бити праћен инфекцијама изазваним различитим микроорганизмима. Није сигурно да ли инфекција панкреаса претходи развоју рака или обрнуто.

Циљ ове студије је да анализира путеве инфекција из дванаестопалачног црева кроз канал панкреаса да би се утврдило које врсте микроорганизама могу да прођу кроз овај канал у панкреас и оближње ткиво.

Методе Код пацијената подвргнутих цефаличној дуоденопанкреатектомији сек. Випл због аденокарцинома папиле Ватери, дванаестопалачног црева или главе панкреаса, узети су брисеви слузнице дванаестопалачног црева, канала панкреаса и панкреаса на линији ресекције. Микроскопски дијапозитиви су припремљени директно од узорака пацијената, а такође и из колонија на плочама за узгој, а оба су обојена по Gramu.

Резултати Кандида је била присутна у све три врсте брисева (дванаестопалачном цреву, каналу панкреаса и ткиву), док су бактерије, зависно од врсте (*Pseudomonas aeruginosa*. *α hemolytic Streptococcus, Coagulase Negative Staphylococcus, Enterococcus spp, Serratia spp)* биле присутне у панкреасном каналу или ткиву, али не и у дванаестопалачном цреву.

Закључак Постоји веза између присуства микроорганизама и патологије аденокарцинома панкреаса. Резултати показују да инфекција кандидом потиче из дуоденума, док бактеријске инфекције потичу директно из крви или из ткивних повреда.

Кључне речи: *Candida albicans*; *Pseudomonas aeruginosa*; аденокарцином панкреаса; инфекције панкреаса; дуоденум

INTRODUCTION

Pancreatic cancer belongs to the group of cancer with high lethal outcome. Symptoms are often detected very late and cure rates are very low. Development of the pancreatic cancer is often preceded by inflammation, such as pancreatitis, which increases the chance of tumor

development. Infections can worsen acute pancreatitis by inflammation expansion and tissue necrosis [1].

It is well known that certain pathological conditions such as acute pancreatitis, necrotic pancreatitis and cysts are caused by Gram-positive bacteria (74%), Gram-negative bacteria (21%), Enterobacter spp. (up to 58%) and Candida albicans (5–24%). Pancreatic infections can also be caused by infected venous catheter, urinary tract, tracheal mucosa and bile. The fact that Candida infection of pancreatic pseudocysts can lead to sepsis or even death due to organ failure shows the perils of infection with this organism [2]. Infections with Candida spp. caused by spontaneous perforation or surgical opening of the gastrointestinal tract may increase general mortality [3].

Pancreatic infections mostly originate from the duodenum. Some studies showed infections of the pancreatic duct with Candida [4, 5]. Data show that microorganisms from the intestine can access the pancreas in three ways: directly from the duodenum via the pancreatic duct [1, 4], penetration into the body cavity due to injuries, and via blood.

So, how do microorganism from the intestine get to the pancreas. There are some pathological conditions that facilitate penetration of microorganisms from the intestine into the pancreas such as decreased secretion of pancreatic juice (in acute and chronic pancreatitis), weakening of the sphincter of Oddi (often present in elderly populations), anatomical changes in the gastrointestinal tract, tumors or abundant and frequent food intake that may cause stretching of the stomach and intestine, thus leaving the sphincter of Oddi opened or dilated [6, 7].

The main aim of this study is to analyze routes of infections from the duodenum through the pancreatic duct to determine what types of microorganism can gain access through this duct into the pancreas and nearby tissue. Another aim is to establish a possible connection between infection and the pathology of pancreatic adenocarcinoma.

METHODS

In patients subjected to Whipple's procedure (duodenopancreatectomy) [8] due to adenocarcinoma of the ampulla of Vater, duodenum or head of pancreas, swabs from the duodenal mucosa at the line of the resection, from the pancreatic duct and pancreatic tissue were taken. All surgical procedures were performed at the First Surgical Clinic, University Clinical Center of Serbia. All subjects enrolled in this research have responded to an informed consent which has been approved by local Ethics Committee on Human Research and that this protocol has been found acceptable by them. From January to June 2018, 24 patients were operated, 16 men and eight women, aged 58–65 years. Patients were from the city of Belgrade. Microbiological analyses were performed by standard procedure at the Institute for Microbiology, Faculty of Medicine, Belgrade. Microbiology isolates were identified on the basis of microscopic, cultural and biochemical properties. Microscopy slides were prepared directly from patient specimens and also from colonies on culture plates, and then Gram stained. We used Columbia blood agar plates (7% sheep blood), MacConkey agar and XLD agar plates, and Sabourand dextrose agar. Depending on the cultural and morphological characteristics of the microorganisms isolated, we prepared a small series of biochemical tests.

RESULTS

Results are shown in Table I. Swab analysis showed no pathogenic microorganisms in duodenum except for Candida in (16.7%) cases. Pancreatic duct was infected with α-hemolytic *Streptococcus,* coagulase-negative *Staphylococcus*, *Enterococcus* spp., *Rhodotorula rubra*, and *Serratia spp*. (8.3%) and in (25%) *Candida albicans* was detected. In pancreatic tissue, the presence of α-hemolytic *Streptococcus*, coagulase-negative *Staphylococcus*, *Enterococcus* spp.

and *Serratia* spp. was established (8.3%). The majority of the cases were infected with *Pseudomonas aeruginosa* (33.3%) and *Candida albicans* (25%).

DISCUSION

Results presented here suggest that pancreatic infection with *Candida* spp. originates from duodenum, as it was detected in all three areas swabbed. Similar results from other studies support this finding [4, 5]. *Candida albicans* has high adaptability as illustrated by possession of different adhesins, as well as the presence of hyphae, pseudohyphae and yeast-like cells that allow colonization and infection of almost all body sites.

Candida albicans is a commensal and a constituent of the normal microflora in 80% of the human population. It predominately colonizes the mucosal surfaces of the gastrointestinal tract, genitourinary tract and, to a lesser extent, the skin. It can cause superficial but also systemic and potentially life-threating infections, especially in immunocompromised patients (e.g., cancer chemotherapy, AIDS) [9] or after long-term antibiotic therapy [10].

Immunological response to *Candida* infection in humans is unclear. It is not likely that the yeast growth correlates directly with activation of gut-associated lymphoid tissue (GALT). Saprophytic bacteria normally present in the intestine have considerable influence and antibiotic use can disrupt this relationship, decreasing the number of saprophytic bacteria that normally do not allow propagation and overgrowth of *Candida*, hence reducing the chance of entry to the duodenum.

Recent studies [11] revealed that the prevalence of fungi increases up to 3000 times in pancreatic ductal adenocarcinoma (PDA) in humans compared to normal pancreatic tissue. *Candida, Saccharomyces* and *Malassezia* predominate in the mycobioma. These fungi were isolated and transferred to experimental mice causing PDA. Ablation of mycobiomes in mice

slowed the growth of invasive forms of the cancer with the simultaneous use of certain chemotherapy. Although the research points out *Malessia spp* as a possible oncogenic factor, the data are not clear. Proposed hypothesis is that Fungal-MBL axis (ligation of mannose-binding lectins) promotes PDA progression by complement activation.

Regarding the presence of bacteria in pancreatic cancer, *Pseudomonas aeruginosa* was present only in pancreatic tissue. This may suggest that bacteria gained entry to the pancreas through injury or through the blood. It is possible that bacteria are retain only in pancreatic tissue and not in the duodenum or pancreatic duct, which were not suitable for their growth. Presence of *Rodotorula rubra* and *Enterococcus* and *Serratia* only in the pancreatic duct does not mean that they will not spread to pancreatic tissue.

Penetration of bacteria from the intestine into the bloodstream is best illustrated in patients who consume excess alcohol. Heavy alcohol use affects gut microflora composition, leading to increased permeability of the intestine, thus permitting pathobionts to gain access to the bloodstream and other, distant organs. For example, alcohol consumption, a common cause of chronic pancreatitis, is related to dysfunction of the intestinal barrier and overgrowth of Gram-negative bacteria. Regular alcohol consumption also affects the normal function of the sphincter of Oddi, preventing its closure.

Commensal microbiota may play a role in the onset of pancreatic inflammation [12]. Moreover, intestinal microbiota has a synergistic, interactive effect during inflammation. Damage to the pancreas increases intestine permeability [13], causing ischemia and also overgrowth of intestinal bacteria and their translocation in the pancreas, where they provoke secondary infections. Furthermore, infection of necrotic pancreatic tissue is one of the main causes of mortality in acute pancreatitis [14, 15].

Substantial numbers of preclinical and clinical results suggest that bacterial influence on this process is by activation of immune receptors and prolongation of cancer-associated

inflammation. The most recent research suggests that disruption in the commensal bacterial population can affect the inflammatory process and some diseases, including carcinogenesis [16, 17]. *Chlamydia trachomatis* infection has been associated with an increased risk of the development of invasive cervical carcinoma [18]. Bacteremia and endocarditis due to *Streptococcus bovis* have likewise been linked with malignancies in the colon [19], and *Helicobacter pylori* infection is considered a causative agent for both gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphomas [20, 21]. Moreover, several mechanisms by which different bacteria may play a role in cancer development have been proposed, such as through the induction of chronic inflammation, by interference, either directly or indirectly, with eukaryotic cell cycle and signaling pathways [22, 23, 24].

CONCLUSIONS

Based on the data presented here, we can assume that microorganisms isolated in this study have a role in the development of pancreatic adenocarcinoma. It is also possible that infections are consequences of pancreatic diseases such as pancreatic cancer. However, further research of these issues is required in order to provide support for these hypotheses.

There are some indications that the presence of certain microorganisms may play a role in the pathogenesis of pancreatic adenocarcinoma. Based on the results presented here, we can conclude that the origin of infection in pancreatic cancer is from the duodenum (i.e., *Candida* infection), through body injuries or via the bloodstream (bacterial infections).

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Table 1. Results of swabs of the duodenum, pancreatic duct and pancreatic tissues; microorganisms were detected in only one swab taken from the site indicated in the table; where there are more positive results, they are expressed as a percentage of the total number of swabs taken

Swabs	I	II	III
Location	Duodenum	Duct	Tissue
Pseudomonas aeruginosa	-	-	33%+
α-hemolytic *Streptococcus*	-	+	+
Coagulase-negative *Staphylococcus*	-	+	+
Enterococcus spp.	-	+	+
Candida albicans	16.7%+	25%+	25%+
Rhodotorula rubra	-	+	-
Serratia spp.	-	+	+

