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Original article

Spectrum of microorganisms in infected walled-off pancreatic necrosis – Impact on organ failure and mortality

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ABSTRACT

Objective: Data on the microbial spectrum in infected pancreatic necrosis are scarce. Only few studies have addressed this issue in a larger, consecutive group of patients treated by a standardized algorithm. Since 2005 endoscopic, transmural drainage and necrosectomy (ETDN) has been the treatment of choice for walled-off necrosis in our centre. The present study evaluated the microbial spectrum of infected pancreatic necrosis and the possible relationship between infected necrosis, organ failure, and mortality. Furthermore, we investigated whether the aetiology of pancreatitis, use of external drainage, and antibiotic treatment influenced the microbial findings.

Methods: Retrospective review of medical charts on 78 patients who underwent ETDN in our tertiary referral centre between November 2005 and November 2011.

Results: Twenty-four patients (31%) developed one or more organ failures, 23 (29%) needed treatment in the intensive care unit (ICU), and 9 (11%) died during hospital admission. The prevailing microbial findings at the index endoscopy were enterococci (45%), enterobacteriaceae (42%), and fungi (22%). There was a significant association between the development of organ failure ($p < 0.001$), need of treatment in ICU ($p < 0.002$), in-hospital mortality ($p = 0.039$) and infected necrosis at the time of index endoscopy. Enterococci ($p < 0.0001$) and fungi ($p = 0.01$) were found more frequently in patients who died during admission as compared to survivors.

Conclusion: Different microbes in pancreatic necrosis may influence the prognosis. We believe that a detailed knowledge on the microbial spectrum in necrotizing pancreatitis may be utilized in the treatment to improve the outcome.

Introduction

The early phase of severe acute pancreatitis (SAP) is characterized by an extensive systemic inflammatory response syndrome, which is the major cause of organ failure and death in this phase [1]. Later follows an anti-inflammatory state characterized by inhibition of the immune system and an increased susceptibility to infectious complications, both bacteraemia and infected pancreatic necrosis. Infected necrosis is a major cause of death in the late phase of severe pancreatitis [2,3].

However, data on the microbial spectrum of infected pancreatic necrosis are scarce. It has been suggested that Gram-negative bacteria are the predominant microbial finding [4], but only few studies have addressed this issue in a larger, consecutive group of patients treated by a standardized algorithm [5]. Recently, a shift from a Gram-negative to a Gram-positive microbial flora has been reported [6]. Furthermore, to our knowledge, no studies have directly addressed the issue of microbial findings in the late phase of necrotizing pancreatitis with development of walled-off pancreatic and peripancreatic necrosis (WON). The aim of the present retrospective, single-centre study was to evaluate the microbial spectrum of infected pancreatic necrosis in patients undergoing endoscopic, transmural drainage and necrosectomy (ETDN) for WON, and to evaluate the possible relationship between infected necrosis, organ failure and in-hospital mortality. Furthermore, we aimed to investigate whether the microbial spectrum

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changes during the course of ETDN treatment, and whether the aetiology of pancreatitis, the use of external drainage, and antibiotic treatment influenced the microbial findings.

Methods

We retrospectively reviewed the medical charts on all patients who underwent ETDN in our tertiary referral centre between November 2005 and November 2011. Concomitantly, we retrieved the charts from the referring hospitals in order to obtain information on the patients' previous health status, disease course, microbiological findings, and antibiotic treatment.

All patients had a well documented episode of acute pancreatitis based on the Atlanta criteria leading to WON [7]. The indication for endoscopic intervention was persistently symptomatic collections despite either optimal conservative treatment, percutaneous drainage, or in few cases also surgical treatment. Symptoms included infection, pain, gastric outlet obstruction, bile duct obstruction, and leakage (e.g. ascites).

Endoscopic procedure

Endosonography-guided, transgastric or transduodenal drainage was performed using a curve-linear echoendoscope (Olympus GF-UCT140-AL5/Aloka SSD-5000) by: 1) needle puncture (ECHO-19; Cook Medical), 2) fluid aspiration for microbiological diagnostics, 3) insertion of a guidewire (0.035 ″ Dreamwire; Boston Scientific) through the needle, 4) needle knife incision over the wire (Huibregtse Triple Lumen; Cook Medical), 5) balloon dilatation of the tract (CRE Wireguided 12–20 mm; Boston Scientific), 6) placement of two double pigtail stents (Zimmon 7 Fr/6 cm, Cook Medical) and a nasocystic catheter (7-Fr nasal biliary drainage set; Cook Medical) for subsequent irrigation of the cavity, and 7) endoscopic debridement of loose necrotic material using a therapeutic gastroscope (Olympus GIF-1TQ160/XTQ160) and either tripod, stone retrieval basket, or polypectomy snare (used in most cases).

A small dilatation balloon diameter was usually chosen at the index procedure and when there was an increased risk of bleeding from collaterals e.g. in cases with splenic vein thrombosis. Endoscopic necrosectomy was usually not performed during the index procedure. Additional placement of percutaneous catheters was done in cases of widely expanding peripancreatic collections that were not accessible by endoscopic route alone. All endoscopies were performed with CO_2 insufflation.

Irrigation of the collections through nasocystic and/or percutaneous catheters was done 3–6 times a day. The irrigation volume depended on the size of the collection, usually 100–250 mL per procedure. The endoscopic sessions with redilatation of the transmural tract and debridement were repeated at weekly intervals until the necrotic cavity was free of debris and vital granulation tissue was seen. At that time the nasocystic and/or percutaneous catheters were removed.

As a part of the endoscopic treatment, fluid from the collections was aspirated for microbiological diagnostics before further endoscopic intervention. The fluid was cultured both for aerobic, anaerobic, and fungal organisms.

The occurrence of organ failure was noted using the following definitions: 1) Circulatory failure: need for inotropic support or temporal pacemaker, 2) respiratory failure: need for mechanical ventilation, 3) renal failure: need for haemofiltration, and 4) gastrointestinal failure: absence of bowel sounds and intestinal paralysis on diagnostic imaging.

The severity of the disease at the time of index endoscopy was assessed by Sequential Organ Failure Assessment (SOFA) [8] and both Computed Tomography Severity Index (CTSI) [9], and modified CTSI [10].

Statistics

All data are expressed as median and interquartile ranges (IQR). Chi-square test was used to analyse categorical data.

Differences were judged as statistically significant if the p value was less than 0.05.

Univariate and multivariate regression was used to evaluate predictors of in-hospital mortality, development of organ failure and need for ICU. The results of the regression analysis are presented as odds ratios (OR) with 95% confidence intervals (CI).

Results

Base-line characteristics and severity assessment

Base-line data at the time of index endoscopy are presented in Table 1.

Twenty-four (31%) patients developed one or more organ failures. Twenty-three (29%) patients needed treatment in the ICU. Nine patients (11%) died during hospital admission.

Procedure-related complications were seen in 10 patients (12%) and included one procedure-related death due to development of peri-procedural septic shock and multi-organ failure. Despite aggressive fluid resuscitation, administration of broad-spectrum antibiotics and inotropics in the ICU, the patient died the day after the index endoscopy. Four patients experienced bleeding from the necrosis cavity managed by embolization, four experienced pneumoperitoneum without need of intervention or treated with needle aspiration, and one patient bleeding from the transmural tract treated with epinephrine injection and red blood cell transfusion.

Microbial assessment at the index endoscopy

Culture proven infected necrosis was demonstrated at the index endoscopy in 55 out of 78 (71%) patients. A single microbial species was found in 27 patients (49%), two species were found in 21

Table 1
The base-line data of 78 patients with walled-off necrosis treated by endoscopic, transmural drainage and necrosectomy.

	Total $N = 78$	Sterile necrosis $N = 23$	Infected necrosis $N = 55$	p-value
Gender, males/females	49/29	13/10	36/19	0.458
Age, median (IQR[a])	54 (40–63)	44 (38–57)	55 (41–65)	0.035
BMI, median (IQR)	27 (24–30)	27 (24–30)	27 (24–31)	0.927
Etiology, *n*				0.779
- alcohol	26	8	18	
- gallstone	33	8	25	
- other	19	7	12	
Time from onset of symptoms to index endoscopy, days, median (IQR)	44 (29–61)	49 (24–85)	44 (29–54)	0.683
Antibiotic treatment prior to index endoscopy, *n*	54	14	40	0.301
External drainage and/or fine-needle aspiration prior to index endoscopy, *n*	40	10	30	0.373
CTSI[b], median (IQR)	7 (5–9)	7 (5–8)	7.5 (5–9)	0.40
Modified CTSI, median (IQR)	9 (8–10)	10 (8–10)	8 (8–10)	0.663
SOFA[c]-score, median (IQR)	3 (0–9)	1 (0–3)	4 (2–10)	0.0004

[a] IQR = interquartile range.
[b] CTSI = computed tomography severity index.
[c] SOFA = sequential organ failure assessment.

patients (38%), three in three patients (5%), four in three patients (5%), and five species in one patient (2%). The prevailing microbial findings at the index endoscopy were enterococci (45%), *Enterobacteriaceae* (42%), and fungi (22%), see Table 2.

Two of the *Escherichia coli* strains were extended spectrum beta-lactamase (ESBL) producing, which was also the case for four of the *Klebsiella pneumoniae* strains. No vancomycin resistant *Enterococcus* strains were found. No methicillin resistant *Staphylococcus aureus* strains were found. No anaerobes were identified at the index endoscopy.

Change in the microbial flora from index to second endoscopy

Of 23 patients with sterile necrosis at the index endoscopy, 11 were infected at the second endoscopy. Another 11 patients were not re-cultured and one continued to have a sterile necrosis. Of the 11 secondarily infected cases, nine received antibiotics between the index and the second endoscopy (four received antibiotics that covered the findings at the second endoscopy and five did not). Four were monomicrobial and seven multimicrobial. Overall 21 microbes were isolated (six fungi, five coagulase negative staphylococci, four non-haemolytic streptococci, three enterococci, one *E. coli*, one *Stenotrophomonas maltophilia*, and one *S. aureus*).

Of 55 patients with infected necrosis at the index endoscopy, 33 were re-cultured and 22 were not. Thirty-one patients were still infected at the second endoscopy, whereas in two patients the subsequent culture was negative. Of the 31 patients with infected necrosis at second endoscopy, 23 had been treated adequately with antibiotics according to the microbiological findings at the index endoscopy. In nine of these, the primary microbe was eliminated but another microbe cultured at second endoscopy, in 10 the same microbe was found, and in four patients additional microbes were cultured. In two cases antibiotics were not given and in both cases the same microbes were found at both index and second endoscopy. The overall change in the isolated microbes from index to second endoscopy is shown in Table 2.

Overall, 14 cases of fungal infection were identified at the second endoscopy. Of these, only one was treated with antifungals between index and second endoscopy. On the other hand, 10 patients received antifungals between index and second endoscopy, and in only one of these fungi were identified at second endoscopy.

Microbial assessment throughout the disease period

Overall, cultures from WON were performed 161 times resulting in 272 different isolates. The median time between two culturing episodes was 11 days (IQR 7–19). Enterococci were the predominant finding (found in 41% of the cultures) followed by *Enterobacteriaceae* (34%), and fungi (25%). The frequency of enterococci and fungi increased during the disease period, see Table 2.

Polymicrobial findings were more common at the second (and third) endoscopy as compared to the index endoscopy (74% vs. 51%, $p = 0.02$).

Table 2
Microbial findings at six consecutive endoscopies in 78 patients treated by endoscopic, transmural drainage and necrosectomy.

	Endoscopy number					
Microbial findings at endoscopy, *N* (%)[a]	1	2	3	4	5	6
Enterococci	25 (45)	20 (48)	8 (53)	5 (63)	6 (86)	1 (25)
Enterobacteriaceae	23 (42)	11 (26)	7 (47)	5 (63)	5 (71)	2 (50)
Fungi	12 (22)	14 (33)	9 (60)	3 (38)	2 (29)	–
Non-haemolytic streptococci	10 (18)	10 (24)	4 (27)	2 (25)	–	1 (25)
Coagulase negative staphylococci and Corynebacterium species	10 (18)	18 (43)	7 (47)	5 (63)	2 (29)	1 (25)
Pseudomonas species and *Stenotrophomonas maltophilia*	4 (7)	4 (10)	1 (7)	2 (25)	1 (14)	–
Various	4 (7)	3 (7)	2 (13)	–	1 (14)	–
Monomicrobial/polymicrobial, N	27/28	11/31	4/11	2/6	1/6	3/1
Number of cases with infected/sterile necrosis	55/23	42/3	15/0	8/2	7/0	4/0

[a] *N* = number of patients with specific microbial finding; % = percentage of patients with infected necrosis with specific microbial finding.

Impact of antibiotic treatment on the microbial spectrum

Fifty-four (69%) patients received antibiotics before the index endoscopy, of which 47 received more than one antibiotic. Metronidazol was given in 37 patients, piperacillin and ciprofloxacin in both 19, meropenem in 18, and cephalosporins in 14 patients. Antifungals were given in 16 patients.

The rate of infected necrosis did not differ between patients who received antibiotics before the index endoscopy as compared to those who did not (74% vs. 63%, $p = 0.64$). This was also the case for patients who were not externally drained or had fine needle aspiration (FNA) before the index endoscopy (69% vs. 58%, respectively, $p = 0.51$). However, in the group treated with antibiotics before the index endoscopy, enterococci were found significantly more often than in the group without antibiotics (41% vs. 13%, $p = 0.01$). Furthermore, fungi were found in 20% of the cases in the antibiotics group as compared to only 4% in the non-antibiotics group, although this difference did not reach statistical significance ($p = 0.07$).

Impact of external drainage on the microbial spectrum

Percutaneous drainage, FNA, or both was done before the index endoscopy in 42 (52%) patients. At the time of external drainage/FNA, the WON was sterile in 29 patients (69%), and infected in 10 patients (24%). In three patients (7%) culture was not performed. There was no significant difference in relation to the infectious state of necrosis at time of the index endoscopy between patients who were externally drained or had FNA performed, as compared to those who did not (75% vs. 66%, $p = 0.37$). Of the 29 cases with sterile necrosis on external drainage/FNA, however, 23 (79%) were found infected at the index endoscopy. The median time from external drainage/FNA to index endoscopy was 27 days (5–103). In 7 out of the 10 cases with infected necrosis at the time of external drainage/FNA, the antibiotic treatment had to be changed because of the microbiological findings.

Impact of aetiology, smoking, and body-mass index (BMI) on infectious state of necrosis and microbial spectrum

The aetiology of pancreatitis, smoking status and BMI did not influence the infectious state of necrosis at the time of index endoscopy, the number of mono- versus polymicrobial findings, or the type of microbial findings.

Association between infectious state of necrosis and microbial spectrum and outcome

Nine patients died during the hospital admission, all with infected necrosis at the time of index endoscopy, as opposed to no deaths in the group with sterile necroses ($p = 0.04$), see Table 3. The presence of enterococci and fungi at the index endoscopy was also significantly associated with in-hospital mortality, see Table 4.

Tventy-three patients with infected necrosis at the index endoscopy developed organ failure, as opposed to only one in the sterile group, see Table 3. Both enterococci (58% vs. 18%, $p = 0.0004$)

Table 3
Association between infected necrosis at time of index endoscopy and outcome.

	Infected necrosis ($n = 55$)	Sterile necrosis ($n = 23$)	p-value
In-hospital mortality	9	0	0.039
Organ failure[a]	23	1	0.001
- respiratory	21	1	
- circulatory	15		
- renal	14		
- gastro-intestinal	7		
Need for ICU[a]	22	1	0.002

[a] In two patients (one with infected and one with sterile necrosis) it was not possible to retrieve data on organ failure and need for ICU.

and fungi (27% vs. 8%, $p = 0.03$) were present more often in patients with organ failure than without.

Tventy-one patients with infected necrosis at the index endoscopy needed treatment at the ICU, as opposed to only one patient in the sterile group, see Table 3. A significant association between the need for ICU and presence of enterococci (66% vs. 12%, $p < 0.0001$) and fungi (33% vs 8%, $p = 0.04$) at the index endoscopy was found.

Predictors of mortality, need for ICU and organ failure

Following variables were included into a multivariate analysis of the predictors of in-hospital mortality, need for ICU and organ failure: age, sex, BMI, ASA, aetiology, CTSI, modified CTSI, SOFA-score, infected necrosis, enterococci and fungi at the time of index endoscopy. The SOFA-score (OR = 1.74, 95%CI: 1.10–2.78, $p = 0.02$) and presence of enterococci (OR = 20.2, 95%CI: 1.10–371, $p = 0.043$) were significant predictors of in-hospital mortality. Also, SOFA-score (1.52, 95%CI: 1.25–1.85, $p < 0.0001$) and enterococci (OR = 8.6, 95%CI:1.88–39.3, $p = 0.005$) were significant predictors of need for ICU, whereas only SOFA-score (OR = 1.57; 1.25–1.98, $p < 0.0001$) was a significant predictor of organ failure.

Microbial findings in blood cultures and on central venous catheters (CVC) and the impact of bacteraemia on organ failure and mortality

Blood cultures were performed in seventy-six patients (94%) during the disease course. One or more episodes of bacteriaemia were found in twenty-three patients (30%). Fourteen patients in the organ failure group compared to only eight patients in the no organ failure group had a positive blood culture (54% vs. 16%, $p = 0.0007$). Six out of nine patients who died during admission had bacteriaemia, as compared to 17 out of 67 survivors (67% vs. 25%, $p = 0.01$).

Only one of the patients with positive blood cultures (coagulase-negative staphylococci and *Enterococcus faecium*) presented with a sterile necrosis. The most common findings in blood cultures were *Enterobacteriaceae* (28%), fungi (26%), coagulase-negative staphylococci (26%), and enterococci (11%).

Overall, 32 positive cultures with 56 microbial findings from CVC were obtained. The most common findings were cutaneous flora (64%), followed by non-haemolytic streptococci (7%), *Enterobacteriaceae* (5%), enterococci (5%), Pseudomonas species (5%), and fungi (5%).

The identified species of enterococci and fungi

In samples from the necroses, *E. faecium* and *Enterococcus faecalis* were identified from 19 and 18 patients, respectively. Two isolates of enterococci from the first endoscopy were not identified further than as *Enterococcus species.* Only two other enterococcal species, one *Enterococcus durans*, and one *Enterococcus avium,* respectively, were found from the necroses. Enterococcal bacteraemia were seen in 8 patients, 6 with *E. faecium* and 2 with *E. faecalis*.

From 30 patients fungi were diagnosed from the necroses, most often *Candida albicans* (24 patients), more rarely *Candida glabrata* (5), and *Candida tropicalis* (1). Eight patients experienced fungi in bloodcultures, 4 with *C. albicans*, 2 with *C. glabrata*, one with *Candida dubliniensis*, and one with *Candida guilliermondii*. In 4 cases more than one species of yeast were found, i.e. two or more of *C. albicans, C. tropicalis*, *Candida krusei*, *Candida famata*, *Candida lusitaniae, or Saccharomyces cerevisiae*.

Table 4
Association between the microbial findings at index endoscopy and mortality in 78 patients undergoing endoscopic, transmural drainage and necrosectomy.

	Alive, $n = 69$	Dead, $n = 9$	p-value
Enterobacteriaceae	19	3	0.72
Enterococci	17	8	0.0001
Fungi	8	4	0.01
Coagulase negative staphylococci and Corynebacterium species	8	2	0.37
Non-haemolytic streptococci	11	0	0.85
Pseudomonas and Stenotrophomonas species	4	0	0.46
Various	4	0	0.46

Discussion

The present study specifically investigated the influence of the microbial findings on mortality and organ failure in a large group of patients with SAP undergoing ETDN. We showed that infected pancreatic necrosis at the time of index endoscopy is associated with both a significantly increased in-hospital mortality and occurrence of organ failure. Recently, a meta-analysis of published clinical studies comprising 1478 patients with acute pancreatitis showed that infected pancreatic necrosis resulted in a 30% mortality rate [11]. A prospective study by Garg et al. [12] showed that patients with infected pancreatic necrosis were more likely to develop organ failure than patients with sterile necrosis (64 vs. 24%). This was confirmed in our study and indicates that special surveillance should be offered patients with infected pancreatic necrosis.

To our knowledge, this is the first study to evaluate the microbiological spectrum in patients with WON throughout the length of disease. Our data indicate that a persistent microbiological evaluation is necessary as the spectrum of bacteria and fungi is changing over time and that the antibiotic treatment should be continuously adjusted according to culture findings.

Interestingly, no significant difference in the rate of infected necrosis was found between the group who received antibiotics before drainage as compared to the group who did not. This adds further weight to previous findings and to current recommendations stating that prophylactic antibiotics do not prevent infection of pancreatic necroses [13,14]. In addition to selecting enterococci, antibiotic treatment before the index endoscopy pointed a trend ($p = 0.07$) towards increasing the rate of fungal infections. Finally, antibiotics given on empirical basis prior to culture had to be changed in 70% of cases as a consequence of the microbial findings.

More than half of our patients had a diagnostic fine needle aspiration (FNA) or were treated with percutaneous drainage before the index endoscopy. In a prospective, randomized trial Zerem et al. [15] showed that percutaneous drainage of initially sterile collections led to colonization in 55% of cases. Even though we did not find any difference in relation to the infectious state of

necrosis at time of the index endoscopy between patients who were externally drained or had FNA performed as compared to those who did not, 79% of those who had a sterile necrosis at the external drainage/FNA prior to endoscopy were later found to be infected. This supports that any puncture or drainage is associated with a risk of superinfection.

The predominant microbial aetiology at the index endoscopy in our study was enterococci followed by Gram-negative bacteria and fungi. This is in contrast to at least some previous studies which found Gram-negative bacteria to be the most common finding in infected necrosis [4,8]. Recently, however, Rische et al. [16] and Negm et al. [17] found Gram-positive bacteria to be more prevalent, though both studies included patients with simple pseudocysts as well as necroses. In the multivariate analysis, enterococci were significant predictors of mortality and need for ICU treatment. This is in contrast to a recent study that could not demonstrate that a shift to Gram-positive flora was associated with an increase in morbidity and mortality [6].

Despite adequate antibiotic treatment, the same or additional microbes were found at subsequent culture in the majority of patients. This raises a question of efficacy of systemic antibiotics in the treatment of infected WON. Interestingly, however, 10 patients received antifungals between the index and second endoscopy, and in only one patient fungi were identified at second endoscopy.

Half of our patients had *Candida* in their necroses or blood, a proportion somewhat greater than described by others [4]. The majority was infected with *C. albicans*, and only few patients experienced other species. We showed a clear association between fungal infection and poor outcome. Other studies have addressed the issue of fungal infections in SAP and their impact on in-hospital mortality and organ failure. Our findings are in accordance with some [18], but not others [19]. However, these studies have used different treatment algorithms (i.e. external drainage, endoscopy, or open surgery), making a comparison difficult.

Surprisingly, we did not find any anaerobes at the index endoscopy. Beger et al. [4] found anaerobes as the only microbial finding in 5% of patients with infected pancreatic necrosis. Bacterial translocation from the gut to pancreatic necrosis has been proposed as an important mechanism by which necroses become infected [20]. It is supposed that bacterial translocation primarily occurs from the small bowel due to its thinner wall compared to the colon [21]. Since anaerobes are rare in the small intestine [22], this may explain the rare finding in pancreatic necroses.

Thirty-two cases with positive cultures from CVC were found in this study, a somewhat high frequency, which partly can be explained by the finding of a cutaneous flora in 64%. Undoubtedly, this can partly be ascribed to contamination. However, our findings suggest an increased awareness on catheter-related bacteriaemia and a need for continuous culturing and timely replacement of CVCs.

A change from sterile to infected necroses in some patients after the index endoscopy and the increase in the proportion of patients with polymicrobial findings at subsequent endoscopies raise a concern of superinfection due to the intervention per se. A superinfection is probably unavoidable since endoscopic procedures cannot be performed in a sterile environment. Even though it may be looked at as an adverse event, it did not seem to influence the outcome as only one patient with sterile necrosis at the index endoscopy developed organ failure/need for ICU and no deaths occurred. However, it is a question that should be addressed in a future prospective study.

Our study has a number of limitations, of which the most important is the retrospective collection of data. We defined organ failure according to the Atlanta criteria, although we only used the definite criteria as need for mechanical ventilation, inotropics, haemofiltration, and intestinal paralysis on diagnostic imaging. This approach was chosen as the retrospective data collection did not allow us to calculate more quantitative criteria because of missing data. The microbiological data were, however, obtained from a central, electronic database with real-time entry of data, minimizing the risk of missing values. A clear relationship between a certain type of microorganism cultured at the index endoscopy and MOF or mortality is very difficult to establish, as the time spectrum is very long from onset of the first symptom to index endoscopy, as well as the patient's clinical condition may vary substantially throughout the disease period. Also, all endoscopic procedures were performed at a single expert centre, rendering it difficult to extrapolate the applicability of our results to daily practice in smaller volume centres.

The strength of our study is the relatively high number of patients. To our knowledge this is the largest single centre study on patients with severe acute pancreatitis undergoing ETDN. The patients in our study were all treated according to a standardized algorithm.

In conclusion, this study has outlined the microbial spectrum of infected pancreatic necroses and its variability during the disease course. Our data call for continuous culturing, as a detailed knowledge on the changing microbiology may be utilized to tailor an adequate antibiotic treatment. We suggest that any empirical therapy in necrotizing pancreatitis should cover the most common microbial findings in our study, i.e. enterococci, *Enterobacteriacea*, and fungi.

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