Prevention and Intervention Strategies in Acute Pancreatitis

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Cover: 1893 edition of the Henley Royal Regatta (unknown artist),
River & Rowing Museum, Henley on Thames, England (with permission)

A rowing regatta provides a striking metaphor for performing multicentre trials. The rowers (the researchers) have to closely coordinate their movements in order to make good progress but also depend on their co-workers (spectator boats) and sponsors (large house boats) in providing them with the conditions needed for rowing an optimal race (multicentre trial). In beautiful contrast with the studies described in this thesis, spectator boats on the Henley Royal Regatta course were a regular hazard for competing crews.

Prevention and Intervention Strategies in Acute Pancreatitis

Preventie- en Interventiestrategieën in Acute Pancreatitis

(met een samenvatting in het Nederlands)

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Aan mijn ouders
Voor Carlien

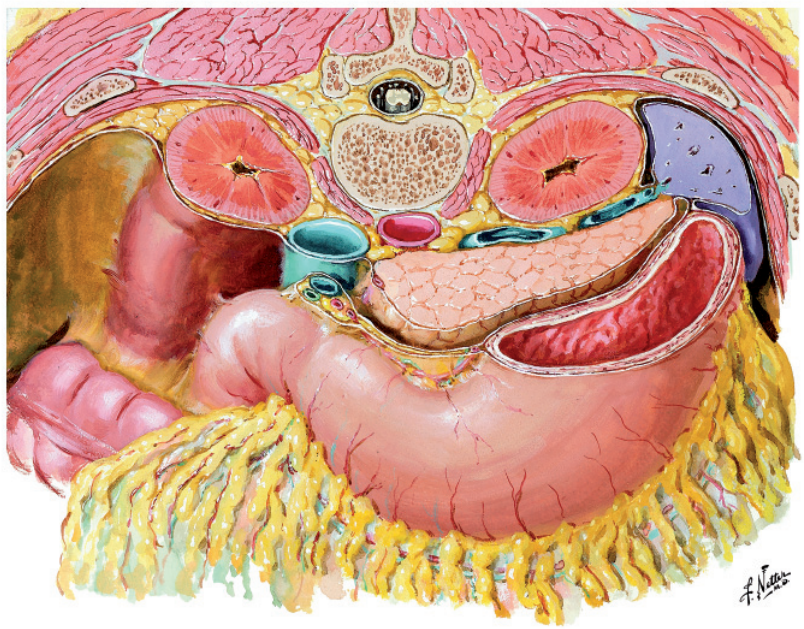
Contents

Chapter 1

General introduction and thesis outline

The pancreas is a 12-15 cm elongated organ, situated in the retroperitoneal space, behind the stomach (*Figure 1*). Acute pancreatitis is the acute inflammation of the pancreas, characterized by severe upper abdominal pain of acute onset. Acute pancreatitis is a common, potentially lethal, costly and poorly understood disease.

Figure 1 The pancreas, located behind the stomach.



Etiology

In Western countries the most common form of acute pancreatitis (app. 50%) is biliary pancreatitis.[1] Biliary pancreatitis is caused by obstruction of the biliary tree by gallstones and/or gall sludge. The second most common form (app. 20-25%) is acute alcoholic pancreatitis, which is caused by alcohol abuse. Other, less common causes are medication, hereditary causes or (yet) unknown factors. In about 10% of cases no cause of acute pancreatitis is found.[1]

Incidence

Acute pancreatitis is the second most common gastrointestinal condition requiring acute hospitalisation in the United States[2], with an estimated annual cost exceeding 2 billion dollar.[3] Both in Europe and in the United States the incidence of acute pancreatitis is increasing rapidly, most likely caused by an increase in biliary pancreatitis.[4,5] In the

Netherlands, the incidence of acute pancreatitis increased by 50% in the last decade and by 100% in the previous two decades. In Dutch non-university hospitals, during a 6-year period, a 75% increase in admissions for acute pancreatitis was observed (from 19 to 33 patients per year, per hospital). In the same time period, university hospitals observed a 14% increase (from 33 to 37 patients per year, per hosptial).[6]

Clinical course

The clinical course of severe pancreatitis varies widely. In the early phase, the inflammation does not remain restricted to the pancreas but, to a varying extent, involves the entire patient.[7] This first inflammatory phase, which usually lasts one to two weeks, is summarized by the term 'systemic inflammatory response syndrome' (SIRS). SIRS is characterized by tachycardia, fever, tachypnoea and high leukocyte blood count. SIRS may progress towards (multi-)organ failure and ultimately death. It is generally believed that bacterial infection plays only a minor role in SIRS. Furthermore, in approximately 20% of patients with acute pancreatitis, parts or the entire pancreas may become necrotic (necrotizing pancreatitis). Patients with necrotizing pancreatitis often also develop large peripancreatic collections containing, in varying amounts, fluid and necrosis. These peripancreatic collections may exist without necrosis of the pancreas parenchyma, a condition commonly referred to as 'extrapancreatic necrosis'.[8] In approximately 33% of patients with (peri-)pancreatic collections, these collections become secondarily infected by bacteria originating from the intestinal lumen.[9] These infections represent the most severe complications of acute pancreatitis, frequently leading to organ failure, and ultimately death. Infection of (peri-)pancreatic necrosis usually does not occur until the third week of disease, after the initial phase of SIRS has subsided.[10] Very early infection of (peri-)pancreatic necrosis, as early as one week after onset of disease, is also possible, making it difficult to differentiate between organ failure caused by SIRS and organ failure caused by infection of pancreatic necrosis. Patients with necrotizing pancreatitis and/or organ failure often require prolonged hospital and intensive care stay, sometimes for several months, with direct medical costs approaching 90.000 euros per patient.[11]

Predicting severity

Because it may take several days for a patient to demonstrate clinical signs of SIRS and/or organ failure, several laboratory scores have been developed to predict the severity of acute pancreatitis in an early stage of the disease.[7,12] The predictive scores qualify a patient as either being 'predicted mild' or 'predicted severe', already in the first 24-48 hours of disease. The most commonly used predictive scores and cut-off values

for 'predicted severe acute pancreatitis' are a) Acute Physiology and Chronic Health Evaluation [APACHE II] score ≥8, b) the Imrie/modified Glasgow score ≥3 or c) serum C-reactive protein >150 mg/L. A large proportion of patients with predicted severe acute pancreatitis will ultimately be diagnosed with mild pancreatitis (40-60%) making these scores not very useful for daily clinical decision making (e.g. intensive care admission in cases of 'predicted severe acute pancreatitis'). In clinical trials, however, the predictive scores can be useful in selecting 'high-risk' patients thus keeping the sample size of the study to a minimum.[13]

Mortality

In the Netherlands, in 2006, 137 of 3413 patients (4.0%) admitted for acute pancreatitis died (Prismant; National Hospital Registration System), mainly from organ failure and/or infectious complications. In the absence of organ failure and/or necrotizing pancreatitis, pancreatitis is usually classified as 'mild acute pancreatitis' and mortality is very low, 1%. The mortality of predicted severe acute pancreatitis has been reported to be 11-18%.[9] About one fifth of patients with acute pancreatitis will develop organ failure and/or (peri-)pancreatic necrosis and hence be diagnosed with 'severe acute pancreatitis', a condition associated with a mortality rate of 17%.[9] In cases of infection of the (peri-) pancreatic necrosis, the mortality rate increases to 30%.[9]

Thesis outline

The clinical studies presented in this thesis focus on prevention and intervention strategies in acute pancreatitis. The majority of studies are multicentre studies, performed by the Dutch Acute Pancreatitis Study Group (www.pancreatitis.nl). This study group, which includes all 8 Dutch university medical centres and large teaching hospitals, was founded in 2002. The study group aims to improve treatment and outcome of patients with severe acute pancreatitis via a combination of research, consultation and centralisation. Currently, 22 centres, over 80 surgeons, gastroenterologists, radiologists, microbiologists and epidemiologists, five MD-PhD students and two research-nurses are involved in this study group. Between March 2003 and May 2008, over one thousand patients were included in the various studies of the Dutch Acute Pancreatitis Study Group.

Part A – Prevention Strategies

Biliary pancreatitis is the most common form of acute pancreatitis in the Western world. A proportion of cases of biliary pancreatitis may be prevented by performing cholecystectomy in patients with symptomatic gallstone disease. However, due to the long waiting lists for cholecystectomy in some centres, patients with symptomatic gallstone disease remain at risk of developing biliary pancreatitis. Ursodeoxycholic acid reduces gallbladder motility and the cholesterol crystal content of bile. In several retrospective studies, ursodeoxycholic acid was claimed to be effective in reducing colics and complications in patients with gallstone disease.[14,15] **Chapter 2** describes a multicentre randomised, double-blind, placebo-controlled trial that focussed on whether ursodeoxycholic acid can prevent biliary colics and complications, such as biliary pancreatitis, in 177 patients on the waiting list for cholecystectomy.

Infectious complications are a major cause of mortality in acute pancreatitis. For many years, researchers have attempted to design prophylactic strategies to prevent these infectious complications. Two recent multicentre randomised, double-blind, placebo-controlled trials[16,17] and two meta-analyses[18,19] showed no beneficial effect of intravenous antibiotic prophylaxis. Because the gut is generally considered as the main source for secondary infection of pancreatic necrosis[20-22], attention has shifted from antibiotics to strategies using food supplements, such as probiotic prophylaxis. **Chapter 3** describes a multicentre randomised, double-blind, placebo-controlled trial on probiotic prophylaxis in 296 patients with predicted severe acute pancreatitis.

Infectious complications in acute pancreatitis are thought to be the result of a sequence of events starting with small bowel bacterial overgrowth, intestinal barrier dysfunction (i.e. mucosal damage, increased intestinal permeability), and bacterial translocation. Clinical evidence for this hypothesis is, however, lacking.[20-22] In **chapter 4** we use markers for intestinal mucosal damage and gastrointestinal permeability in 141 patients included in the randomised trial described in chapter 3 to focus on the relationship between intestinal barrier dysfunction, probiotic prophylaxis and infectious complications.

If the various prophylactic strategies aiming to prevent intra- and infectious complications are to be effective the treatment should, by definition, be started prior to the onset of infectious complications. In **chapter 5**, we analyse the time of onset and impact of infections in a prospective cohort of 732 patients with a first episode of acute pancreatitis.

Part B – Intervention Strategies

It is obvious that the remarkably high mortality rate for infected necrotizing pancreatitis (app. 30%) represents a major challenge for the health care system. In **chapter 6**, the outcome for the various surgical techniques being used in the Netherlands for (suspected) infected necrotizing pancreatitis is described.

Severe acute pancreatitis is associated with a wide spectrum of intra- and peripancreatic collections, each of which requires a specific intervention strategy. The 1992 Atlanta classification is the worldwide standard used to describe intra- and peripancreatic collections on computed tomography.[23] **Chapter 7** describes the results of the first interobserver study ever on the Atlanta classification.

Minimally invasive intervention strategies for infected necrotizing pancreatitis are gaining popularity.[24] All minimally invasive strategies have in common that collections have to be accessible by percutaneous drain. The feasibility of percutaneous drain placement depends on the intra-abdominal localization of the collection. **Chapter 8** describes an interobserver study focussing on the localization and the feasibility of percutaneous drain placement in patients with infected necrotizing pancreatitis.

In recent years, the timing of surgical intervention in infected necrotizing pancreatitis has greatly shifted from early, immediate intervention in cases of sterile necrosis to delayed intervention in cases of infected encapsulated (peri-)pancreatic necrosis. Current guidelines advocate intervention between day 14 and 30 of admission.[25] **Chapter 9** describes the timing and outcome of surgical intervention for necrotizing pancreatitis in the University Medical Center Utrecht as well as a systematic review of large published series on surgical intervention for necrotizing pancreatitis.

Endoscopic retrograde cholangiopancreaticography (ERCP) is capable of clearing the common bile duct of gallstones and/or sludge in case of biliary pancreatitis. Cholangitis is widely recognized as an indication for urgent ERCP.[7] Controversy exists on whether patients with biliary pancreatitis without cholangitis (app. 90% of patients) benefit from urgent ERCP.[7] **Chapter 10** is a meta-analysis of all randomised controlled trials performed in patients with biliary pancreatitis without cholangitis.

Table 1 Summary of study questions and methods used to answer these questions

Chapter	Study question	Method
2.	Can ursodeoxycholic acid prevent biliary colics and complications in patients with symptomatic gallstone disease awaiting cholecystectomy?	Randomised, double-blind, placebo-controlled, multicentre trial
3.	Can a multispecies probotic (Ecologic 641) prevent infectious complications in patients with predicted severe acute pancreatitis?	Randomised, double-blind, placebo-controlled, multicentre trial
4	What is the relationship between intestinal barrier dysfunction in acute pancreatitis, infectious complications and probiotic prophylaxis?	Prospective, multicentre study
5.	What is the time of onset and impact of infections in acute pancreatitis?	Prospective, multicentre study
6.	What are the results of the various surgical strategies using for infected necrotizing pancreatitis in the Netherlands?	Retrospective, multicentre study
7.	What is the interobserver agreement when using the Atlanta classification to describe computed tomography findings in acute pancreatitis?	Interobserver, multicentre study
8.	What is the feasibility of minimally invasive approaches in patients with infected necrotizing pancreatitis?	Interobserver, multicentre study
9.	What is the impact of the timing of surgical intervention in necrotizing pancreatitis?	Retrospective study and systematic review
10.	What is the role of early endoscopic retrograde cholangiopancreatography in acute biliary pancreatitis without cholangitis?	Meta-analysis

Answers to these questions are listed in Chapter 11: 'General discussion and summary', table 1, page 187.

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Part A

Prevention Strategies

Chapter 2

Ursodeoxycholic acid exerts no beneficial effect in patients with symptomatic gallstones awaiting cholecystectomy: a randomised, double-blind, placebo-controlled trial

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Hepatology 2006; **43**: 1276-83.

Abstract

Background

Ursodeoxycholic acid (UDCA) and impaired gallbladder motility purportedly reduce biliary pain and acute cholecystitis in patients with gallstones. However, the effect of UDCA has not been studied prospectively. This issue is important, as in several countries (including the Netherlands) scheduling problems result in long waiting periods for elective cholecystectomy.

Methods

We conducted a randomised, double-blind, placebo-controlled trial on effects of UDCA in 177 highly symptomatic patients with gallstones scheduled for cholecystectomy. Patients were stratified for colic number in the preceding year (< 3: 32 patients; ≥ 3: 145 patients). Baseline postprandial gallbladder motility was measured by ultrasound in 126 consenting patients.

Results

23 patients (26%) receiving UDCA and 29 (33%) receiving placebo remained colic-free during the waiting period (± 3 months) before cholecystectomy (p=0.3). Also, actuarial analysis of colic-free intervals did not reveal differences. Number of colics, non-severe biliary pain, analgesics intake and number of patients with colics during a 6-month period after cholecystectomy were comparable. Low numbers of prior colics were associated with higher likelihood to remain colic-free (59% *vs* 23%, p< 0.001), without effects on complication risk. In patients evaluated for gallbladder motility, 57% were weak and 43% were strong contractors (minimal gallbladder volume > resp. ≤ 6 mL). Likelihood to remain colic-free was comparable in strong and weak contractors (31% *vs* 33%). In weak contractors, UDCA decreased likelihood to remain colic-free (21% *vs* 47%, p=0.02). In the placebo group, 3 pre-operative, and 2 post-cholecystectomy complications occurred. In contrast, all 4 complications in the UDCA group occurred after cholecystectomy.

Conclusion

Ursodeoxycholic acid does not reduce biliary symptoms in highly symptomatic patients awaiting cholecystectomy. Early cholecystectomy is warranted in patients with symptomatic gallstones.

Introduction

Gallstone disease is very common with an estimated prevalence of 10-15% in the adult Western population.[1,2] Approximately 90% of gallstone carriers are asymptomatic. The annual risk of biliary colic in asymptomatic gallstone carriers has been suggested to be approximately 1%.[3,4] Also, asymptomatic gallstone carriers are at risk for acute pancreatitis, choledocholithiasis and acute cholecystitis. Although accurate prospective data are lacking, annual incidences of these potentially lethal complications are estimated to be approximately 0.2%[5-7], 0.2%[4,8] and 0.3%[4,8], respectively.
One can easily imagine that migration of gallbladder stones into the common bile duct may lead to biliary pain. How gallbladder stones remaining in the gallbladder lead to biliary symptoms is not entirely clear. Strong gallbladder contraction, with temporary impaction of the stone in the orifice of the cystic duct might cause biliary pain. Although impaired gallbladder motility could therefore theoretically protect against colics, symptomatic gallbladder stone patients often have complaints despite coexistent impaired gallbladder emptying.[9-11]

Ursodeoxycholic acid (UDCA) has been claimed to reduce the risk of biliary pain, regardless of gallstone dissolution.[12-14] In a large study by Tomida *et al.*, risks of biliary pain or gallstone complications (acute cholecystitis) were reduced in both symptomatic and asymptomatic gallstone carriers, although gallstones were generally not dissolved.[15] Also, biliary pancreatitis might be prevented by long-term UDCA treatment.[16,17] Increased fasting and residual postprandial gallbladder volumes during UDCA treatment[18,19], less cholesterol crystals[20] or decreased mucin contents in bile[21] could be the underlying mechanisms for these beneficial effects. However, prospective studies on effects of UDCA on symptoms or complications in highly symptomatic patients are lacking.

In the Netherlands, symptomatic gallstone patients are admitted to a waiting list for elective cholecystectomy by general surgeons. The waiting period may last several months because of logistic reasons. During this waiting period gallstone patients are at risk for biliary pain and complications. Therefore, we conducted a randomised, double-blind, placebo-controlled trial on effects of UDCA on biliary pain and complications in highly symptomatic gallstone patients scheduled for cholecystectomy. We also evaluated potential beneficial effects of impaired gallbladder motility.

Patients and Methods

Patients

All patients who were referred for symptomatic gallstones to one of the participating centres, and who were between 18 and 75 years of age, were potential candidates for inclusion in the study (n=379; Fig. 1). Patients were not admitted to the study if any of the following criteria were present: a) contra-indication for general anaesthesia or surgery (n=1); b) current or previous acute cholecystitis (n=10), obstructive jaundice (n=6) or pancreatitis (n=2); c) previous papillotomy, because of preferential flow of UDCA-enriched bile to the intestine rather than to the gallbladder (n=14); d) pregnancy or lactation (n=2); e) current or previous use of UDCA (n=1); f) participation in another study; g) inability to speak Dutch or English (n=2) or mental disability (n=1). Patients became eligible upon admission to the waiting list for elective cholecystectomy for symptomatic cholecystolithiasis defined as: a) presence of gallbladder stones or sludge, without bile duct stones or bile duct dilatation at ultrasonography; b) at least one episode of severe right upper quadrant or midline epigastric pain of at least 30 min. duration, with radiation and/or movement urge, or at least three episodes of severe right upper quadrant or midline epigastric pain of at least 30 min. duration without such radiation or movement urge, in the previous 12 months. Sixty-three of the referred patients did not meet these eligibility criteria. Also, patients were not admitted to the study if they preferred surgery in another hospital or if urgent cholecystectomy was deemed necessary (n=24). Seventy-three of the eligible patients refused to participate for various reasons. One-hundred-eighty patients were finally included in the study. Approval of the protocol was obtained from the Ethical Committees of the three participating centres, and written informed consent from all included patients.

Study protocol

After inclusion, patients were randomised to receive either ursodeoxycholic acid (Ursofalk®) 250 mg or placebo (Ursofalk®-placebo) 3 capsules once daily taken at bedtime. Ursofalk®-placebo was identically supplied and formulated as Ursofalk® (same appearance, smell and taste) except that it contained no ursodeoxycholic acid. Patients took the first dosage at the day of inclusion and the last dosage the night before cholecystectomy. Patients were evaluated at baseline, and for primary or secondary endpoints (see further) by one of the trial physicians (NGV, MGHB and YCAK) after 6 weeks of treatment, and thereafter at 3 month intervals, until cholecystectomy. Patients were also evaluated at the day of cholecystectomy, 6 months after cholecystectomy, and in case of withdrawal at the end of treatment. Pill counts were performed at all

evaluation visits to check compliance. Presence of gallstone complications was assessed by one of the participating surgeons (PMNYHG, IAMJB and MAB).

Patients were clearly instructed to urgently contact one of the trial physicians in case of any potential adverse events. In case of significant adverse events (*e.g.* diarrhea), possibly related to the trial drug, patients were allowed to temporarily reduce their drug intake to one capsule daily, but were asked to resume the drug intake to the original three capsules at the earliest time possible. Treatment was ended in case of gallstone complications (acute cholecystitis, choledocholithiasis or pancreatitis), in case of cholecystectomy, in case of 12 months of treatment and subsequent refusal of elective cholecystectomy, or in case of earlier refusal of elective cholecystectomy and wish to end the study medication. Patients who refused elective cholecystectomy and wished to end the study medication were followed up to 12 months after start of treatment.

Pre-defined primary endpoints of the current study were incidence of episodes of severe right upper quadrant or midline epigastric pain of at least 30 min. duration, and colic-free intervals.

Pre-defined secondary endpoints were: a) number of gallstone complications (acute cholecystitis, choledocholithiasis or acute pancreatitis), b) episodes of non-severe right upper quadrant or midline epigastric pain, c) number of (non-prescription) analgesics taken for right upper quadrant or midline epigastric pain. Analyses were based on intention-to-treat principle.

We estimated that the incidence of episodes of severe right upper quadrant or midline epigastric pain in patients with highly symptomatic gallbladder stones awaiting cholecystectomy would decrease from 50% in the placebo group to 25% in the Ursofalk® group. Based on 0.9 power (β=0.1) to detect a significant difference (α=0.05, two-sided), 85 patients were required for each study group. To compensate for potential non-evaluable patients, we planned to enrol 90 patients per group. An independent pharmacist from the University Medical Center (UMC) Utrecht without any patient contact dispensed either Ursofalk® or Ursofalk®-placebo according to computer generated randomization into permuted blocks of six patients, with stratification for number of colics in the preceding year (< 3 or ≥ 3). Trial physicians and patients were blinded to treatment assignment for the entire study period.

Gallbladder motility studies

Baseline postprandial gallbladder motility was studied by ultrasonography in 126 consenting patients (67 receiving Ursofalk® and 59 receiving Ursofalk®-placebo). Fasting and postprandial gallbladder volumes were measured by real-time ultrasonography using the sum-of-cylinders method.[22] The standard semi-solid mixed meal consisted of

30 g fat, 30 g protein and 70 g carbohydrate (2,815 kJ). Fasting gallbladder volumes were defined as means of three measurements at 5 min. intervals in the fasting state. After subsequent meal ingestion, gallbladder volumes were determined at 15 min. intervals during 120 min. The following indices of gallbladder motor function were determined: fasting volumes (in mL), postprandial volumes (in mL and in % of fasting volume) including the minimal postprandial volumes (in mL and in % of fasting volume), and maximal decrease of gallbladder volumes (in mL). Patients were characterized as strong (minimal postprandial volume ≤ 6 mL) or weak (minimal postprandial volume > 6 mL) contractors.[9,11]

Statistical analysis

All data analysis was carried out according to a pre-established analysis plan. Results were analyzed in both treatment groups and in pre-defined subgroups (low or high number of preceding colics, and strong or weak contractors). Results are shown as means ± SEM and, in case of marked non-parametric distribution, also as medians with ranges. Differences were tested for statistical significance with Student's *t* test, Mann Whitney U test, χ^2 test as appropriate, with the aid of NCSS software (Kaysville, UT, USA). Multiple comparisons were tested by GLM ANOVA. Time to first colic after initiation of treatment was computed by actuarial life-table analysis according to Kaplan and Meier[23], and differences between subgroups were compared by the log rank test. Multivariate regression analyses were performed to identify factors related to biliary colics and complications. Statistical significance was defined as two-tailed probability ≤ 0.05.

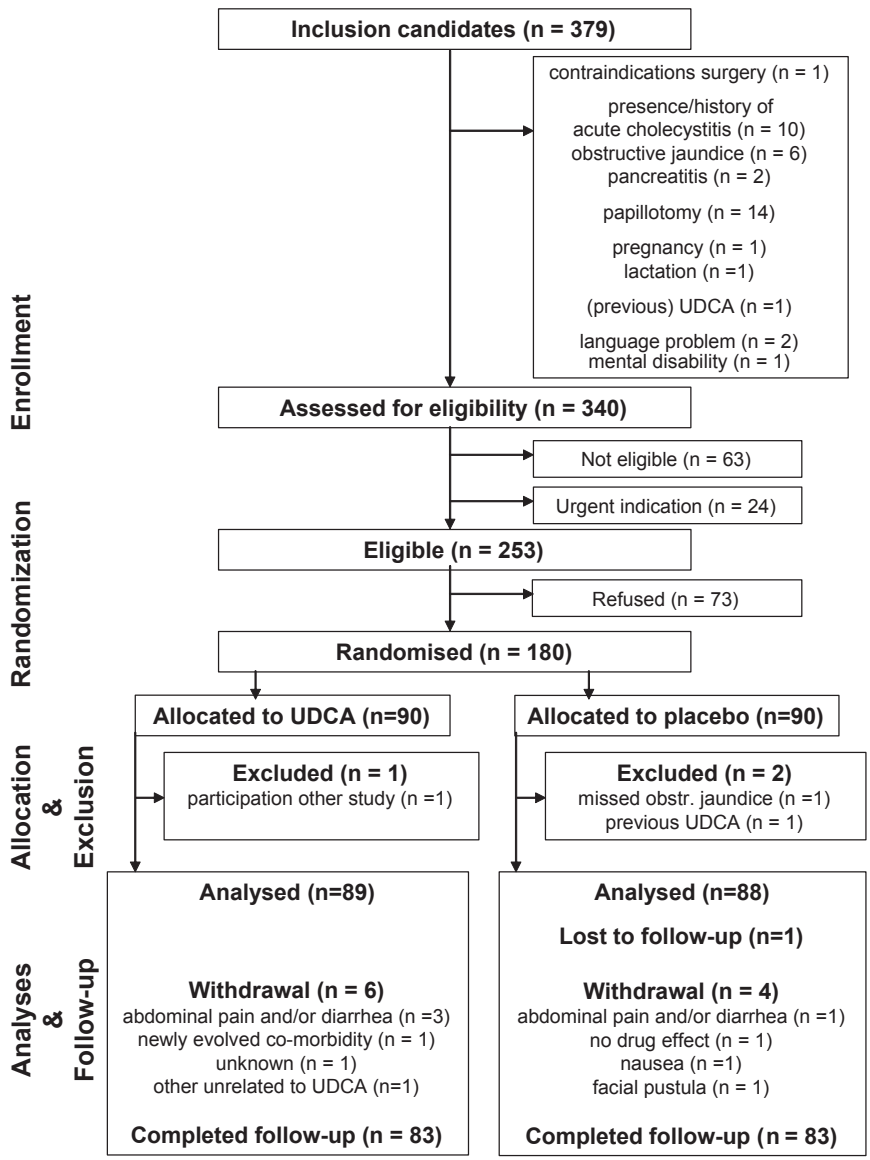
Results

Patients

Eligible patients were recruited from November 2001 to October 2003 when the target of 180 included patients was reached. Follow-up ended in October 2004, 6 months after the last cholecystectomy. Three patients proved to be erroneously included because of participation in another study, previous use of UDCA, or missed pre-existent diagnosis of choledocholithiasis (with subsequent cholangitis) combined with age > 75 years. These patients were excluded from our analyses after consultation of our statistician. Their inclusion would not have changed results. One patient was lost-to-follow-up after 80 days. Ten patients withdrew from the study after 43 ± 16 days for: abdominal cramps/pain and/or diarrhea (n=4), nausea (n=1), facial pustula (n=1), newly evolved co-

morbidity (n=1), lack of trial drug effect (n=1), unknown (n=1), other but unrelated to drug effects (n=1). The latter patients and the lost-to-follow-up patient were included in our analysis until the last day of intake of Ursofalk® or placebo. The flow diagram in *Figure 1* shows progress through the trial.

Figure 1 Flow diagram of gallstone patients referred for pain of potentially biliary origin. Indicated are the various stages of enrolment, randomization, allocation, follow-up and analyses. Also, exclusion and withdrawal of patients are indicated.



Effects of UDCA on biliary colics and complications

As shown in *Table 1*, patients receiving Ursofalk® or placebo were comparable for the most important demographic and clinical characteristics. In both groups, pain localization during colic was in the right upper quadrant in approximately 50% of patients, and in the epigastric region in the other patients. Proportion of patients with impaired gallbladder motility and proportion of patients with low number of previous colics were similar in both groups (*Table 1*).

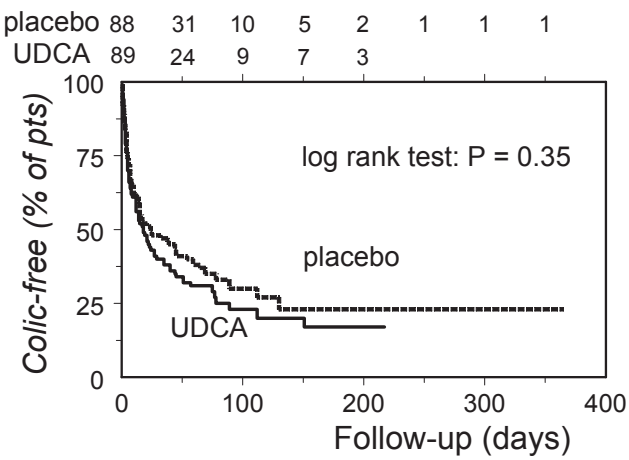
Table 1 Baseline demographic and clinical characteristics

	UDCA (n=89)	Placebo (n=88)	p value
Age (yrs)	47 ± 1	45 ± 1	0.47
Males (% total pts)	21	28	0.28
Body Mass Index (kg/m^2)	28 ± 1	29 ± 1	0.84
Previous gastroscopy (% total pts; always without significant pathology)	31	25	0.34
Time elapsed since US detection of gallstones (months)	16 ± 5 [2; 0-300]	7 ± 2 [3; 0-90]	0.64
Smallest gallbladder stone diameter (mm)	6 ± 1 [4; 0.5-33]	8 ± 1 [5; 0.5-28]	0.33
Gallbladder sludge (% total pts)	18	16	0.74
Number colics in preceding year	14 ± 2	12 ± 2	0.47
Low number previous colics (% total pts)	16	20	0.41
Good gallbladder motility (% of pts)*	43	42	0.92
Pain localization (RUQ/epigastric, % of total pts)^	53/47	47/53	0.49
Maximal duration of colic (hrs)	7 ± 1	6 ± 1	0.64
Movement urge during colic (% total pts)	75	78	0.62
Radiation of pain during colic (% total pts)	88	88	0.98
Episode after food intake (% total pts)	60	50	0.50
Fat intolerance (% total pts)	40	45	0.50
Coffee intolerance (% total pts)	19	13	0.23
Nausea (% total pts)	85	80	0.31
Vomiting (% total pts)	35	47	0.11
Episodes of non-severe biliary pain (% total pts)	64	58	0.41
Defecation frequency (per week)	8 ± 1	8 ± 1	0.43
Number of NSAID's in preceding year	6 ± 2 [0; 0-120]	11 ± 3 [2; 0-203]	0.11
Number of paracetamol in preceding year	8 ± 4 [0;0-300]	3 ± 1 [0; 0-90]	0.56

Data are given as mean ± SEM and, in case of marked non-parametric distribution, also as medians with ranges, unless otherwise indicated. *126 patients were evaluated. ^in 5% of patients, localization of pain could not be differentiated between RUQ or epigastric pain. RUQ, right upper quadrant; US, ultrasonography

Also, fasting and residual gallbladder volumes, as well as other parameters of gallbladder motility did not differ (results not shown). The follow-up period in patients receiving Ursofalk® or placebo did not differ (93 ± 6 *vs* 87 ± 6 days, p=0.58, table 2). The number of patients that (temporarily) reduced their drug-intake, was higher among patients receiving Ursofalk® (15 (17%) *vs* 5 (6%) in patients receiving placebo, p=0.02). Overall trial drug intake was therefore slightly, but significantly, lower in patients receiving Ursofalk® (89 ± 2% *vs* 94 ± 1% of total number of trial-drugs prescribed, p=0.03). Defecation frequency during follow-up on the other hand was comparable in both patient groups.

Figure 2 Actuarial analysis of biliary colics in 89 patients receiving Ursofalk® and 88 patients receiving placebo. Numbers of patients at risk are given in the upper part of the graph. Ursofalk® treatment does not protect against episodes of severe right upper quadrant or midline epigastric pain during follow-up. UDCA, ursodeoxycholic acid.



During follow-up, 23 patients (26%) receiving Ursofalk® and 29 patients (33%) in the placebo group remained entirely colic-free (p=0.30). The colic-free interval, depicted in *Figure 2* as an actuarial analysis, was highly similar in patients receiving Ursofalk® or placebo. Also, the incidence of colics was comparable (2.9 ± 0.6 *vs* 2.4 ± 0.5 colics per month, p=0.36). Furthermore, no significant differences occurred with respect to radiation and/or movement urge during episodes of severe pain (*Table 2*). When we restricted analysis to those episodes with radiation and/or movement urge, actuarial analyses again revealed no differences between both groups in colic-free interval (data not shown). Numbers of (non-prescription) NSAID's, paracetamol, and (not shown) spasmolytics or other medication taken for right upper quadrant or midline epigastric pain were also comparable (*Table 2*).

Table 2 Effects of UDCA on biliary colics and complications

	UDCA (n=89)	Placebo (n=88)	p value
Follow-up (days)	93 ± 6 [77; 7-244]	87 ± 6 [78; 4-365]	0.58
% of pts. < 3 colics in preceding year	16	21	0.41
Trial drug-intake (% total prescribed)	89 ± 2	94 ± 1	0.03
Reduction drug-intake (% total pts)	17	6	0.02
Colic-free (% total pts)	26	33	0.30
Nr. colics/month	2.9 ± 0.7	2.4 ± 0.5	0.36
Colic-free interval (days)	22 ± 4 [8.5; 1-151]	21 ± 4 [7.5; 0-130]	0.69
Movement urge during colic (% total pts)	57	44	0.08
Radiation of pain during colic (% total pts)	62	53	0.26
Defecation frequency (per week)	9 ± 1	9 ± 1	0.63
Number of NSAID's/month	3.1 ± 1.1	3.2 ± 1.0	0.71
Number of paracetamol/month	1.7 ± 0.7	1.7 ± 0.8	0.70
Episodes of non-severe pain (% total pts)	69	58	0.14
Nr. episodes non-severe pain/month	7.7 ± 1.1	5.7 ± 1.0	0.12
GS complications (Nr.)	4	5	0.72
Nr. pts with acute cholecystitis	-	1	
Nr. pts with choledocholithiasis	1	2	
Nr. pts with acute pancreatitis	3	2	
GS complications before/after cholecystectomy (Nr.)*	0/4	3/2	0.16

Data are given as mean ± SEM and, in case of marked non-parametric distribution, also as medians with ranges, unless otherwise indicated. *for comparison of pre- *vs* post-cholecystectomy *vs* no complications in placebo *vs* UDCA group. GS, gallstone

Furthermore, incidence of non-severe right upper quadrant or midline epigastric pain during follow-up was similar in both groups (7.7 ± 1.1 *vs* 5.7 ± 1.0 episodes per month, p=0.12). In the placebo group there were 3 pre-operative complications (pancreatitis, cholecystitis, choledocholithiasis), and 2 post-cholecystectomy complications (pancreatitis, choledocholithiasis). In contrast, all 4 complications in the UDCA group occurred after cholecystectomy (pancreatitis (n=3), choledocholithiasis, p=0.16, for comparison of pre- *vs* post-cholecystectomy *vs* no complications in placebo *vs* UDCA group). All complications resolved after surgery or ERCP, without mortality.

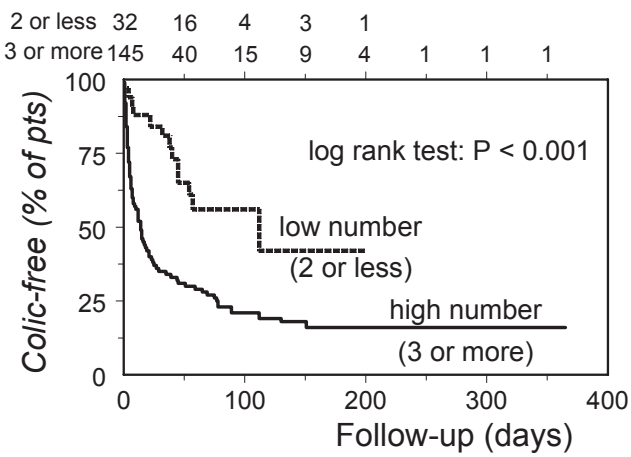
Eleven (14% of 81 evaluable) patients in the Ursofalk® group, and six (7% of 81 evaluable) patients in the placebo group exhibited episodes of severe right upper quadrant or midline epigastric pain during the 6 months after cholecystectomy (p= 0.20). Frequency of these episodes was similar in both patient groups (data not shown). Furthermore,

17 (21%) and 10 (12%) patients in the Ursofalk® and placebo groups, respectively, exhibited episodes of non-severe right upper quadrant or midline epigastric pain during the 6 months after cholecystectomy (p=0.14).

Relation between number of biliary colics before the study and colics or complications during the study

When patients were divided into pre-defined subgroups according to number of colics during the 12 months prior to the study, 32 (18%) exhibited a low number of colics (2 or less) and 145 (82%) exhibited a high number (3 or more). Patients with low or high number of prior colics were comparable for the most important demographic and clinical characteristics, except for the number of NSAID's taken prior to the study (2 ± 1 *vs* 10 ± 2 in pts. with high number of episodes, p=0.004). The number of ingested NSAID's correlated to the number of colics prior to the study (*R*=0.45, p< 0.001).

Figure 3 Actuarial analysis of biliary colics in 32 patients with low numbers of prior colics (≤ 2) and 145 patients with high numbers of colics (≥ 3) in the 12 months prior to the study. Numbers of patients at risk are given in the upper part of the graph. The episode-free interval is significantly longer in patients with low number of prior colics.



More patients with a low number of prior colics remained colic-free during follow-up (59% *vs* 23% in patients with high number of prior episodes, p< 0.001, table 3). The colic-free interval, depicted in *Figure 3* as an actuarial analysis, was significantly longer in patients with low number of prior colics. Also, the number of colics during follow up was much lower among patients with low number of prior colics (0.7 ± 0.3 *vs* 3.1 ± 0.4 colics per month, p=0.02). Furthermore, radiation and/or movement urge during episodes of severe pain were significantly less frequent among patients with

low number of prior colics (*Table 3*). Accordingly, when we restricted episodes to those with radiation and/or movement urge, actuarial analyses revealed significantly longer colic-free intervals in patients with low number of prior colics (data not shown). The numbers of (non-prescription) NSAID's,paracetamol, and (not shown) spasmolytics or other medication taken for right upper quadrant or midline epigastric pain tended to be higher in patients with high number of preceding colics, without reaching significance (*Table 3*).

Table 3 Effects of number of previous colics on biliary colics and complications

	Low number (2 or less) (n=32)	High number (3 or more) (n=145)	p value
Follow-up (days)	87 ± 9 [72; 13-202]	91 ± 5 [77; 4-365]	0.67
Trial drug-intake (% total prescribed)	92 ± 3	92 ± 1	0.89
Colic-free (% total pts)	59	23	<0.001
Nr. colics/month	0.7 ± 0.3	3.1 ± 0.4	0.02
Colic-free interval (days)	45 ± 10 [40; 4-116]	19 ± 3 [7; 0-151]	<0.001
Movement urge during colic (% total pts)	28	56	0.005
Radiation of pain during colic (% total pts)	25	65	<0.001
Number of NSAID's/month	2.3 ± 1.3	3.3 ± 0.4	0.44
Number of paracetamol/month	0.1 ± 0.1	2.0 ± 0.6	0.08
Episodes of non-severe pain (% total pts)	66	63	0.76
Nr. episodes non-severe pain/month	5.0 ± 1.3	7.0 ± 0.9	0.75

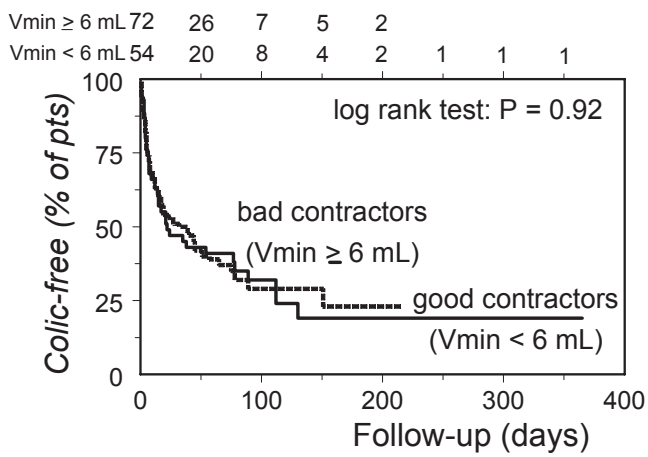
Data are given as mean ± SEM and, in case of marked non-parametric distribution, also as medians with ranges, unless otherwise indicated.

The number of episodes of non-severe right upper quadrant or midline epigastric pain during follow-up was similar in both groups (5.0 ± 1.3 *vs* 7.0 ± 0.9 episodes per month, p=0.75). In the low number of prior colics group, one pre-operative (choledocholithiasis), and one post-cholecystectomy complication (choledocholithiasis) occurred. Two complications (cholecystitis and pancreatitis) in the high number of prior colics group occurred before and five complications occurred after cholecystectomy (pancreatitis (n=4), choledocholithiasis, p=0.78, for comparison of pre- *vs* post-cholecystectomy *vs* no complications in patients with low *vs* high number of prior colics). There was no difference in post-cholecystectomy pain between patients with low or high number of prior colics.

Effects of gallbladder motility on biliary colics and complications

When 126 patients consenting to undergo gallbladder motility studies were divided into pre-defined subgroups according to the minimal postprandial volume[9,11], 54 patients were good contractors (minimal postprandial volume ≤ 6 mL) and 72 patients were bad contractors (minimal postprandial volume > 6 mL). Patients with good or impaired gallbladder motility were comparable for the most important demographic and clinical characteristics.

Figure 4 Actuarial analysis of biliary colics in 54 patients with good gallbladder motility (Vmin ≤ 6 mL) and 72 patients with impaired gallbladder motility (Vmin > 6 mL) placebo. Numbers of patients at risk are given in the upper part of the graph. Occurrence of colics is similar in both groups. Vmin, minimal postprandial gallbladder volume.



Thirty-one % of patients with good gallbladder motility and 33% of patient with impaired gallbladder motility remained colic-free during follow-up (p=0.83). The colic-free interval, depicted in *Figure 4* as an actuarial analysis, was similar in patients with good or impaired gallbladder motility. Also, the number of episodes of severe right upper quadrant or midline epigastric pain was comparable during follow-up (2.0 ± 0.5 *vs* 2.5 ± 0.5 colics per month, p=0.74). No significant differences existed in the number of patients with radiation and/or movement urge during episodes of severe pain (*Table 4*). When we restricted episodes to those with radiation and/or movement urge, actuarial analyses revealed no differences in the colic-free interval between patients with good or impaired gallbladder motility (data not shown). Numbers of (non-prescription) NSAID's, paracetamol, and (not shown) spasmolytics or other medication taken for right upper quadrant or midline epigastric pain tended to be higher in patients with impaired gallbladder motility, without approaching significance (*Table 4*).

Table 4 Effects of gallbladder motility on biliary colics

Nr	Good contractors (Vmin ≤ 6 mL) (n=54)	Bad contractors (Vmin > 6 mL) (n=72)	p value
Fasting GB volume (mL)	15.2 ± 0.8	26.6 ± 1.7	<0.001
Vmin (mL)	3.7 ± 0.2	13.8 ± 1.0	<0.001
Vmin (% of fasting GB volume)	28 ± 2	55 ± 2	<0.001
Fasting volume – Vmin (mL)	11.4 ± 0.8	12.8 ± 1.2	0.80
Follow-up (days)	100 ± 9 [82.5; 4-365]	85 ± 6 [71.5; 7-215]	0.25
Trial drug-intake (% total prescribed)	92 ± 2	92 ± 2	0.63
Colic-free (% of pts)	31	33	0.83
Nr. colics/month	2.0 ± 0.5	2.5 ± 0.5	0.74
Colic-free interval (days)	29 ± 6 [12; 1-130]	23 ± 4 [10; 0-151]	0.54
Movement urge during colic (% total pts)	54	49	0.57
Radiation of pain during colic (% total pts)	48	60	0.20
Number of NSAID's/month	1.4 ± 0.3	3.4 ± 1.5	0.68
Number of paracetamol/month	0.5 ± 0.2	2.9 ± 1.2	0.62
Episodes of non-severe pain (% total pts)	61	72	0.19
Nr. episodes non-severe pain/month	4.8 ± 1.1	8.3 ± 1.2	0.11

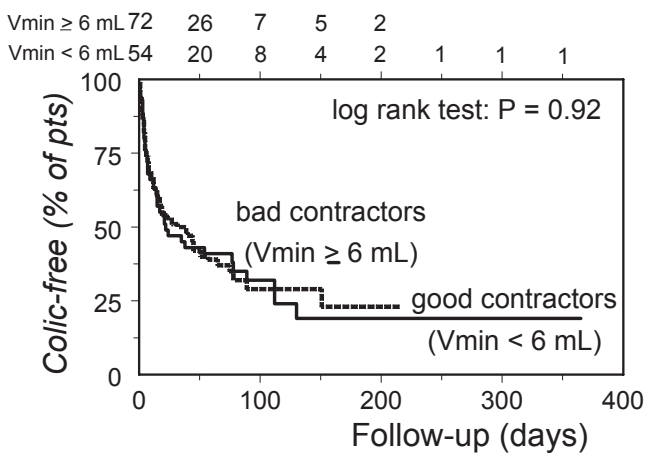
Data are given as mean ± SEM and, in case of marked non-parametric distribution, also as medians with ranges, unless otherwise indicated. GB, gallbladder; Vmin, minimal postprandial gallbladder volume

The number of episodes of non-severe right upper quadrant or midline epigastric pain during follow-up also tended to be higher in patients with impaired gallbladder motility (4.8 ±1.1 *vs* 8.3 ±1.2 episodes per month, p=0.11). Within the subgroup of patients with impaired gallbladder motility, fewer patients receiving Ursofalk® remained colic-free (21% *vs* 47% in patients receiving placebo, p=0.02). Also, actuarial analysis revealed that the colic-free interval in patients with impaired gallbladder motility receiving Ursofalk® was significantly reduced (*Figure 5*). Furthermore, when we restricted episodes to those with movement urge and/or movement urge and radiation, actuarial analyses revealed significantly shorter colic-free intervals in patients with impaired gallbladder motility, receiving Ursofalk® than those receiving placebo (data not shown).

In patients with good gallbladder motility, Ursofalk® did not influence risk of biliary colics or colic-free interval (not shown). There was no difference in complication rate or incidence of post-cholecystectomy pain between patients with good or impaired gallbladder motility.

Additional calculations on biliary colics and complications, applying 50% of the fasting gallbladder volume as the cut-off value to discriminate between patients with good or impaired gallbladder motility, did not reveal any difference in biliary colics or complications (data not shown). Also, with this cut-off value, in the impaired motility group, fewer patients receiving Ursofalk® remained colic-free (19% *vs* 54% in patients receiving placebo, p=0.02), whereas Ursofalk® did not affect risk of colics in the good motility group. Fasting gallbladder volumes neither affected risk of biliary colics nor complications (data not shown).

Figure 5 Actuarial analysis of biliary colics in 38 patients receiving Ursofalk® and 34 patients receiving placebo, in the subgroup with impaired gallbladder motility. Numbers of patients at risk are given in the upper part of the graph. In patients with impaired gallbladder motility, risk of biliary colics is increased by Ursofalk®. UDCA, ursodeoxycholic acid.



Multivariate analysis on biliary colics and complications

With multivariate regression analysis on risk of biliary colics, with UDCA, number of prior colics, and quality of gallbladder motility as potential factors, number of prior colics was identified as the only independent factor predictive for biliary colics (R=0.30, p< 0.001). No independent risk factor predicted biliary complications.

Discussion

Ursodeoxycholic acid (UDCA) has been claimed to reduce the risk of biliary pain.[12-14] Tomida *et al*. found in a large study that risks of biliary pain or acute cholecystitis were reduced by UDCA in both symptomatic and asymptomatic gallstone carriers compared to an untreated control group, regardless gallstone dissolution.[15] Also, long-term UDCA treatment may prevent recurrence of biliary pancreatitis.[16,17] Increased fasting and residual postprandial gallbladder volumes during UDCA treatment could be the underlying mechanism for these beneficial effects.[18,19] However, convincing prospective studies on effects of UDCA on symptoms or complications in highly symptomatic patients are lacking.

The main finding of our prospective, randomised, double-blind, placebo-controlled trial is that UDCA does not exert a beneficial effect on biliary pain or complications in highly symptomatic gallstone patients. Also, the number of prior colics rather than quality of gallbladder motility appears to predict future colics.

Different findings in the present and previous studies may be explained by differences in study design, patient selection, or treatment length and intensity. In the non-randomised study by Tomida *et al.*, 527 patients referred for symptomatic or asymptomatic gallbladder stones, were asked to be treated with 600 mg UDCA per day. Those who refused served as a control group, thus introducing potential bias.[15] In line with our study, number of prior colics was also the only predictor of recurrent biliary colics in symptomatic gallstone patients. Incidence of biliary colics was however very low compared to the present study (38% in 66 months *vs* 71% in 3 months), while the follow-up period was much shorter in the present study. Patients in the present study may have been more symptomatic because of our strict in- and exclusion criteria. Also, in the study by Tomida *et al*, no information is given on the number of biliary colics or the time from colic to treatment start.[15] Patients who were assigned as symptomatic may have had only one biliary colic, years before treatment start, whereas patients in the present study had an average of 13 biliary colics in the 12 months prior to the study. UDCA may theoretically be more beneficial in patients who have only minimal symptoms, possibly related to non-biliary effects of the bile salt (*e.g.* improved bowel transit). The clinical relevance of such an effect is however questionable. In the present study, patients were selected on the basis of symptoms rather than on likelihood of stone dissolution as in previous studies.[12,15] Also, in contrast to the current study, gallbladder opacification on oral cholecystography was an entry criterium in most previous studies.[12,13] Ultrasonographic evidence of good

gallbladder motility (>50% decrease of gallbladder volume after meal stimulation) has been used as alternative evidence of functioning gallbladder in gallstone dissolution studies.[24] We found no indication of any beneficial effects of UDCA in the subgroup of patients with good gallbladder motility. Interestingly, multivariate regression analysis on risk of biliary colics revealed that the number of prior colics was the only independent factor predictive for biliary colics.

In asymptomatic gallstone carriers, the annual incidence rates of symptomatic choledocholithiasis, acute cholecystitis and acute pancreatitis have been estimated to be 0.2%[8,25], 0.3%[3,8,25] and 0.05-0.2%[5-7], respectively. In contrast, we found a 5% complication rate during the three-month follow-up period. In the present study, UDCA could not prevent gallstone complications. Absence of beneficial effects of UDCA on complications may theoretically be explained by type 2 error, because of the relatively short treatment period and the relatively small number of patients included. The number of patients included in the present study was in fact based on estimated incidence of biliary colics, rather than on gallstone complications. Nevertheless, our data suggest no beneficial effects of UDCA on gallstone complications.

No independent risk factors were identified to predict biliary complications. Therefore, early cholecystectomy appears indicated in highly symptomatic gallstone patients, in order to prevent these complications, regardless severity or frequency of previous biliary symptoms.

Surprisingly, 6 out of 9 gallstone complications (of which 4 (all pancreatitis or choledocholithiasis) in UDCA treated patients) occurred after cholecystectomy. Most likely, these complications occurred through gallbladder stones that migrated into the common bile duct before or during elective cholecystectomy. One could imagine that UDCA facilitated stone migration into the common bile duct, through partial dissolution of gallbladder stones and/or altered gallbladder motility. Pancreatitis was the main post-operative complication in the UDCA group (3 of 4 patients). During the treatment period with UDCA, pancreatitis could be prevented by the hydrophilic bile salt composition in bile refluxed into the pancreatic duct. Alternatively, decreased amounts of cholesterol crystals during UDCA treatment could have been the explanation.

Previous studies have suggested that increased fasting and residual postprandial gallbladder volumes during UDCA treatment may prevent biliary colics.[18,19] However, baseline gallbladder motility did not affect the risk of biliary colics or complications in our

study. The distribution of patients with good or impaired gallbladder motility was similar to previous studies from our group.[11,26] Remarkably, within the subgroup of patients with impaired gallbladder motility, UDCA tended to increase rather than decrease the risk of biliary colics. In the present study we applied a cut-off value of 6 mL (minimal postprandial volume) to differentiate between good or bad contractors.[9,11] One may criticize that this is an arbitrary cut-off value. Nevertheless, this cut-off value appears to be particularly relevant for gallstone formation and recurrence after extra-corporeal shockwave lithotripsy.[9,11] In one study, risk for gallstone recurrence was increased nine-fold if minimal postprandial volumes exceeded 5 mL.[27] Also, calculations on biliary colics and complications applying 50% of the fasting gallbladder volume as the cut-off value for discrimination between patients with good or impaired gallbladder motility yielded comparable results. In addition, fasting gallbladder volume did not predict biliary colics or complications. Although we did not actually measure gallbladder motility during UDCA treatment, our findings indicate that occurrence of biliary colics is not a simple mechanical problem.

In conclusion, the current study indicates that UDCA does not exert a beneficial effect on biliary pain or complications in highly symptomatic gallstone patients. Also, the number of prior colics rather than quality of gallbladder motility predicts future colics.

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Chapter 3

Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial

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Abstract

Background

Infectious complications and associated mortality are a major concern in acute pancreatitis. Enteral administration of probiotics allegedly prevents infectious complications, but convincing evidence based is scarce. Our aim was to assess the effects of probiotic prophylaxis in patients with predicted severe acute pancreatitis.

Methods

In this multicentre randomised, double-blind, placebo-controlled trial, 298 patients with predicted severe acute pancreatitis as defined by an Acute Physiology and Chronic Health Evaluation [APACHE II] score ≥8, or Imrie score ≥3, or C-reactive protein >150 mg/L were randomly assigned within 72 hours after onset of symptoms to receive a multispecies probiotic preparation (n=153) or placebo (n=145), administered enterally twice daily for 28 days. The primary endpoint was the composite of infectious complications, *i.e.*, infected pancreatic necrosis, bacteraemia, pneumonia, urosepsis, or infected ascites, during admission and a 90-day follow-up. Secondary endpoints included mortality and adverse events. Analyses were by intention to treat. This study is registered, number ISRCTN38327949.

Results

One person in each group was excluded from analysis because of incorrect diagnosis of pancreatitis; thus 152 individuals in the probiotics group and 144 in the placebo group were analysed. Groups were much the same at baseline in terms of patients' characteristics and disease severity. Infectious complications occurred in 30% in the probiotics group (46 of 152 patients) and 29% in the placebo group (41 of 144 patients), resulting in a relative risk of 1.06 (95% confidence interval 0.75-1.51). 24 (16%) patients in the probiotics group died, compared with nine (6%) in the placebo group (relative risk 2.53, 95% confidence interval 1.22-5.25). Nine patients in the probiotics group developed bowel ischaemia (eight with fatal outcome), compared with none in the placebo group (p=0.004).

Conclusion

In patients with predicted severe acute pancreatitis, probiotic prophylaxis with this composition of probiotic strains did not reduce the risk of infectious complications and was associated with an over two-fold increased mortality. Probiotic prophylaxis should therefore not be administered in this category of patients.

Introduction

The incidence of acute pancreatitis in Europe and the United States is increasing by about 5% per year, mainly owing to an increase in biliary pancreatitis.[1-3] About a fifth of patients will develop necrotizing pancreatitis which is associated with a 10-30% mortality rate, mostly attributed to infectious complications and infection of (peri)pancreatic necrosis in particular.[1] These infections are considered the sequelae of a cascade of events starting with small bowel bacterial overgrowth, mucosal barrier failure and a pro-inflammatory response leading to bacterial translocation of intestinal bacteria.[4-6] Systemic antibiotic prophylaxis has long been studied as a measure to prevent secondary infection in acute pancreatitis.[1] Today, however, after two recent double-blind placebo-controlled trials[7,8] and two meta-analyses[9,10] failed to demonstrate a beneficial effect, many clinicians have abandoned this strategy. In the two recent antibiotic trials the incidence of extra-pancreatic infections (*e.g.* bacteraemia, pneumonia) and pancreatic infection remained as high as 28 and 11%, respectively.[7,8] Consequently, there is a clear need for alternative strategies to prevent infectious complications in patients with acute pancreatitis.

Probiotics, as an adjunct to enteral nutrition, have raised high expectations and are currently gaining worldwide popularity for their presumed health-promoting effects.[11,12] Certain strains of probiotic bacteria may prevent infectious complications by reducing small bowel bacterial overgrowth, restoring gastrointestinal barrier function, and modulating the immune system.[11,12] A number of clinical studies using probiotics in patients undergoing elective abdominal operations[13,14] and in patients with acute pancreatitis[15] have indeed reported a reduction of infectious complications. Unfortunately, due to their small size and methodological quality these studies do not justify global implementation of probiotics as a preventive measure in acute pancreatitis. Accordingly, we embarked on a nationwide multicentre randomised, double-blind, placebo-controlled trial – the PRObiotics in PAncreatitis TRIAL (PROPATRIA) – to assess the effects of probiotic prophylaxis in patients with predicted severe acute pancreatitis.

Methods

Study design

The design and rationale of the study have previously been described in detail.[16] Adult patients admitted with a first episode of acute pancreatitis were enrolled in 8 Dutch university medical centres and 7 major teaching hospitals. Acute pancreatitis was defined as abdominal pain in combination with serum amylase and/or lipase levels

elevated to at least three times the institutional upper limit of normal. Patients with acute pancreatitis and an Acute Physiology and Chronic Health Evaluation [APACHE II] score ≥8 [17], or Imrie/modified Glasgow score ≥3 [18], or C-reactive protein >150 mg/L [19], predicting a severe course of disease, were randomised. They received either a multispecies probiotic preparation or a placebo at the first possible occasion but no later than 72 hours after onset of symptoms of pancreatitis. Patients were not enrolled in the study if any of the following criteria were present: post-endoscopic retrograde cholangiopancreaticography (ERCP) pancreatitis, suspected malignancy of the pancreas or biliary tree, non-pancreatic infection/sepsis caused by a second disease, diagnosis of pancreatitis first made at operation, medical history of immune deficiency.

All patients or their legal representatives gave written informed consent. This study was investigator-initiated and investigator-driven and conducted in accordance with the principles of the Declaration of Helsinki and 'good clinical practice' guidelines. The institutional review board of each participating hospital approved the protocol. Randomisation was performed with a computer-generated permuted-block sequence and balanced by participating centre and by presumed aetiology (biliary *vs* non-biliary) in blocks of four. The study was double-blinded. Both the probiotic and placebo preparation were packed in identical, numbered sachets which were stored in identical, numbered containers. The study product and placebo were both white powders, identical in weight, smell and taste. All doctors, nurses, research staff and patients involved remained unaware of the actual product administered during the entire study period. An independent monitoring committee was informed in cases of serious adverse events possibly associated with the study product. At the time of a pre-specified interim-analysis[16], the monitoring committee advised about whether to continue the trial.

Study product

The rationale for the design of the multispecies probiotic preparation has been previously described in detail.[20] In brief, the study product consisted of six different strains of freeze-dried, viable bacteria: *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus salivarius, Lactococcus lactis, Bifidobacterium bifidum* and *Bifidobacterium lactis* (previously classified as *Bifidobacterium infantis*), in a total daily dose of 10^{10} bacteria, and cornstarch and maltodextrins. The individual probiotic cultures are being sold by the major probiotic producers as ingredients for probiotic supplements or dairy food and carry the European Union 'Qualified Presumption of Safety (QPS)' and the United States 'Generally Recognized As Safe (GRAS)' status. The individual strains were selected on the basis of their capacity to inhibit growth of pathogens most often cultured from

infected necrotizing pancreatitis *in vitro*.[20,21] Probiotic species that were ever reported to have been associated with an infectious complication, regardless of underlying disease, were excluded.[20] The placebo sachets contained only cornstarch and maltodextrins but no bacteria. Both the probiotic and placebo preparations were checked according to national regulations for any contamination with known pathogens and for the presence of endotoxins. Throughout the study three different batches of probiotics and placebo were produced, tested and used.

Treatment protocol

After randomisation, patients received a nasojejunal feeding tube. The study product (Ecologic® 641, Winclove Bio Industries, Amsterdam, the Netherlands) or placebo was administered twice daily and added to the continuously running fibre-enriched tube feeding (Nutrison Multi Fibre®, Nutricia, Zoetermeer, the Netherlands). The study product or placebo was dissolved in sterilised distilled water and administered for a maximum of 28 days. If placement of the nasojejunal tube was delayed for more than 12 hours, the first dose of the study product or placebo was taken orally. Nasojejunal tubes were placed by either upper gastrointestinal endoscopy or under fluoroscopic guidance. When nasojejunal tubes would become blocked or were pulled out, a new tube was re-inserted at the first possible occasion, generally within 24 hours. The amount of tube feeding was gradually increased over the first days with an energy target of 30 kcal/kg (up to 90 kg) on day 4 after start of enteral nutrition. When patients started oral intake, the nasojejunal tube was removed and the study product or placebo were dissolved in tap water and ingested orally for the remainder of the 28 days. Administration of the study product or placebo was stopped when a patient was diagnosed with infected pancreatic necrosis. Patients discharged prior to 28 days were only allowed to stop treatment once computed tomography (CT) demonstrated absence of pancreatic necrosis and/or fluid collections. During the study, patients were not allowed to use any commercially available product containing probiotics. During administration of the study product or placebo, nursing staff recorded the number of sachets administered and registered any potential side effect such as abdominal complaints.

Antibiotic prophylaxis was not given routinely in patients with necrotizing pancreatitis. The use of antibiotics was recorded, regardless of indication. When ERCP was indicated in cases of biliary pancreatitis, antibiotic prophylaxis was allowed. A standard 'baseline' (intravenous) contrast-enhanced CT scan was performed seven days after admission to detect pancreatic necrosis. One experienced radiologist (TLB), unaware of treatment allocation, re-read all CT-scans to assess the CT severity index (CTSI).[22] In cases of a

clear clinical diagnosis of infected (peri)pancreatic necrosis (persistent fever and clinical deterioration in the third or fourth week of disease in the presence of documented necrosis or 'air bubbles' in the collections with necrosis on CT, while other sources of infection were absent), fine needle aspiration of (peri)pancreatic collections was not mandatory to confirm the clinical suspicion. For the endpoint 'infected necrosis' a positive culture was mandatory, *Table 1*. During surgical intervention or percutaneous drainage for (suspected or documented) infected necrosis, tissue and/or fluid samples were sent for routine microbiological evaluation. Body temperature was measured at least twice daily and, in cases of fever, blood cultures were drawn. Further diagnostic and therapeutic measures were left to the treating clinicians' discretion.

Table 1 Definitions of the primary endpoint

Endpoint	Definition
Infected pancreatic necrosis	Positive culture of peripancreatic fluid or pancreatic necrosis obtained by either fine needle aspiration, during the first percutaneous drainage or during the first surgical intervention
Bacteraemia	Positive blood culture*
Pneumonia	Coughing, dyspnoea, chest film showing infiltrative abnormalities, lowered arterial blood gas with positive sputum culture. If in the intensive care unit a positive endotracheal culture is mandatory
Urosepsis†	Dysuria with bacteraemia on the same day, without a urinary catheter in situ
Infected ascites‡	Bacteria detected in aspirate of intra-peritoneal fluid or abdominal fluid sampled during surgical exploration

*For non-pathogens like coagulase-negative staphylococci at least two samples had to be positive.
†Prior to any analysis the adjudication committee restricted the definition of urinary tract infection to urosepsis.
‡Prior to any analysis the adjudication committee added this group of infections to the endpoint 'infectious complications'.

End points

The primary endpoint was the composite of one of the following infectious complications: infected pancreatic necrosis, bacteraemia, pneumonia, urosepsis, or infected ascites, during admission and a 90-day follow-up. Table 1 lists the definitions of infectious complications. All infections were weighted equally; multiple infections in the same patient were considered as one endpoint. Secondary endpoints (during admission and 90-day follow-up) were mortality, sequential organ failure assessment (SOFA) scores, (multi-)organ failure during admission, onset of (multi-)organ failure after randomisation, need for surgical intervention because of (documented or suspected) infected necrosis or intra-abdominal catastrophe, hospital stay, intensive care stay, the use of antibiotics and abdominal complaints (nausea and abdominal fullness with visual analogue scales (VAS); cut-off 3.0 on a 10.0 scale, and presence of diarrhoea as assessed by the patient

(at days 5, 10, 14, 21, 28 and 35)). Per patient, the percentage intake of the study product or placebo was calculated and categorised as <80%, 80-89%, 90-95% and >95%. Microbiological data of the initial positive culture for each of the infectious complications of the primary endpoint were collected.

Organ failure was defined as PaO_2 < 60 mmHg despite FiO_2 30%, or the need for mechanical ventilation (pulmonary insufficiency); serum creatinine > 177 mmol/L after rehydration or need for haemofiltration or haemodialysis (renal failure), and systolic blood pressure < 90 mmHg despite adequate fluid resuscitation or need for vasopressor support (cardiocirculatory insufficiency), adapted from the Atlanta classification.[23] Multi-organ failure was defined as failure of at least two organ systems on the same day. Organ failure prior to randomisation was defined as any organ failure that started prior to the day of randomisation. Because the administration of the study product or placebo could start at any time during the day of randomisation, start of organ failure on that day was left out of this definition. Onset of organ failure after randomisation was defined as initial (for the first time) onset of organ failure after the day of randomisation.

Statistical analysis

We calculated that 200 patients with predicted severe acute pancreatitis would be required to detect a 20% absolute risk reduction in the occurrence of infectious complications (from 50% to 30% of patients during admission and 90-days follow-up) for the study to attain an 80% statistical power, at a two-sided alpha of 0.05. This sample size calculation took into account the fact that up to 40% of patients with predicted severe pancreatitis are ultimately diagnosed with mild pancreatitis (i.e. no local or systemic complications) and thus do not progress to severe and/or necrotizing pancreatitis. After the first 100 patients were randomised and had completed follow-up, the number of infectious complications was determined in the total group of randomised patients without unblinding the data. On the basis of the lower-than-expected rate of infectious complications (28%), the monitoring committee advised increasing the total sample size from 200 to 296 patients in order to maintain statistical power. After 184 patients had been randomised and had completed follow-up, a blinded interim-analysis was performed for the primary endpoint and mortality. Although a non-significant difference in mortality was observed (p=0.10), it was tentatively concluded that this had been caused by 'skewed randomisation' because more patients in the group with higher mortality required intensive care admission within 72 hours after admission (p=0.15), whereas the overall mortality was well within the expected range (11%). According to the predefined stopping rule[16] the monitoring committee recommended that the study should be completed.

Data are presented as mean (±SD) or as median (range). All data analyses were carried out according to a pre-established analysis plan. The incidence of the primary endpoint was compared between the groups and the results are presented as relative risk with exact 95% confidence intervals (CI). The Kolmogorov-Smirnov test was used to determine whether continuous data were normally distributed ($p>0.05$). For continuous variables, differences between groups were tested with Student's T-test for normally distributed data or Mann-Whitney U test for non-normally distributed data. Fisher's exact test was used for proportions in all cases. In cases of significant difference in the incidence of either the primary endpoint or mortality between groups, Kaplan-Meier curves with log-rank tests were generated.
All analyses were performed on the basis of the intention-to-treat principle. Pre-specified subgroup analyses were performed for aetiology and for presence of pancreatic parenchymal necrosis. We used logistic regression models to perform a formal test for interaction to determine whether treatment effects differed significantly between these subgroups. A two-sided P value <0.05 was considered statistically significant.

Data collection and endpoint assessment

Local physicians completed the case-record forms. During the study an independent data monitor performed a cross-check in at least 10% of the individual patients' data against the primary source data, on site in the participating centres. After completion of the follow-up of the last patient but prior to any analysis or unblinding, the two primary authors (MGHB and HCvS) checked all primary and secondary endpoints on site with primary source data. Prior to any analysis and without knowledge of treatment allocation, the blinded adjudication committee judged all exclusions, endpoints that were not fully specified in the protocol in individual patients and serious adverse events. Only after agreement was reached on all endpoints, were analyses performed while maintaining blinding regarding the products administered (group 0 *vs* 1). Finally, after the results of the blinded analyses were presented to the monitoring committee, the randomisation code was broken on October 26th 2007 (group 0 = probiotics).

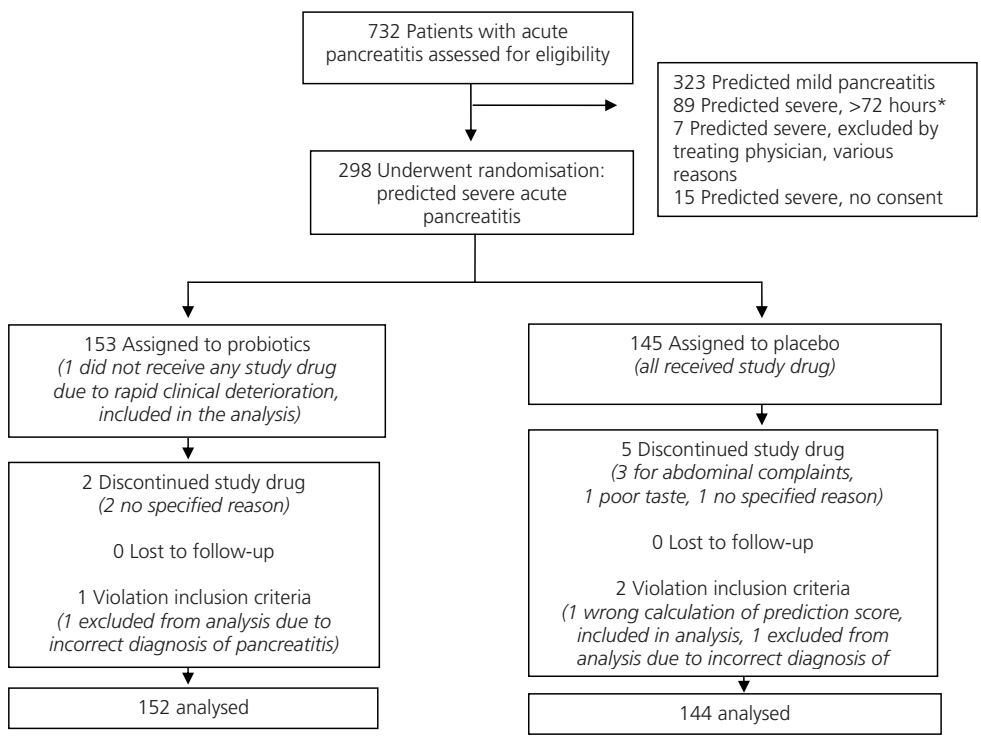
Role of the funding source

The sponsor of the study had no role in the study design, data collection, data-analysis, interpretation of the study results and writing of the manuscript. The corresponding author had full access to all the data and coordinated the decision to submit for publication.

Results

Between March 2004 and March 2007, 732 consecutive patients with a first episode of acute pancreatitis were prospectively registered (*Figure 1*). Of 298 patients with acute pancreatitis and an APACHE II score ≥8 (n=135), or Imrie score ≥3 (n=204), or C-reactive protein >150 mg/L (n=252)(*i.e.,* predicted severe acute pancreatitis), 153 were randomly assigned to receive probiotics and 145 to receive a placebo. Two patients, one in each group, were excluded from final analysis because of an incorrect diagnosis of acute pancreatitis; they were ultimately diagnosed with acute cholecystitis (n=1) and post-pancreatic surgery anastomotic leakage (n=1). One patient who did not receive any study product and one patient who, in retrospect, suffered from predicted mild pancreatitis were included in the final analysis (*Figure 1*). Study groups were comparable for all baseline characteristics as shown in *Table 2*.

Figure 1 Trial profile



*Not randomised due to clinical symptoms of pancreatitis for more than 72 hours at time of diagnosis of 'predicted severe acute pancreatitis'. Patients were either (a) initially missed for randomisation, (b) were transferred from other hospitals more than 72 hours after onset of symptoms or (c) already had complaints for more than 72 hours on admission.

Table 2 Baseline characteristics

	Probiotics (n=152)	Placebo (n=144)
Age (years)	60.4 (±16.5)	59.0 (±15.5)
Sex (male)	91 (59.9%)	83 (57.6%)
Body-mass index (kg/m^2)	27.1 (±6.1)	27.8 (±5.9)
Aetiology of pancreatitis		
Biliary	92 (60.5%)	75 (52.1%)
Alcohol	27 (17.8%)	28 (19.4%)
Unknown	21 (13.8%)	28 (19.4%)
Medication	4 (2.6%)	6 (4.2%)
Hypertriglyceridaemia	4 (2.6%)	3 (2.1%)
Other	4 (2.6%)	4 (2.8%)
American Society of Anaesthesiologists class		
I (healthy status)	62 (40.8%)	62 (43.1%)
II (mild systemic disease)	76 (50.0%)	64 (44.4%)
III (severe systemic disease)	14 (9.2%)	18 (12.5%)
Severity of pancreatitis		
APACHE II score*	8.6 (±4.4)	8.4 (±4.5)
Imrie score (first 48hrs)	3.3 (±1.7)	3.4 (±1.6)
C-reactive protein level (highest first 48hrs)	268 (±127)	270 (±122)
SOFA (on admission)†	2.1 (±2.0)	1.9 (±1.6)
MODS (on admission)‡	1.6 (±1.6)	1.5 (±1.5)
Organ failure prior to randomisation§	9 (5.9%)	5 (3.5%)
Multi-organ failure prior to randomisation	5 (3.3%)	1 (0.7%)
Endoscopic sphincterotomy	48 (31.6%)	35 (24.3%)
Interval first symptoms to admission (days)	0 (0-3)	0 (0-3)
Interval admission to first dose (days)	2 (0-4)	2 (0-3)
Interval admission to enteral nutrition (days)	2 (0-7)	2 (0-7)
Contrast-enhanced computed tomography		
Necrotizing pancreatitis‖	46 (30.3%)	34 (23.6%)
≤30% pancreatic parenchymal necrosis	16 (10.5%)	14 (9.7%)
>30% pancreatic parenchymal necrosis	30 (19.7%)	20 (13.9%)
No contrast-enhanced CT performed	6 (3.9%)	12 (8.3%)
CT severity index**	4 (0-10)	4 (0-10)

*APACHE II, Acute Physiology and Chronic Health Evaluation score, determined on admission.
†SOFA, sequential organ failure assessment, ranges from 0 to 24, with higher scores indicating more severe disease.
‡MODS, multiple organ dysfunction score, ranges from 0 to 24, with higher scores indicating more severe disease.
§Patients with multi-organ failure are included in the group 'patients with organ failure'.
‖ Performed on day 7-10 after admission.
**The CT severity index ranges from 0 to 10, with higher scores indicating more extensive pancreatic parenchymal necrosis and peripancreatic fluid collections. Continuous data are presented as mean (±SD) or median (range).

Study progress

All but 5 patients started treatment within 72 hours after onset of symptoms. The median intake of probiotics or placebo per patient was 100% (25% lower limit: 91%). No difference in the categorised intake between the groups was found (<80%: 65 patients, 80-89%: 26 patients, 90-95%: 50 patients and >95%: 155 patients), p=0.78. No infections were confirmed to be caused by any of the probiotic strains administered. During the study, two serious adverse events were reported, both of patients with fatal outcome. The monitoring committee convened on both occasions: in one patient, a ruptured cecum with ischaemia was found during emergency laparotomy and a second patient suffered from small bowel ischaemia diagnosed at emergency laparotomy. In both cases, the randomisation code was broken (both patients had received probiotics). This information was only revealed to members of the monitoring and the steering committee. An instant review of the literature did not reveal any evidence of a relationship between bowel ischaemia and the use of probiotics. The monitoring committee subsequently advised to continue the study. The institutional review board was informed on both occasions.

Primary endpoint

Infectious complications occurred in 30.3% of the patients in the probiotics group (46 of 152 patients) and in 28.5% of the patients in the placebo group (41 of 144 patients), resulting in a relative risk of 1.06 (95% CI 0.75-1.51) (*Table 3*). There were no significant differences between the groups for the individual components of the primary endpoint. *Table 4* shows the pathogens cultured from the 87 patients with an infectious complication, no significant differences between the groups were observed.

Secondary endpoints

Table 3 also lists the secondary endpoints. Mortality was 15.8% in the probiotics group (24 of 152 patients) *vs* 6.3% in the placebo group (9 of 144 patients), resulting in a relative risk of 2.53 (95% CI 1.22-5.25). *Figure 2* shows the Kaplan-Meier curve for time to mortality (log-rank test, p=0.01). A follow-up of longer than 90 days was obtained in 89% of patients. Most patients died of multi-organ failure, 83.3% in the probiotics group (20 of 24 patients) and 77.9% in the placebo group (7 of 9 patients). Other causes of death were respiratory failure after aspiration (n=1) and cerebral infarction/bleeding (n=3) in the probiotics group, and ruptured aneurysm (n=1) and cerebral infarction (n=1) in the placebo group.

Table 3 Endpoints

	Probiotics (n=152)	Placebo (n=144)	p value
Primary endpoint: infectious complication*	46 (30.3%)	41 (28.5%)	0.80
Infected necrosis	21 (13.8%)	14 (9.7%)	0.29
Bacteraemia	33 (21.7%)	22 (15.3%)	0.18
Pneumonia	24 (15.8%)	16 (11.1%)	0.31
Urosepsis	1 (0.7%)	2 (1.4%)	0.61
Infected ascites	4 (2.6%)	0 (0.0%)	0.12
Secondary endpoint			
Use of antibiotics, any indication	75 (49.3%)	76 (52.8%)	0.56
Percutaneous drainage	14 (9.2%)	8 (5.6%)	0.23
Surgical intervention, any indication	28 (18.4%)	14 (9.7%)	0.05
Necrosectomy	24 (15.8%)	14 (9.7%)	0.16
Intensive care admission	47 (30.9%)	34 (23.6%)	0.19
Intensive care stay (days)	6.6 (±17.1)	3.0 (±9.3)	0.08
Hospital stay (days)	28.9 (±41.5)	23.5 (±25.9)	0.98
Organ failure during admission, any onset†‡	41 (27.0%)	23 (16.0%)	0.02
Multi-organ failure during admission, any onset‡	33 (21.7%)	15 (10.4%)	0.01
Organ failure, onset after randomisation†§	21 (13.8%)	16 (11.1%)	0.60
Multi-organ failure, onset after randomisation§	18 (11.8%)	11 (7.6%)	0.25
Nausea	20 (13.2%)	23 (16.0%)	0.51
Abdominal fullness	36 (23.7%)	43 (29.9%)	0.24
Diarrhoea	25 (16.4%)	28 (19.4%)	0.55
Bowel ischaemia	9 (5.9%)	0 (0.0%)	0.004
Mortality	24 (15.8%)	9 (6.3%)	0.01

*Patients with one or more infectious complication.
†Patients with multi-organ failure are included in the group 'organ failure'.
‡Included are patients with organ failure present at any time during admission, regardless of the date of onset of organ failure.
§Included are patients in whom organ failure developed (for the first time) after the day of randomisation. Patients in whom organ failure (in any organ) started prior to the day of randomisation or on the day of randomisation are not included in this parameter.
Continuous data are presented as mean (±SD). All continuous data were non-normally distributed and hence analyzed with the Mann-Whitney U test.

Bowel ischaemia was detected during operation and/or autopsy in nine patients in the probiotics group, with fatal outcome in eight, and not once in the placebo group (p=0.004), *Table 5*. The nine cases of bowel ischaemia were all diagnosed within the first 14 days of admission in seven different hospitals; four university and three teaching

hospitals. The patient who survived was a 22-year-old male. In all 9 patients contrast-enhanced CT (either the baseline CT or an earlier CT) showed unequivocal evidence of acute pancreatitis. All these patients had experienced an early onset of organ failure (median 2 days after admission, range 1-6 days). At the time of diagnosis 6 patients had vasopressor support (in the placebo group a total of 14 patients had vasopressor support in the first 14 days). Patients had received a median of 6 doses of probiotics (range 4-22 doses) prior to diagnosis of bowel ischaemia. In eight of the nine patients (including the survivor) the small bowel was involved. During autopsy (n=6), five patients with small bowel ischaemia had no sign of occlusive disease in the mesenteric vessels.

Table 4 Pathogens isolated from 87 patients with an infectious complication*

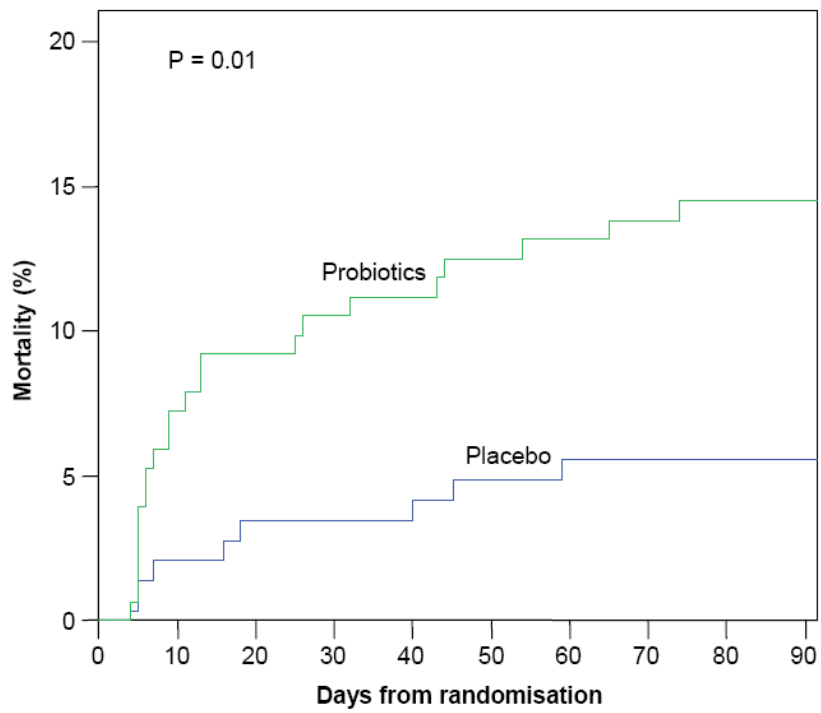
	Probiotics (n=152)	Placebo (n=144)
Gram-positive bacteria		
Staphylococcus spp.	20	20
Staphylococcus aureus	10	11
Coagulase-negative *Staphylococcus*	9	5
Enterococcus spp.	10	3
Streptococcus spp.	3	3
other gram-positive microorganisms†	3	3
Gram-negative bacteria		
Enterobacteriaceae	28	20
Escherichia coli	17	7
Klebsiella spp.	8	8
other gram-negative microorganisms‡	4	8
Fungi§	6	2

*Per patient only the first positive culture results of each infection consistent with the primary endpoint was used. If, in one patient, different organisms were cultured from different sites (e.g. from the initial positive blood culture and from pancreatic necrosis) these are all listed. If, in one patient, the same organism was cultured from different sites, this organism was listed only once.
†*Bacillus* spp. (2), *Clostridium* sp. (1), *Corynebacterium striatum* (1), *Propionibacterium* sp. (1) and unknown (1)
‡*Aeromonas* spp. (1), *Bacteroides* spp. (2), *Moraxella catarrhalis* (1), *Neisseria meningitidis* (2), *Pasteurella multocida* (1), *Pseudomonas auruginosa* (1) , *Stenotrophomonas maltophilia* (3) and *Veillonella* sp. (1).
§*Candida* spp. (6), *Chrysosporium* sp. (1) and unknown (1)

Apart from the patients with bowel ischaemia, 11 patients died in the first two weeks after admission, eight in the probiotics group and three in the placebo group. These patients died of multiple organ failure without signs of bowel ischaemia.
No significant differences were observed between the groups for the serial SOFA scores (data not shown). Although overall more patients in the probiotics group developed

organ failure during the study, there was no difference between the groups regarding organ failure that started after the day of randomisation (p=0.6). During the study, 118 patients (39.9%) developed the most severe form of acute pancreatitis (organ failure and/or pancreatic parenchymal necrosis). A total of 18 patients did not undergo a CT. In 17 patients the treating physician deemed CT unnecessary or the patient refused because of good clinical condition and 1 patient died on day 4 (placebo group) before CT could be performed. The latest point in time a 'baseline' CT was performed was 10 days after admission.

Figure 2 Time to mortality



Number still at risk

Probiotics	152	141	138	136	135	133	132	131	130	130
Placebo	144	141	139	139	138	137	136	136	136	136

Kaplan-Meier time-to-event analysis for mortality in the first 90 days after randomisation. Three deaths occurred after 90 days: two in the probiotics group (day 112 and 125) and one in the placebo group (day 140). Overall mortality was 15.8% in the probiotics group and 6.3% in the placebo group. The P-value represents log-rank test.

Table 5 Clinical characteristics of 9 patients with bowel ischaemia in the probiotics group

SSN*	Sex	Age (years)	Day of diagnosis	Days of treatment prior to diagnosis	Vasopressor support at day of diagnosis	Day of onset of organ failure	Day of death	Findings
10	F	40	5	3	0	1	5	Emergency laparotomy day 5: perforated cecum with adjacent ischaemia. At autopsy: mucosal ischaemia 80 cm of small bowel.
93	M	61	12	11	0	1	13	Emergency laparotomy day 12: resection ischemic small bowel. At autopsy: necrosis and inflammatory changes of the small bowel wall.
121	M	62	4	2	1	2	4	At autopsy: only the proximal jejunum vital, rest of the small bowel ischemic.
124	F	88	6	4	1	6	6	At autopsy: inflammatory changes of the duodenum wall and necrotizing esophagitis.
160	F	62	4	2	1	1	4	Emergency laparotomy day 4: ischaemia of most of the small bowel.
202	M	60	12	10	1	2	26	Emergency laparotomy day 12: necrosis of 40 cm jejunum. At autopsy: necrotizing jejunitis.
235	M	57	9	9	1	2	125	Emergency laparotomy day 9: showing 90cm of ischemic ileum resected. Patient died 4 months later from cerebral infarction.
243	M	22	4	3	0	2	survived	Emergency laparotomy day 4: proximal jejunal ischaemia.
297	M	57	3	3	1	2	6	Emergency laparotomy day 3: ischaemia and inflammation of the entire small and large bowel.

*SSn=Sequential study number, patient number 1 was the first patient in the trial.

Subgroup analyses

Pre-defined subgroup analyses were performed for the presence of pancreatic parenchymal necrosis (any extent) and aetiology (biliary *vs* non-biliary) for both the primary endpoint and mortality. The tests for interaction were not significant, *i.e.*, we could not confirm an interaction between probiotic administration and pancreatic necrosis or underlying aetiology, for either the primary endpoint or for mortality. In the subgroup of patients with pancreatic parenchymal necrosis one or more infectious complication consistent with the primary endpoint occurred in 69.6% (32 of 46 patients) of patients in the probiotics group *vs* 52.9% (18 of 34 patients)) in the placebo group, p=0.16. In patients with pancreatic parenchymal necrosis, mortality in the probiotics group was 41.3% (19 of 46 patients) *vs* 14.7% (5 of 34 patients) in the placebo group, p=0.01.

Discussion

This randomised, double-blind, placebo-controlled trial in patients with predicted severe acute pancreatitis showed no beneficial effect of probiotic prophylaxis on the occurrence of infectious complications. However, mortality in the probiotics group was about twice as high as in the placebo group. Thus, this combination of strains should not be administered routinely in patients with predicted severe acute pancreatitis, and such preparations can no longer be considered to be harmless adjuncts to enteral nutrition.

The rate of infectious complications in our study (29%, 87/296) is in line with a previous large German multicentre study (31%, 35/114) on antibiotic prophylaxis in predicted severe acute pancreatitis.[8] Although antibiotic prophylaxis was strongly discouraged in our study, antibiotics were used in about half the patients whereas only a third of all patients had a documented infection. This is explained by the fact that antibiotics were sometimes started pre-emptively, based on clinical suspicion of infection prior to bacterial culture results becoming available. Obviously, this clinical indication for antibiotic treatment leads to false-positive diagnoses of infectious complications. The overall rate of antibiotic use in our study was not different from that in the placebo-arms of recent trials on antibiotic prophylaxis in acute pancreatitis.[7,8]

Until now, no single study on probiotics has reported any adverse effects of probiotics comparable to the effects observed in this large RCT. In fact, several studies have associated probiotics with a reduction in infectious complications.[13,14] Most of these studies have

been performed in patients undergoing elective abdominal operations. Conversely, one randomised study in 90 critically ill patients demonstrated a non-significant increase in septic complications in the probiotics group.[24] Recently, a randomised study in 61 children admitted to a paediatric intensive care unit was discontinued prematurely because of a non-significant increase in infections in the probiotics group.[25,26] To date, the main criticism on most RCTs regarding probiotic prophylaxis is the methodological shortcomings like no intention-to-treat analysis and sample sizes too small to provide convincing evidence on relevant clinical endpoints.

In patients with acute pancreatitis, two small placebo-controlled RCTs on probiotic prophylaxis have been performed. The first study randomised 45 patients with both predicted mild and predicted severe pancreatitis of solely non-biliary causes.[15] The infection rate was reduced in the probiotics group, whereas no effect on mortality was observed. This study was criticised because of excluding biliary pancreatitis and for methodological shortcomings including small sample size and not performing an intention-to-treat analysis.[27,28] In the second trial by the same group in 62 patients with predicted severe pancreatitis, the difference in the rate of infectious complications initially observed could not be reproduced.[29] This second study used a probiotic preparation previously found to be effective in preventing infectious complications in patients undergoing abdominal operations.[13,14]

Because the findings of our trial are in marked contrast with the previous reports, we closely scrutinised our results for explanations other than a deleterious effect of probiotics. First of all, randomisation was successful as there were no significant differences in baseline characteristics between groups. In the probiotics group there was a (non-significant) higher proportion of patients with 'organ failure prior to randomisation' as well as '>30% pancreatic parenchymal necrosis'. When we assessed this unbalance using logistic regression the (adjusted) mortality remained significantly higher in the probiotics group (data not shown). We also found no indication that treatment effects differed in the subgroup analyses on necrotizing pancreatitis and aetiology. Next, we considered whether the composition of the product or the dosages used explained for the effects observed. The daily dosage was similar to dosages used in previous studies and, although the combination of probiotic strains administered was different from the preparations used so far, the individual strains have an unblemished reputation as probiotics, both in (smaller) clinical studies and in daily practice in the food industry. The six probiotic strains used in this study were selected from 69 different probiotic lactic acid bacteria based on their capacity to inhibit growth of gut derived

pathogens and to modulate immune responses.[20] The combination of strains was shown to result in a superior antimicrobial spectrum, induction of interleukin 10 and silencing of pro-inflammatory cytokines as compared to the individual components.[20] The combination of strains was found capable of inhibiting *in vitro* growth of a wide variety of pathogens cultured from pancreatic necrosis.[21] Again, the combination of strains had better growth-inhibiting capacities than the individual stains.[21] In addition, when the preparation was administered prior to induction of a severe acute pancreatitis in rats, a significant reduction of both infectious complications and late mortality was attained.[30] The same preparation was also used in three small clinical studies under elective circumstances in healthy volunteers, patients with ileostomy, patients about to undergo pancreaticoduodenectomy and patients with primary sclerosing cholangitis, and no adverse events were noted (unpublished data, trial registry ISRCTN45167712, ISRCTN71637623 and NCT00161148). However, obviously, these patients were less ill than the patients in the present study.

All previous randomised trials with probiotics have been of much smaller sample size and with fewer critically ill patients as compared to the present study. Consequently, the power of these studies was too small to detect differences in mortality or uncommon adverse events such as bowel ischaemia as found in our study. In our study, probiotics caused a significant increase in mortality, most likely due to deleterious effects on the (small) bowel wall. After administering probiotics, no significant increase in new onset organ failure was observed. Possibly, probiotics especially exert their deleterious effects in patients already suffering from organ failure. Because the exact mechanism responsible for bowel ischaemia is unknown at present we cannot exclude nor confirm that another product, i.e., combination of strains or single strain, would have resulted in similar results. Considering the fatal character of the complications observed, before the mechanism of the complications observed has been unravelled, the administration of any type of probiotic in this category of patients must strongly be advised against.

This is the first study to report on bowel ischaemia as a complication in the severely ill patient using probiotics. Non-occlusive mesenteric ischaemia is well-known in critically ill patients[31] and several cases of non-occlusive mesenteric ischaemia have been reported in acute pancreatitis.[32] This may explain why only two of the nine cases of mesenteric ischaemia were reported as a 'serious adverse event'. There is evidence to suggest that intestinal blood flow at the mucosal level is generally reduced in acute pancreatitis. An experimental study in rats found a reduction in the blood flow to the intestinal mucosa of up to 85%.[33] A clinical study in patients with severe pancreatitis demonstrated a

significant increase in a biological marker for enterocyte death and small bowel ischaemia.[34] Some have suggested that in the severely ill patient going through a phase of severe systemic inflammation or organ failure, an already critically reduced blood flow and oxygen supply in the small bowel mucosa may be further compromised by the administration of enteral feeding known for its increased demand for local oxygen.[35,36] It has been hypothesised that this is a local effect, as ischaemia usually occurred at the site of administration of enteral feeding.[35,36] In none of the reports until now has this phenomenon been recognised as an argument to refrain from enteral nutrition in critically ill patients because the beneficial effects outweigh the small risk of developing ischaemia.

With only these recent clinical observations available, we can only speculate as to the mechanism of bowel ischaemia in the probiotics group. The administration of 10 billion probiotic bacteria per day on top of enteral nutrition may have even further increased local oxygen demand, with a combined deleterious effect on an already critically reduced blood flow. A second possible explanation may be that the presence of probiotics caused local inflammation at the mucosal level. It is known from experimental studies that gut epithelial cells under metabolic stress react to commensal bacteria with an inflammatory response.[37] One could hypothesise that increasing the bacterial load in the small bowel could lead to aggravation of local inflammation again with a further reduction of capillary blood flow and ultimately ischaemia. Notably, three of the six autopsy reports of patients with bowel ischaemia indeed mentioned 'inflammatory changes of the small bowel wall',

A speculative parallel with immunonutrition can be drawn from a recent meta-analysis showing that whereas immunonutrition in elective surgical patients reduced the infection rate, it increased mortality in critically ill patients.[38] This effect was only seen in studies of high methodological quality and the reasons for the increased mortality could not be identified. Experimental studies in rats demonstrated that pre-treatment with glutamine protects against the effects of bowel ischaemia[39], whereas mortality increased when glutamine was administered after the induction of a low flow state.[40] Apparently, there is reason for concern regarding administration of potent immuno-nutritional supplements in the presence of a low flow state, or more generally, in the critically ill.

Our findings show that probiotics should not be administered routinely in patients with predicted severe acute pancreatitis, and that the particular composition used here should

be banned for the present indication. Whether other (combinations of) strains might have resulted in different results is debatable, but, until the underlying mechanism is actually revealed, administering probiotics in predicted severe acute pancreatitis must be regarded as unsafe. Most importantly, probiotics may no longer be considered as 'harmless' adjuncts to enteral nutrition, especially in critically ill patients or patients at risk for non-occlusive mesenteric ischaemia.

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Contributors

MGHB, EB, MAB, HvG, HMT, VBN, BvR, BJMW, RJP, AFMS, CHCD, CHJvE, LMAA, HGG and several other members of the study group participated in the design of the study. MGHB, HCvS, TLB collected the data.

MGHB and EB performed the statistical analysis. MGHB drafted the first and subsequent versions of the manuscript with input from all authors. HGG supervised the current study. All authors read and approved the final manuscript.

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Conflict of interest statement

HM Timmerman is an employee of Winclove Bio Industries, Amsterdam and the University Medical Center Utrecht. None of the other authors have any conflict of interest to declare.

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Chapter 4

Intestinal barrier dysfunction in a randomised placebo-controlled trial of probiotic prophylaxis in acute pancreatitis

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Submitted

Abstract

Background

Intestinal barrier dysfunction (i.e. mucosal damage and increased intestinal permeability) is thought to be associated with infectious complications, organ failure and mortality in acute pancreatitis. Enteral administration of probiotics could mitigate intestinal barrier dysfunction and hereby prevent infectious complications. Clinical evidence for both these suggestions is however lacking.

Methods

During a randomised, placebo-controlled, multicentre trial on probiotic prophylaxis in patients with predicted severe acute pancreatitis, intestinal mucosal damage was assessed in 141 patients by measuring the excretion of intestinal fatty acid binding protein (IFABP), a protein located specifically in small bowel enterocytes, in urine collected 24-48 hrs after onset of probiotic or placebo treatment. Intestinal permeability was determined in 101 patients at the same time and seven days thereafter by measuring the recovery of enterally administered polyethylene glycols (PEGs) with varying molecular weights (400, 1500, 4000 and 10000 kDa).

Results

IFABP concentrations in the first 72 hours were higher in patients who developed bacteraemia ($p=0.03$), infected pancreatic necrosis ($p=0.01$), pancreatic necrosis ($p=0.001$) or organ failure ($p=0.008$). PEG-recovery in the first 72 hours was higher in patients who developed organ failure (PEG 4000, $p<0.0001$), bacteraemia (PEG 4000, $p=0.001$) or died (PEG 4000, $p=0.009$). Probiotic prophylaxis was associated with an increase in mucosal damage (median IFABP 362 vs 199 pg/ml; $p=0.02$) but did not influence intestinal permeability. This effect was restricted to patients with organ failure.

Conclusion

Intestinal barrier dysfunction early in the course of acute pancreatitis was associated with bacteraemia, infected necrosis, organ failure, and mortality. The probiotics administered did not alter intestinal permeability but increased mucosal damage in patients with organ failure.

Introduction

Infectious complications are the major cause of morbidity and mortality in acute pancreatitis.[1] Intestinal barrier dysfunction (i.e. mucosal damage and increased intestinal permeability) and subsequent bacterial translocation are held responsible for the majority of these infectious complications.[2-4] However, the association of intestinal barrier dysfunction and clinical infections, particularly in acute pancreatitis, has not been established. Furthermore, no study has demonstrated a direct positive effect of antibiotic or probiotic prophylactic treatment on either mucosal damage or intestinal permeability.

From animal studies it is known that in acute pancreatitis the blood flow to the intestinal mucosa is reduced up to 85%, leading to relative or absolute hypoperfusion.[5] The resulting mucosal damage can be assessed, in the clinical situation, by measuring the urinary excretion of intestinal fatty acid binding protein (IFABP). IFABP is a protein specifically located in the apical villi of small bowel mucosa and is released into the circulation in the event of enterocyte death.[6] In previous clinical studies, IFABP was considered a valuable early marker for mucosal damage and intestinal ischaemia.[7-10] Moreover, it was found to be increased in patients with severe pancreatitis and associated with organ failure but not with infectious complications.[11] It has been reported that increased intestinal permeability, assessed by recovery of enterally administered polyethylene glycols (PEGs) with varying molecular weights, is associated with endotoxemia, organ failure and mortality but not with infectious complications in patients with acute pancreatitis.[2,11]

Evidence from animal studies suggests that probiotic prophylaxis stabilizes the intestinal barrier function and minimises bacterial translocation and subsequent infectious complications.[12-15] However, there is no clinical evidence of a positive effect of probiotic prophylaxis on intestinal barrier function in severely ill patients. Two small placebo-controlled trials studied probiotic prophylaxis in patients with acute pancreatitis. The first study demonstrated a reduction of infectious complications[16], the second trial could not confirm these findings.[17]

In the present study, we assessed intestinal barrier function in 141 of 296 patients included in a randomised, placebo-controlled trial on probiotic prophylaxis in predicted severe acute pancreatitis (PROPATRIA; probiotics in pancreatitis trial).[18] Completely unexpectedly, a significant, more than twofold increase in mortality was observed in patients receiving probiotic prophylaxis (16% vs placebo 6%), whereas probiotics did

not alter the rate of infectious complications.[18] Furthermore, 9 patients in the probiotics group developed macroscopic bowel ischaemia (8 with fatal outcome), whereas none was observed in the placebo group (p=0.004).[18] These results initiated a widespread discussion focussing on a) the potential role of probiotics in the development of bowel necrosis, and b) the subgroup of patients potentially at risk for developing bowel necrosis when receiving probiotics.[19-22] Because the PROPATRIA study included more patients with organ failure than any previous trial it was suggested that the negative effect of probiotics could have been restricted to this subgroup of patients.[22]

Based on the previous experimental studies we hypothesised that: (a) intestinal barrier dysfunction in acute pancreatitis is associated with infectious complications, organ failure and mortality. Based on the abovementioned findings that probiotics did not reduce the incidence of infectious complications but appeared to be involved with bowel necrosis in some patients with severe acute pancreatitis, we additionally hypothesised that: (b) the administered probiotics do not influence intestinal permeability, but (c) potentially aggravate mucosal damage in the subgroup of patients with organ failure.

Methods

Study design

This study was a sub-study of a randomised, placebo-controlled, double-blind, multicentre trial on probiotic prophylaxis in patients with predicted severe acute pancreatitis (PROPATRIA; probiotics in pancreatitis trial). The protocol and the results of PROPATRIA were published recently.[18,23] All patients or their legal representatives gave written informed consent. This study was conducted in accordance with the principles of the Declaration of Helsinki. It was investigator-initiated and investigator-driven. The ethical review boards of the participating hospitals approved the protocol. Patients were enrolled in PROPATRIA between March 2004 and March 2007. Once the trial was well underway and the trial's infrastructure had proven to be sound, and after an amendment regarding this sub-study had been approved by the ethical review board of the University Medical Center Utrecht, we started enrolling consecutive patients from January 2005 to March 2007.

Adult patients with a first documented episode of acute pancreatitis were enrolled in 8 Dutch university medical centres and 7 major teaching hospitals. Patients with predicted severe acute pancreatitis, defined as Acute Physiology and Chronic Health Evaluation

[APACHE II] score ≥8[24], or Imrie/modified Glasgow score ≥3[25], or C-reactive protein >150 mg/L[26], were eligible for randomisation between probiotics or placebo. The randomised patients received a multispecies probiotic preparation[27] or a placebo (both: Winclove Bio Industries, Amsterdam) at the first possible occasion but always within 72 hours after onset of symptoms of pancreatitis. All patients received enteral nutrition (Nutrison Multi Fibre®, Nutricia, Zoetermeer, the Netherlands) administered by a nasojejunal feeding tube.

Patients were not eligible for this sub-study if any of the following criteria were present: renal failure (serum creatinine >177 mmol/L after rehydration or need for haemofiltration or haemodialysis) or gastric retention >250 cc with no nasojejunal feeding tube in situ. Reasons for exclusion from PROPATRIA were: post-endoscopic retrograde cholangiopancreatography pancreatitis, suspected malignancy of the pancreas or biliary tree, non-pancreatic infection/sepsis caused by a second disease, diagnosis of pancreatitis first made at operation, or medical history of immune deficiency.

Measurement of intestinal permeability

PEGs with molecular weights 400, 1500, and 4000 kDa were obtained from Bufa Chemical Company (Uitgeest, the Netherlands) and PEG with molecular weight 10,000 kDa from Sigma Chemical Company (St Louis, MO). The PEG solution contained 5 g PEG 400, 1.5 g PEG 1500, 5 g PEG 4000, and 10 g PEG 10,000 dissolved in 100 mL water, adapted from Parlesak *et al*.[28] Sorbate (0.1 %) was added as a preservative. The PEG solution was prepared and quality- and purity-controlled as a pharmaceutic by the Department of Pharmacy of the UMC Utrecht. Within 72 hours after admission, but always 24-48 hours after randomisation, the mixture of PEGs was enterally administered via a nasojejunal feeding tube. Twenty-four hours urine output was collected and stored at -20 ^{0}C. This procedure was repeated after 7 days.

Upon analysis, urine samples were homogenised and 25 mL were centrifuged at 1000 x g for 10 minutes. Two mL of clear supernatant were desalted by treatment with an ion-exchange resin (Bio-Rad RG 501-X8, Hercules, CA, USA). The resin was removed by centrifugation and 50 μL supernatant were analysed by reverse-phase high performance liquid chromatography (HPLC) (Shimadzu SCL-10A VP, Shimadzu Benelux, 's-Hertogenbosch, the Netherlands) using a 25 cm 5μm Lichrospher 100-RP 18E column equipped with a 1.5 cm similar guard column (Li Chro Cart 2504 mm, Merck KgaA, Darmstadt, Germany), and evaporative light-scattering detection (Alltech 500, Grace Alltech Applied Science, Breda, the Netherlands). The mobile phase consisted of a gradient of 40-80% methanol in water. Detection limits were 0.05 mg/mL for PEG

400 and 0.005 mg/mL for PEGs 1500, 4000, and 10,000. Analytical recovery of all four PEGs was 100 ± 4 %, reproducibility 97 ± 1 %. It is assumed that PEG 400 can freely pass the intact intestinal mucosal barrier, whereas larger PEGs can only pass if paracellular intestinal permeability is compromised. Therefore, as PEG 400 acted as a 'positive control', tests were excluded in cases where the recovery of PEG 400 was less than 10%.

Measurement of urinary IFABP

IFABP concentrations were determined in 100 µL urine samples, taken from the 24 hours urine collected for the PEG permeability test, using the human IFABP enzyme-linked immunosorbent assay (ELISA) kit (HyCult Biotechnology bv Uden, the Netherlands). Assays were performed in accordance with the manufacturer's instructions. To compensate for differences in urinary volume, the total amount of IFABP excreted was calculated by multiplying the concentration with the total 24-hour urine volume. The detection limit of the essay was 20 – 5000 pg/ml.

Study parameters

Bacteraemia was defined as a positive blood culture. In cases of a positive blood culture with non-pathogens like coagulase-negative staphylococci, this had to be confirmed by a second positive culture. Infected necrosis was defined as a positive culture of (a) a fine needle aspirate or (b) a peripancreatic fluid sample or (c) necrosis removed during first surgical intervention. Organ failure was defined as (a) PaO_2 < 60 mmHg despite FiO_2 30%, or (b) the need for mechanical ventilation (pulmonary insufficiency); (c) serum creatinine > 177 mmol/L after rehydration or need for haemofiltration or haemodialysis (renal failure), or (d) systolic blood pressure < 90 mmHg despite adequate fluid resuscitation or need for vasopressor support (cardiocirculatory insufficiency). All definitions are adapted from the Atlanta classification.[29] Onset of organ failure, bacteraemia and infected necrosis were recorded in days after admission (day 0 = day of admission). All patients underwent a contrast-enhanced computed tomography (CECT) 7-10 days after admission. All CECTs were re-evaluated by one radiologist (TLB) unaware of the treatment administered. Severe acute pancreatitis was defined by the presence of either pancreatic necrosis and/or organ failure.

Statistic analysis

We investigated whether intestinal barrier dysfunction, as determined in the first 72 hrs of admission, was associated with clinical outcome (bacteraemia, infection of pancreatic necrosis, (extent of) pancreatic necrosis, organ failure, severe pancreatitis,

and mortality). Next we investigated the effect of probiotic prophylaxis on the intestinal barrier function both in the first 72 hrs of admission and seven days thereafter. IFABP levels were compared for patients that died and/or developed macroscopic bowel necrosis. Finally we correlated IFABP levels with PEG recovery.

Unless specifically stated otherwise, all reported results concern the initial IFABP and PEG test, done within the first 72 hours of admission. Recovery of the PEG molecules with molecular weights 400, 1500, 4000 and 10,000 PEG in urine samples was presented as a percentage of the quantity administered. Differences between groups in PEG recovery were analysed for the individual components as continuous variables. When significant differences were detected for more than one PEG molecule, the p values were provided for the largest PEG molecule.

Normally distributed data were presented as mean ± standard deviation. All non-normally distributed data were presented as median with interquartile range (IQR: P25-P75). Differences in proportions were tested by Fisher's exact test in all cases. The differences in PEG-recovery and IFABP concentration (or total amounts) were tested with the Mann-Whitney U test when comparing two groups, and Kruskal Wallis test when comparing multiple groups. The relationship between PEG-recovery and IFABP concentrations were assessed with Pearson (rank) correlation. The differences between outcome for the initial and the second intestinal dysfunction tests were tested with the Wilcoxon signed ranks test. All statistical analyses were performed using SPSS for Windows version 12.0.0.1 (SPSS Inc, Chicago, Illinois). A two-sided P value < 0.05 was considered statistically significant.

Results

Between January 2005 and March 2007, 141 patients with a first episode of predicted severe acute pancreatitis were included (*Figure 1*). Baseline characteristics of the 141 patients with a IFABP-test and the 101 patients with a valid PEG-test are shown in *Table 1*. Reasons for excluding non-valid test results are listed in *Figure 1*.

Comparability of included and excluded patients

The 141 patients in whom an IFABP-test was performed had a lower mortality than the remaining 155 patients in the trial (8% *vs* 14%, p=0.1). The incidence of infections was similar (bacteraemia, p=0.65, infected necrosis p=0.47).

The 101 patients with a valid initial PEG-test had a lower mortality rate than the remaining 195 patients in the trial (4% *vs* 15%, p=0.03). The incidence of infections was similar (bacteraemia 19% *vs* 19%, infected necrosis 9% *vs* 13%, p=0.34).

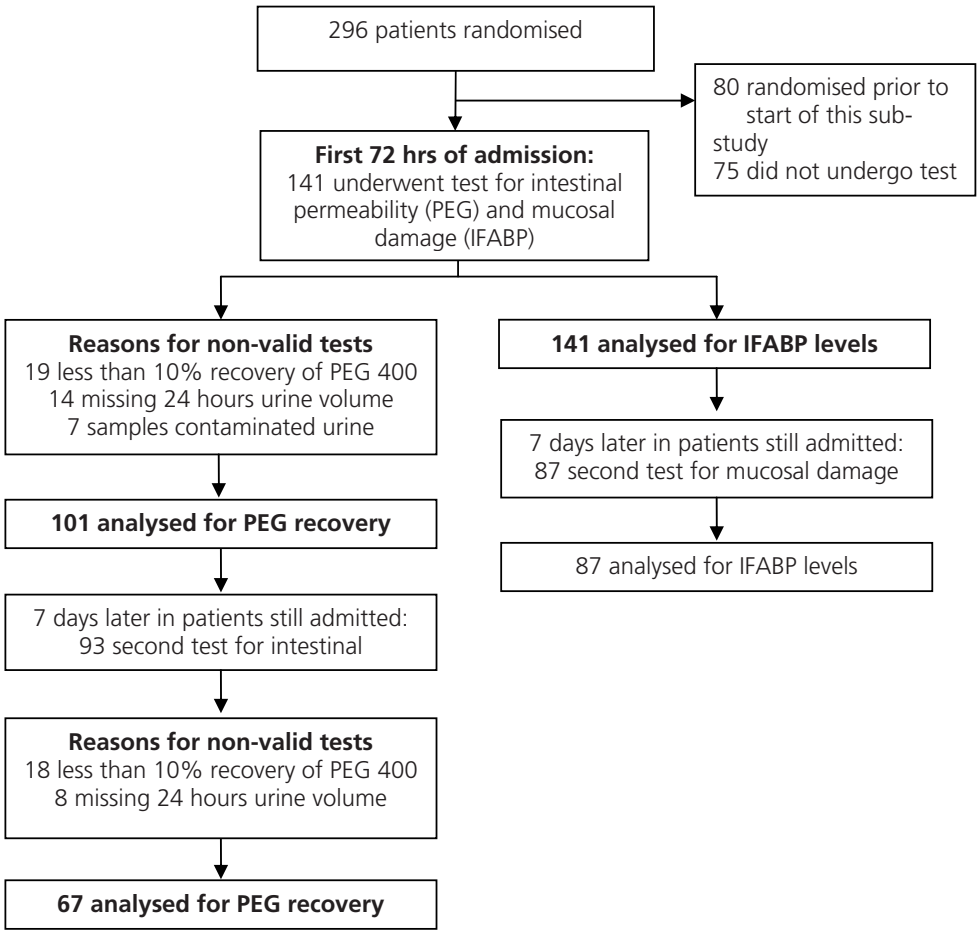
Table 1 Clinical characteristics of patients with predicted severe acute pancreatitis on whom tests for intestinal barrier dysfunction were performed

	Mucosal damage/ IFABP test (n=141)	Intestinal permeability/ PEG test (n=101)*
Male, n (%)	85 (60)	64 (63)
Age	60.5 (± 160.0)	61.9 (± 150.0)
Body-mass index (kg/m^2) (median, IQR)	26.1 (24.2-29.8)	25.8 (23.4-29.1)
Etiology of pancreatitis, n (%)		
Biliary	83 (59)	61 (60)
Alcohol	23 (16)	19 (19)
Other	9 (6)	6 (6)
Unknown	26 (18)	15 (15)
American Society of Anesthesiologists class, no (%)		
I (healthy status)	56 (40)	40 (40)
II (mild systemic disease)	75 (53)	57 (56)
III (severe systemic disease)	10 (7)	4 (4)
Severity of pancreatitis		
APACHE-II score†	8.6 (± 4.7)	8.0 (± 4.2)
Imrie score	3.3 (± 1.6)	3.1 (± 1.5)
CRP, highest first 48hrs (mg/L)	265 (± 119)	245 (± 114)
Time in days between: (median, IQR)		
first symptoms and 24hr urine collection	3.0 (2.0-3.0)	2.0 (2.0-3.0)
admission and 24hr urine collection	2.0 (1.0-3.0)	2.0 (1.0-3.0)
start probiotics/placebo and 24hr urine collection	1.0 (0.0-1.0)	1.0 (0.0-1.0)
admission and enteral nutrition	2.0 (1.0-2.0)	1.0 (1.0-3.0)
Contrast-enhanced computed tomography – no (%)		
Necrotising pancreatitis	37 (26)	20 (20)
<30% pancreatic necrosis	11 (8)	7 (7)
>30% pancreatic necrosis	26 (18)	13 (13)
No contract-enhanced CT performed	7 (5)	6 (6)
CT severity index (median, IQR)	4 (2-6)	4 (2-4)
Randomisation: probiotics / placebo	69 / 72	50 / 51
Severe acute pancreatitis‡	46 (33)	24 (24)

*These patients are all also included in the IFABP group

†Highest score at day of admission, ‡Organ failure and/or pancreatic necrosis

Figure 1 Study flowchart



Relation between intestinal barrier dysfunction and clinical outcome

IFABP levels and clinical outcome

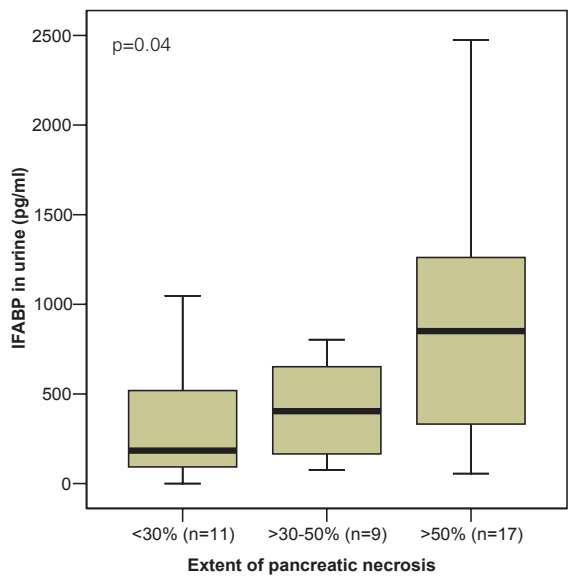
In *Table 2* we show that IFABP levels, representing mucosal damage, were significantly higher in patients who developed bacteraemia, infected necrosis, organ failure, severe acute pancreatitis, but not in those who died. IFABP levels were higher in patients who developed pancreatic necrosis (n=37), than patients who did not (470 (161-990) *vs* 203 (50-454) pg/ml, p=0.001). In patients with pancreatic necrosis, IFABP levels correlated positively with the extent of pancreatic necrosis (<30%, 30-50% and>50%, p=0.04, *Figure 2*).

Table 2 IFABP concentrations, reflecting mucosal damage, within 72 hours of admission in 141 patients with predicted severe acute pancreatitis

Event	Onset (in days)*	N event (%)	IFABP concentrations†		p value
			Event	No event	
Bacteraemia	7 (3-14)	28 (20)	403 (144-911)	203 (66-547)	0.03
Infected necrosis	33 (22-59)	19 (14)	377 (162-1044)	255 (57-525)	0.01
Organ failure	2 (1-4)	26 (18)	438 (182-1044)	203 (67-502)	0.008
Severe pancreatitis	n.a.	46 (33)	370 (129-840)	203 (50-455)	0.009
Mortality	18 (6-65)	11 (8)	69‡ (0-652)	249‡ (82-570)	0.47

Values are proportions and medians (P25-P75)
*Median onset in days after admission (P25-P75)
†Median concentration (pg/ml, P25-P75)
‡Mean concentrations were 565 (deceased) *vs* 383 pg/ml (survivors)
n.a. not applicable

Figure 2 IFABP levels according to the extent of pancreatic necrosis.



*Kruskal Wallis test (multiple groups, non-parametric test), overall p=0.04.
IFABP levels <30% vs >50%, p=0.02, >30-50% vs >50%, p=0.08.

PEG recovery and clinical outcome

In *Table 3* we show that PEG recovery, representing intestinal permeability, was significantly increased in patients who developed bacteraemia, organ failure, severe acute pancreatitis or died, but not in those who developed infected necrosis.

Table 3 PEG recovery, reflecting intestinal permeability, within 72 hours of admission in 101 patients with predicted severe acute pancreatitis

Event	Onset (in days)*	N event (%)	PEG recovery (%)†		p value
			Event	No event	
Bacteraemia	4 (3-11)	19 (19)	0.1 (0.0-0.5)	0.0 (0.0-0.03)	0.001
Infected necrosis	36 (23-64)	9 (9)	1.4 (0.0-4.1)	0.0 (0.0-3.1)	0.57
Organ failure	3 (2-6)	15 (15)	0.3 (0.04-0.5)	0.0 (0.0-0.03)	0.0001
Severe pancreatitis	n.a.	24 (24)	0.1 (0.0-0.4)	0.0 (0.0-0.03)	0.001
Mortality	7 (6-107)	4 (4)	0.2 (0.1-0.9)	0.0 (0.0-0.1)	0.009

Values are proportions and medians (P25-P75)

*****Median onset in days after admission (P25-P75)

†Median PEG recovery (P25-P75). Results are given for the largest PEG-molecule that differed significantly between the groups. For bacteraemia, organ failure, severe pancreatitis and mortality this was PEG 4000 and for infected necrosis PEG 1500.

n.a. not applicable

Relation between intestinal barrier dysfunction and probiotic prophylaxis

IFABP levels and probiotic prophylaxis

Median IFABP levels (24- 48 hours after start of probiotic prophylaxis) were higher in the probiotic group than in the placebo group (362 (108-748) vs 199 (50-449) pg/ml, p=0.02). This difference was the greatest in 26 patients with organ failure (probiotics (n=13): 837 (437 - 1342) vs placebo (n=13): 278 (44-594) pg/ml, p=0.01, *Figure 3a*). In patients with cardiocirculatory insufficiency, this difference was 886 vs 200 pg/ml (p=0.04). In the 115 patients without organ failure IFABP levels were not different between patients receiving probiotic or placebo treatment (232 (84-552) vs 166 (48-453), p=0.18, *Figure 3b*). When comparing the total amount of IFABP excreted similar results were found; in patients with organ failure (p=0.04), and in patients without organ failure (p=0.26). There was no difference in onset of organ failure between patients in the probiotic and placebo group (day 2 (0-3.5) vs 2 (0-4.0) after admission, p=0.74).

IFABP tests were performed on 11 patients who ultimately died (probiotic 6 *vs* placebo 5). Median IFABP levels were 17 times higher in deceased patients who had received probiotics as compared to those who had received placebo (561 (52-2193) vs 33 (0-209) pg/ml, p=0.08).

IFABP tests were performed on three of the patients who developed macroscopic small bowel necrosis in the probiotics group (none did in the placebo group). In these patients, the median IFABP concentration was more than eight times higher than in patients without small bowel necrosis (2099 vs 254 pg/ml, p=0.005).

Figure 3a IFABP levels according to the use of probiotics in patients with organ failure

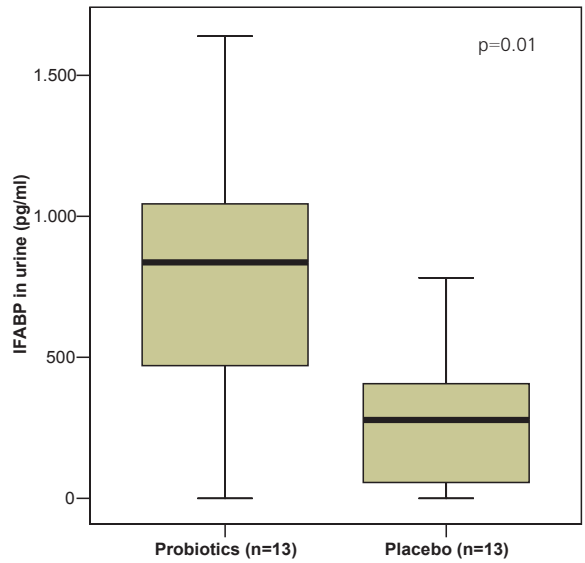
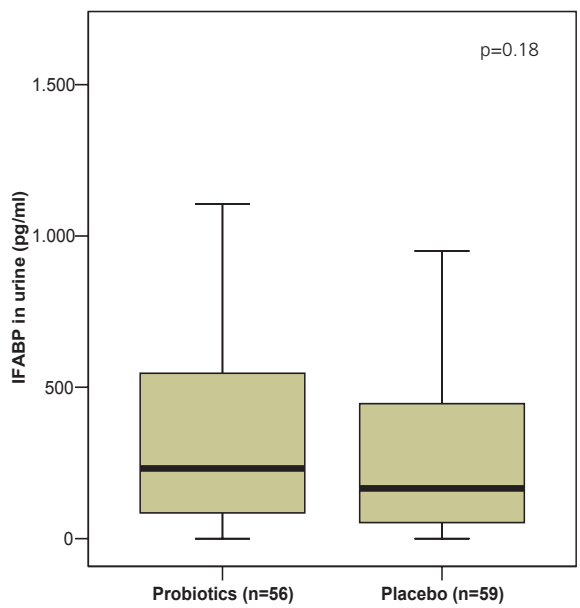


Figure 3b IFABP levels according to the use of probiotics in patients without organ failure



In the patients on whom the IFABP test was repeated 7 days later (n=87), the median IFABP concentration in both the probiotics and placebo groups had significantly decreased (344 to 77 pg/ml, p=0.0001 and 214 to 63 pg/ml, p=0.007). At that point in time IFABP levels were no longer significantly different between the probiotic and placebo groups (probiotic (n=45): 77 (37-246) vs placebo (n=42): 63 (1-223) pg/ml, p=0.42). In this early disease period (day 9 of admission) 11 patients (7%) had died in the probiotics group vs 3 (2%) in the placebo group (p=0.05).

PEG recovery and probiotic prophylaxis

Probiotic prophylaxis did not affect intestinal permeability as assessed by PEG excretion. The median recovery of the smallest passing molecule at the initial measurement (PEG 1500) did not differ between the two groups (probiotics group 0.0 (0.0-3.6%) vs placebo 0.0 (0.0-3.1%), p=0.99). One week later the permeability test was repeated in 35 patients who had received probiotics and 32 patients who had received placebo, again PEG 1500 recovery was similar across treatment groups (1.1 (0.0-2.8%) vs 0.6 (0.0-2.9%), p=0.85). Within both the probiotics and placebo groups no significant difference in recovery of PEG had occurred over time (PEG 1500: p=0.54 and p=0.25; Wilcoxon signed ranks test).

Correlation of IFABP levels with PEG recovery

At the time of the greatest disturbance in intestinal dysfunction, at the initial test, no significant correlation could be demonstrated between PEG- and IFABP-recovery (PEG 1500: Pearson correlation 0.17, p=0.1).

Discussion

This study is the first to demonstrate a relationship between intestinal barrier dysfunction and infectious complications such as bacteraemia and infected pancreatic necrosis in humans. The use of probiotic prophylaxis added to bowel mucosal damage in patients with organ failure, but did not affect intestinal permeability in this subgroup. This is in concordance with the clinical findings of macroscopic (small) bowel necrosis in the probiotic group.[18] In patients without organ failure, probiotic prophylaxis did not increase mucosal damage and did not influence intestinal permeability either.

IFABP has previously been identified as a useful marker for intestinal ischaemia.[8,9] To date, only one study demonstrated that early in the course of severe acute pancreatitis urinary

IFABP levels are significantly increased.[11] This study also showed a relationship between IFABP levels and multi-organ failure and concluded that in severe acute pancreatitis a certain extent of intestinal hypoperfusion is present. We confirmed the relationship between IFABP levels and severe pancreatitis and added a relation between increased IFABP levels and bacteraemia/infected pancreatic necrosis. Surprisingly, median IFABP levels in patients who died were not higher than those of survivors.

There are no studies available to investigate the effects of probiotics on IFABP excretion. In this study, IFABP levels in patients who died in the probiotics group were higher than those who died in the placebo group, possibly suggesting that probiotics added to mucosal damage and induced a more severe course of the disease. Interestingly, IFABP levels were further related, not only to the presence, but also to the extent of pancreatic necrosis. Apparently, a similar mechanism responsible for mucosal hypoperfusion and enterocyte cell death plays a role in pancreatic perfusion, leading to more pancreatic necrosis in patients suffering from severe intestinal hypoperfusion

Our findings regarding intestinal permeability are corroborated by a previous study in patients with severe acute pancreatitis that reported on a relation between early increase in intestinal permeability (as assessed with PEGs) and endotoxemia, organ failure and mortality, but not between increased permeability and infectious complications.[30] Studies with other markers for intestinal permeability than PEG, such as lactulose/mannitol excretion ratio, have also reported a relationship between increased permeability and severity of acute pancreatitis.[31] A recent study of 26 patients with acute pancreatitis failed to correlate increased intestinal permeability to the presence of microbial DNA in peripheral blood.[2] However, in that study only two patients developed bacteraemia as compared to 19 patients with bacteraemia in the present study.

In line with the previous studies we could not demonstrate a relation between increased intestinal permeability and infected pancreatic necrosis, although a relation between increased intestinal permeability and bacteraemia was found. An important difference between bacteraemia and infected necrosis is the time of onset. Bacteraemia was identified at a median of 4 days after admission, while infected necrosis was not diagnosed until four weeks later. So either alternative routes are responsible for late infection of necrosis or the necrosis gets contaminated with micro-organisms early in the course of the disease, i.e. around day 4, and symptoms only develop in the later phase of immuno-suppression or, in some patients, due to successful host defence mechanisms, not at all. In contrast to PEG recovery, the IFABP levels were correlated

with infected pancreatic necrosis. So, one may postulate that bacterial translocation does occur in the absence of a (with PEGs) detectable increase of intestinal permeability. This is also reflected by the absence of a significant relation between increased IFABP levels and increase in intestinal permeability. With their PEG-mixture, Rahman and Ammori were able to demonstrate a positive correlation between IFABP levels and PEG recovery.[11] However, also in their analysis, most patients with high IFABP concentrations (>1000 pg/ml) had a normal intestinal permeability. In our study only 4 of 7 patients with IFABP levels >1000 pg/ml had a detectable PEG 1500 recovery (mean 4.4%). It is therefore apparent that mucosal damage does not necessarily lead to increased intestinal permeability, assuming that PEG 1500 recovery is sensitive enough to adequately detect an increase in permeability. One could hypothesise that in the presence of mucosal damage, the other 'lines of defense' of the intestinal barrier may still be intact thus maintaining permeability at a normal level.[32]

Probiotic prophylaxis has been suggested to be capable of preventing infections when administered prophylactically to patients scheduled for abdominal operations.[33,34] Results of probiotic prophylaxis in clinical acute pancreatitis, however, have been conflicting.[16-18] Two previous randomised studies on (nasogastric) probiotic prophylaxis in elective surgical and critically ill patients also failed to demonstrate a reduction in bacterial translocation to mesenteric lymph nodes, intestinal permeability and nasogastric colonisation with enteric organisms.[35,36] It is important to realise that in the studies on probiotic prophylaxis different patient groups, different mixtures of probiotics, different nutrition and different routes of administration (nasojejunal *vs* nasogastric) were used. In the present randomised trial, probiotic prophylaxis (and fibre enriched nasojejunal feeding) did not reduce the rate of infectious complications but was associated with a (significant) more than two-fold increase in mortality as well as nine cases of bowel necrosis, eight of whom died.[18] The results of this study may explain the apparent paradox between the favourable results of nasojejunal probiotic administration in the elective pre- and postoperative setting (in the absence of organ failure) *vs* the deleterious effects observed in this randomised trial (with large numbers of patients with organ failure). When probiotics were administered to patients with organ failure a significant increase of urinary IFABP levels was observed, suggesting an increase in mucosal damage. In contrast, the administration of probiotics to patients without organ failure did not cause increased IFABP levels. Apparently, the nasojejunal administration of 10^{10} probiotic bacteria to patients with developing organ failure, with an inevitable reduction in mesenteric blood flow caused, via a yet unknown mechanism, clinically relevant worsening of mucosal damage.[18] In the absence of organ failure, apparently

blood flow and/or immune function were not critically altered and probiotic prophylaxis therefore was not associated with detectable mucosal damage.
The strength of the current study is that it was performed in the controlled setting of a large randomised clinical trial of probiotic *vs* placebo treatment. All patients received a similar regime of fibre-enriched tube feeding. These factors allow for adequate comparison between groups, such as IFABP levels between the probiotic and placebo groups. Patients with IFABP and valid permeability tests were less ill than the remaining patients in the trial, as demonstrated by a lower mortality. However, it is likely that if more ill patients (and more clinical endpoints) had been included in the present study the observed correlations would only have become stronger. The recovery of PEG molecules in our study was mostly rather low (generally <5%). Although Ammori *et al* used much larger amounts of PEG 3350 (40 gram), as compared to the amounts of PEG 1500 (1.5 gram) PEG 4000 (5 gram) in our mixture, they still found median recovery of <1% in patients with severe acute pancreatitis.[30]

In summary, intestinal barrier dysfunction early in the course of acute pancreatitis was related to infectious complications (e.g. bacteraemia and infection of (peri)pancreatic necrosis), organ failure, severe acute pancreatitis and mortality. The probiotics used in this study did not alter intestinal permeability as measured with the different PEGs. In patients with acute pancreatitis and concomitant organ failure, probiotic prophylaxis was associated with an increase in mucosal damage as measured with IFABP. Moreover, in patients without organ failure, probiotic prophylaxis was not associated with an increase in mucosal damage. Future studies aiming at preventing infectious complications by improving intestinal barrier function should take into account the possibility that (nasojejunal) probiotic prophylaxis may have deleterious effects in patients with organ failure.

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920-03-368). Neither sponsor had any involvement in any stage of the study design, data collection, data-analysis and interpretation of the study results.

Authors' contributions

MGHB, EB, MAB, HvG, HMT, VBN, BvR, BJMW, RJP, AFS, CHCD, CHJvE, LMAA, HGG and several other members of the study group participated in the design of the study.
MGHB, HCvS coordinated the study and the collecting of samples.
WR and MBMdS developed and performed the PEG analyses.
UAA performed the IFABP analyses.
MGHB and KF performed the statistical analyses.
MGHB drafted the manuscript.
HGG supervised the current study.
All authors co-authored the writing of the manuscript, read and approved the final manuscript.

Competing interests

HM Timmerman is an employee of Winclove Bio Industries, Amsterdam and the University Medical Center Utrecht.

Investigators

In addition to the authors, the following clinicians participated in this study. In parentheses the number of patients that participated. St. Antonius Hospital, Nieuwegein (43): BL Weusten, R Timmer; University Medical Center Utrecht (18); KJ van Erpecum, GA Cirkel, V Zeguers, A Roeterdink, HG Rijnhart, MP Schwartz, MS van Leeuwen, BU Ridwan; Gelderse Vallei Hospital, Ede (12): PhM Kruyt; Leiden University Medical Center, Leiden (12): A Haasnoot; University Medical Center Groningen (11): HS Hofker, VB Nieuwenhuijs, MR Kruijt Spanjer, HT Buitenhuis, SU van Vliet, S Ramcharan; Radboud University Nijmegen Medical Centre, Nijmegen (11): A Nooteboom, JB Jansen, GT Bongaerts, HC Buscher; St. Elisabeth Hospital, Tilburg (10): TA Drixler; Canisius Wilhelmina Hospital, Nijmegen (9): C Rosman, L Ootes, B Houben; Meander Medical Center, Amerfoort (8): M Mundt, R Frankhuisen, EC Consten; Academic Medical Center, Amsterdam (7): O van Ruler, DJ Gouma, MJ Bruno; Erasmus Medical Center, Rotterdam (7): JBC van der Wal; G van 't Hof, EJ Kuipers, Rijnstate Hospital, Arnhem (7): EJ Spillenaar Bilgen, P van Embden; Medical Center Rijnmond Zuid, Rotterdam (3): JF Lange, NA Wijffels, LA van Walraven, FJ Kubben; University Hospital Maastricht (1): JP Rutten.

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Chapter 5

Timing and impact of infections in acute pancreatitis

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Submitted

Abstract

Background

Because prophylactic strategies should be initiated prior to the onset of infections, data regarding the onset of infections are crucial. While infected necrosis is an established cause of mortality in acute pancreatitis, data regarding bacteraemia and pneumonia are lacking.

Methods

This prospective multicentre study in 15 Dutch hospitals included 731 patients with a primary episode of acute pancreatitis in the period 2004-2007. Presence and time of onset of bacteraemia, pneumonia, infected pancreatic necrosis, persistent organ failure (>48hr) and mortality were recorded.

Results

The initial infection in 173 patients was diagnosed after a median of 8 days (infected necrosis: day 7, extrapancreatic: day 26) of admission (interquartile range 3-20 days). Eighty percent of 61 deceased patients had been diagnosed with an infection. In 154 patients with pancreatic necrosis, bacteraemia was associated with increased risk of developing infected necrosis (65 *vs* 38%, p=0.002). In 98 patients with infected necrosis, bacteraemia was associated with higher mortality (40 *vs* 16%, p=0.014). After multivariate analysis, only persistent organ failure (Odds ratio (OR) 18.0), bacteraemia (OR 3.4) and age (OR 1.1) were associated with mortality.

Conclusions

Infections occur very early in the course of acute pancreatitis, and have a significant impact on mortality, especially in cases of bacteraemia. The focus of prophylactic strategies should be shifted to very early intervention.

Introduction

The incidence of acute pancreatitis, both in Europe and the United States, is increasing rapidly.[1,2] In Dutch non-university hospitals, during a 6-year period, a 75% increase in admissions for acute pancreatitis was observed.[3] Despite the increasing incidence, the mortality of acute pancreatitis remains as high as 5-10%, mainly due to infectious complications; not only infection of pancreatic necrosis, but also bacteraemia, pneumonia and (persistent) organ failure.[4-7]

In the past decades, many authors have attempted to reduce mortality in acute pancreatitis by preventing infectious complications with prophylactic antibiotics or probiotics, but the majority of these trials failed to show any benefit.[8,9] If future prophylactic strategies are to be effective, they should, by definition, be instituted prior to the onset of the infectious complications. Although several studies have addressed the timing of the onset of infected pancreatic necrosis[4,10], data on the time of onset and impact of extrapancreatic infectious complications, i.e. bacteraemia and pneumonia, in acute pancreatitis are essentially lacking.

A recent multicentre study on probiotic prophylaxis in acute pancreatitis performed in the Netherlands[8] provided us with the opportunity to study the impact and timing of intra- and extrapancreatic infections in a large prospective cohort of patients with a primary episode of acute pancreatitis. We aimed to answer the following three questions: (a) What is the time of diagnosis of infections in acute pancreatitis?; (b) What percentage of deceased patients had been diagnosed with an infection; (c) What is the impact of infections on outcome?

Methods

Study design

This study was a sub-study of a randomised, double-blind, placebo-controlled multicentre trial on probiotic prophylaxis - the PRObiotics in PAncreatitis TRIAL (PROPATRIA).[8] In PROPATRIA, only patients with predicted severe acute pancreatitis were randomised. During the study, data of patients with predicted mild pancreatitis were also prospectively included in a database. This study describes both patients with predicted mild and predicted severe acute pancreatitis. Sixteen patients refused informed consent for the present study. All 15 patients that refused informed consent for randomisation in PROPATRIA did provide consent for the present study.

All patients or their legal representatives gave written informed consent. This study was conducted in accordance with the principles of the Declaration of Helsinki and was investigator-initiated and investigator-driven. The ethical review board of each participating hospital approved the protocol of this sub-study.

Adult patients with a first episode of acute pancreatitis were enrolled in eight Dutch university medical centres and seven major teaching hospitals. Patients with post-endoscopic retrograde cholangiopancreaticography pancreatitis, suspected malignancy of the pancreas or biliary tree, non-pancreatic infection/sepsis caused by a second disease, diagnosis of pancreatitis first made at operation, or medical history of immune deficiency were not included.

Treatment protocol

Patients randomised in PROPATRIA were treated as previously described.[8] All randomised patients received enteral fibre-enriched nutrition (Nutrison Multi Fibre, Nutricia, Zoetermeer, the Netherlands) via a nasojejunal tube or oral nutrition when possible. Antibiotic prophylaxis was not administered routinely in patients with necrotizing pancreatitis. Contrast-enhanced computed tomography (CECT) was performed seven days after admission. Body temperature was measured at least twice daily and, in the event of fever, blood cultures were drawn. Routine fine needle aspiration was not performed in patients with pancreatic necrosis. Intervention was considered mandatory in cases of clear clinical diagnosis of infected (peri-)pancreatic necrosis (persistent fever and clinical deterioration in the third or fourth week of disease in the presence of documented necrosis or gas bubbles in the collection(s), while other sources of infection were absent). Fine needle aspiration (FNA) of (peri-)pancreatic collections to confirm the clinical suspicion was not mandatory prior to intervention.[8] At all primary procedures cultures were done to confirm the diagnosis of infected necrosis. For the non-randomised (predicted mild) patients no strict treatment protocol was dictated. However, these patients were generally treated according to the same strategy as instituted in PROPATRIA with the difference that a CECT was only done in cases of lack of clinical improvement after the first week of admission. Jejunal tube feeding was only started if resumption of oral intake was not possible after the initial days of hospitalisation.

Definitions

Bacteraemia was defined by a positive blood culture. For non-pathogens like coagulase-negative staphylococci at least two blood culture samples had to be positive. Infected

pancreatic necrosis was defined as a positive culture of peripancreatic fluid or pancreatic necrosis obtained by either fine needle aspiration, during the first percutaneous drainage or during the first surgical intervention. Only the initial culture, either via percutaneous aspiration or during surgery, was considered. Pneumonia was defined by coughing, dyspnoea, chest film showing infiltrative abnormalities and lowered arterial blood gas with positive sputum culture. If in the intensive care unit, a positive endotracheal culture was mandatory. Severe acute pancreatitis was defined by the presence of organ failure and/or pancreatic parenchymal necrosis. Persistent organ failure was defined as the presence of organ failure on at least three consecutive days (i.e. >48hrs).

Data collection

In patients randomised in PROPATRIA, data were collected as previously described.[8] Data on the non-randomised patients were also prospectively entered in a database. All CECTs were re-read by one experienced radiologist (TLB) to assess the presence of pancreatic necrosis and determine the CT severity index.[11] Referring hospitals (n=44) were contacted if data were missing on referred patients (n=88).

The presence and time of onset of bacteraemia, infected pancreatic necrosis, pneumonia (including both hospital and ventilator acquired pneumonia), persistent organ failure and mortality were recorded. The initial infectious complication was the first infection that occurred in an individual patient. Bacteria cultured at the initial infection were registered as previously described.[8] Post-mortem cultures were not allowed. Day 0 was the day of admission. After the study had ended, two of the authors (MGHB and HCvS) visited all hospitals and checked all endpoints of the present study with the primary source data.

Statistic analysis

We calculated mortality in patients with mild and severe acute pancreatitis. Time of onset of infections were presented as median with interquartile range (P25-P75). The impact of infections was expressed as the percentage of deceased patients with an infectious complication. The pathogen(s) cultured in the initial positive culture were listed. In patients with infectious complications, mortality-rates associated with the various pathogens were compared. With multivariate analysis the impact of intra- and extrapancreatic infections on mortality was determined. The impact of bacteraemia on mortality and risk of infected necrosis in patients with pancreatic necrosis was determined. Pathogens responsible for infected pancreatic necrosis and bacteraemia, in the individual patient, were compared.

Non-normally distributed data were presented as median with interquartile range (P25-P75). Differences between groups were tested with Mann-Whitney U test for non-

normally distributed data. Fisher's exact test was used for proportions in all cases. End-model building with forward stepwise backward regression was used to determine which factors contributed to mortality. All statistical analyses were performed using SPSS for Windows version 12.0.1 (SPSS Inc, Chicago, Illinois). A two-sided P value <0.05 was considered statistically significant.

Table 1 Clinical characteristics of 731 patients with a first episode of acute pancreatitis

	n=731
Men	399 (55)
Age*	58 (42-71)
Aetiology of pancreatitis	
Biliary	396 (54)
Alcohol	133 (18)
Other	64 (9)
Unknown	138 (19)
American Society of Anaesthesiologists class	
I (healthy status)	321 (44)
II (mild systemic disease)	329 (45)
III (severe systemic disease)	81 (11)
Severity of pancreatitis	
APACHE-II score†*	6 (4-10)
Imrie/Modified Glasgow score*	2 (1-4)
CRP highest first 48hrs*	201 (91-300)
Contrast-enhanced computed tomography	
Necrotizing pancreatitis	154 (21)
<30% pancreatic necrosis	54 (7)
>30% pancreatic necrosis	100 (14)
CT severity index*	4 (2-6)
Time between onset of symptoms and admission (days)*	0 (0-1)
Time between admission and enteral nutrition (days)*	2 (1-3)
Randomised to receive probiotics or placebo	152 (21) / 144 (20)

Values in parentheses are percentages unless indicated otherwise
*Values are median (P25-P75)
†Highest score at day of admission

Results

Between March 2004 and March 2007, 731 patients with a first episode of acute pancreatitis were included. Baseline characteristics are shown in *Table 1*. Clinical outcome is shown in *Table 2*. Mortality in 203 patients with severe acute pancreatitis was 28% as compared to 1% in 528 patients with mild acute pancreatitis.

Table 2 Outcome of 731 patients with a first episode of acute pancreatitis

Infectious complications (one or more)	173 (24)
Infected necrosis	98 (13)
Bacteraemia	107 (15)
Pneumonia	84 (12) †
Organ failure	129 (18)
Persistent organ failure	115 (16)
Multi-organ failure	94 (13)
Persistent multi-organ failure	78 (11)
Intensive care admission	168 (23)
Intensive care stay (days)*	11 (3-31)
Hospital stay (days)*	12 (7-25)
Severe acute pancreatitis‡	203 (28)
Mortality	61 (8)

Values in parentheses are percentages unless indicated otherwise
*Values are median (P25-P75)
†Including 49 cases of ventilator-acquired pneumonia
‡Defined as organ failure and/or pancreatic necrosis

Table 3 Time of onset of complications

	Day of onset*
Organ failure	2 (1-6)
Initial infectious complication	8 (3-20)
Pneumonia	9 (4-17)
Bacteraemia	10 (3-23)
Infected necrosis	26 (17-37)
Mortality	25 (7-45)

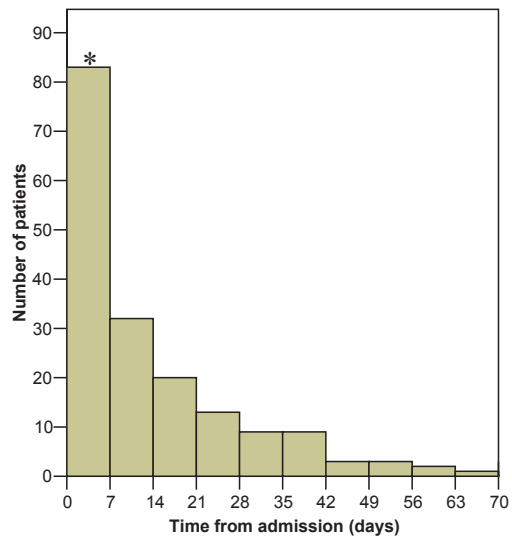
*Values are median (P25-P75), day 0 is the day of admission.

Time of onset of infectious complications

The median time onset of diagnosis of the initial infectious complications in 173 patients was day 8. Twenty per cent of infections were diagnosed within 2 days (day 0-2),

36% within 4 days and 47% within the first 7 days of admission. *Table 3* illustrates that extrapancreatic infections were generally diagnosed earlier than infected necrosis. *Figure 1* shows the time of diagnosis of the initial infectious complication per patient in days after admission. *Figure 2* shows the time of diagnosis of infected necrosis. In 18% (18 of 98 patients) infected necrosis was detected within the first 14 days of admission.

Figure 1 Time of diagnosis of the initial infectious complication in 173 patients during a first episode of acute pancreatitis.



*Forty-seven per cent of initial infectious complications occurred in the first week of admission: bacteraemia in 50, pneumonia in 37 (ventilator acquired in 25) and infected necrosis in five, patients. Nine patients had both bacteraemia and pneumonia in the first week.

Mortality and infectious complications

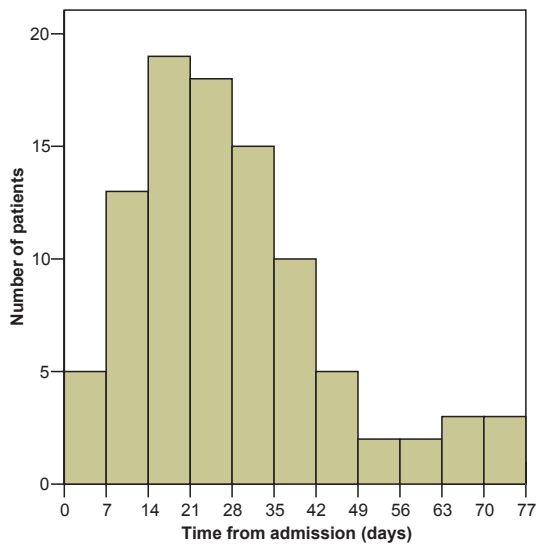
Of the 61 deceased patients, 80% had been diagnosed with an infectious complication, 77% with persistent organ failure, 61% with bacteraemia, 59% with pancreatic necrosis and 48% with infected necrosis. *Table 4* shows the relationship between mortality and infectious complications in the first two weeks of acute pancreatitis and thereafter. Forty-one% of the patients that died did so in the first two weeks of admission.

Microbiology

Table 5 shows the pathogens isolated at the time of the initial infections complication in 173 patients. In patients with infectious complications, the mortality-rate in 20 patients

with fungal infections was higher than in patients in whom other pathogens were cultured (65 *vs* 23%, p<0.0001). Mortality was also higher in 16 patients in whom *Candida* was cultured at the initial infection (56 *vs* 23%, p=0.016).

Figure 2 Time of diagnosis of infected necrosis in 98 patients during a first episode of acute pancreatitis



Impact of extrapancreatic infections on outcome

Mortality was higher in patients with pneumonia (36 *vs* 5%, p<0.0001), bacteraemia (35 *vs* 4%, p<0.0001), infected necrosis (30 *vs* 5%, p<0.0001) and pancreatic necrosis (23 *vs* 4%, p<0.0001). In the multivariate analysis of predictors for mortality, the following parameters were entered: sex, age, persistent organ failure, bacteraemia, infected necrosis and pneumonia. *Table 6* shows the results.

Table 4 Relation between time of death, infection and persistent organ failure during a first episode of acute pancreatitis

	Mortality	Mortality and infectious complication	Mortality and persistent organ failure
Day 0-14	25 (41)	15 (31)	15 (32)
Day 15 -	36 (59)	34 (69)	32 (68)
Total	61 (100)	49 (100)	47 (100)

Values in parentheses are percentages unless indicated otherwise

Table 5 Pathogens cultured from initial infectious complications in 173 patients with a primary episode of acute pancreatitis

Gram-positive bacteria	
Staphylococcus spp.	64
Staphylococcus aureus	37
Coagulase-negative *Staphylococcus*	23
Enterococcus spp.	29
Streptococcus spp.	16
other gram-positive microorganisms	9
Gram-negative bacteria	
Enterobacteriaceae	86
Escherichia coli	48
Klebsiella spp.	20
other gram-negative microorganisms	26
Fungi	20
Candida spp	16

Table 6 Clinical predictors for mortality during a first episode of acute pancreatitis

	Odds ratio	95% CI	p value
Persistent organ failure	18.0	8.5-38.3	<0.0001
Bacteraemia	3.4	1.6-7.1	0.001
Age	1.1	1.0-1.1	<0.0001

After backward stepwise logistic regression the following parameters had no independent significant relation with mortality: sex, infected necrosis and pneumonia.

Forty- seven per cent (72 of 154) of patients with pancreatic necrosis developed infected necrosis. Previous bacteraemia in patients with pancreatic necrosis (n=51) was associated with increased risk of developing infected necrosis (65% (33 of 51) *vs* 38% (39 of 103), p=0.002). The risk of developing infected necrosis was also higher in patients with extensive (more than 30%) pancreatic necrosis than in patients with less than 30% necrosis (56% (56 of 100) *vs* 30% (16 of 54), p=0.002). The increased risk of infected necrosis in patients with previous bacteraemia was however not explained by a difference in the extent of pancreatic necrosis (less or more than 30%) (p=0.858). In 31 of 51 patients with both a previous bacteraemia and infected necrosis the same pathogen was cultured from both initial positive cultures. These pathogens were *Escherichia coli* (n=11), other Enterobacteriaceae (n=7) and various gram-positive cocci (n=13).

In patients with infected necrosis (n=98), mortality was 2.5 times higher if bacteraemia was diagnosed during admission (40% (22 of 55) *vs* 16% (7 of 43), p=0.014).

Discussion

This study describes the largest series thus far on the timing and impact of infections, especially bacteraemia and pneumonia, in acute pancreatitis. The most important findings were (a) half of all infections occurred within the first week of admission; (b) bacteraemia was an independent predictor of mortality; (c) in patients with pancreatic necrosis, bacteraemia was associated with an increased risk of developing infected necrosis and (d) in the subgroup of patients with infected necrosis, bacteraemia was associated with a higher mortality.

The present study represents a large prospective cohort of patients included in a short time period. The PROPATRIA trial allowed for adequate data collection and strict protocol adherence. Since the focus of PROPATRIA was on patients with predicted severe pancreatitis, some patients with predicted mild pancreatitis were likely missed for inclusion. This is reflected by the 28% of patients in this series that suffered from severe pancreatitis whereas, according to the literature, one would expect this number to be 20%.[12] The limitations possibly resulting from this slight under-representation of patients with mild disease appear only minor. Most likely, the timing of infections and the related mortality (i.e. the main outcome of this study) would remain unchanged if more patients with mild disease had been included because patients with mild disease only rarely develop infectious complications. Furthermore, the mortality of mild pancreatitis was only 1% in this study.

Only one previous study focussed on the time of extrapancreatic infections in acute pancreatitis. Burgeaux *et al* found, in a retrospective French single-centre study of some 212 patients, that infectious complications (both infected necrosis and extrapancreatic infections) occurred at a median of 4 days after admission.[13] In their study, after multivariate analysis, only a non-significant association between bacteraemia and mortality could be demonstrated. The present study, however, was prospective, was conducted during a randomised controlled trial with 'infectious complications' as primary outcome measure and included twice as many patients with bacteraemia, and hence was able to identify bacteraemia as an independent predictor of mortality.

In 1997, Beger *et al* demonstrated that 80% of patients that died had been diagnosed with infected necrosis.[10] In contrast, only half of the deceased patients in our study had been diagnosed with infected necrosis. When also taking extrapancreatic infections (bacteraemia, pneumonia) into account, again 80% of deceased patients had been diagnosed with an infectious complication.

Most interestingly, bacteraemia was identified as a risk factor for infected necrosis in patients with pancreatic necrosis. The cultured pathogens point toward the gut as the source of both bacteraemia and infected necrosis. From the present study it cannot be concluded whether there is a causal relationship between bacteraemia and the risk of infected necrosis. However, bacteraemia can be used as a prognostic marker in the sense that bacteraemia should increase the level of suspicion for infected necrosis in a patient already diagnosed with pancreatic necrosis. In patients already diagnosed with infected necrosis, bacteraemia is associated with increased mortality.

Several studies have assessed the time of diagnosis of infected pancreatic necrosis.[4,14] In accordance with these studies we found that infection of necrosis was diagnosed at a median of 26 days after admission. It is important to realise that this only reflects the 'day of diagnosis'. The actual infection of pancreatic necrosis may have occurred at an earlier stage. With routine use of percutaneous FNA, Gerzof *et al* and Rau *et al* independently detected infection of necrosis in the majority of cases already within 14 days after onset of symptoms.[15,16] Apparently, with the use of routine FNA in patients with pancreatic necrosis, infection can be detected much earlier than 26 days after admission. Even though there is ongoing controversy whether FNA should be performed in patients with pancreatic necrosis[17,18], it provides the opportunity to tailor antibiotic treatment. This may be relevant since certain pathogens, fungi and *Candida*, as confirmed in the present study, are associated with increased mortality rates and early targeted treatment may improve outcome.[19,20]

From the present and previous studies[7,21] it is clear that early, persistent organ failure remains a major determinant of mortality in acute pancreatitis, irrespective of the suggested improvement of intensive care management.[22] Our data support the view of McKay and Imrie who outlined that in acute pancreatitis early mortality due to organ failure constitutes up to 40-60% of deaths.[23] It is currently unclear what the contribution of infectious complications to early, persistent organ failure is. One may hypothesize that early bacterial invasion aggravates the systemic inflammatory response syndrome frequently observed in the first days of acute pancreatitis, thus making the patient more

liable to develop organ failure. Since the organ failure was generally diagnosed earlier than infectious complications it is likely that the early form of organ failure cannot be prevented by any form of infection-prophylaxis. This concept is supported by a recent randomised trial from Russia where it was demonstrated that enteral nutrition, when compared to parenteral nutrition, prevented both organ failure related to infectious complications and mortality but not early (inflammation related) organ failure in acute pancreatitis.[24]

Since it has now become clear that 50% of the relevant infections occur within the first days of acute pancreatitis, new prophylactic strategies should preferably start immediately after admission. Conceivably, the recent randomised controlled trials on antibiotic prophylaxis, starting treatment somewhere in the first 72-120 hours after onset of symptoms[14,25] should be repeated with a much earlier start of prophylaxis. Results from a recent randomised trial from Italy showing a significant reduction of extrapancreatic infections by starting antibiotic prophylaxis immediately on admission[26], are in support of this suggestion.

In contrast, many are shifting their attention away from antibiotic (or probiotic) prophylaxis toward the use of early enteral nutrition.[27,28] A recent meta-analysis showed that in patients with acute pancreatitis, enteral nutrition, as compared to parenteral nutrition, is associated with a significant reduction of both infectious complications and mortality.[29] Start of enteral nutrition immediately on hospital admission (as compared to 72-120 hours after admission) may further reduce infections in acute pancreatitis as was shown in a large meta-analysis of studies performed in intensive care units.[30]

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Authors' contributions

MGHB, EB, MAB, HvG, HMT, VBN, BvR, BJMW, RJP, AFMS, CHCD, CHJvE, LMAA, HGG and several other members of the study group participated in the design of the study.
MGHB and KF performed the statistical analysis.
MGHB drafted the manuscript.
All authors co-authored the writing of the manuscript.
All authors read and approved the final manuscript.
HGG supervised the current study.

Competing interests

HM Timmerman is an employee of Winclove Bio Industries, Amsterdam and the University Medical Center Utrecht.

Clinical centres and investigators

In addition to the authors, the following clinicians participated in this study. In parentheses the number of patients included. St. Antonius Hospital, Nieuwegein (112): BL Weusten, R Timmer; University Medical Center Utrecht (84); GA Cirkel, V Zeguers, A Roeterdink, HG Rijnhart, MP Schwartz, MS van Leeuwen, BU Ridwan; Gelderse Vallei Hospital, Ede (72): PhM Kruyt; St. Elisabeth Hospital, Tilburg (67): TA Drixler; University Medical Center Groningen (51): RJ Ploeg, HS Hofker, MR Kruijt Spanjer, HT Buitenhuis, SU van Vliet, S Ramcharan; Radboud University Nijmegen Medical Centre, Nijmegen (46): A Nooteboom, JB Jansen, GT Bongaerts, HC Buscher; Meander Medical Center, Amerfoort (45): M Mundt, R Frankhuisen, EC Consten; Academic Medical Center, Amsterdam (43): O van Ruler, DJ Gouma, MJ Bruno; University Hospital Maastricht (40): JP Rutten; Canisius Wilhelmina Hospital, Nijmegen (36): C Rosman, L Ootes, B Houben; Leiden University Medical Center, Leiden (37): A Haasnoot; Erasmus Medical Center, Rotterdam (31): JBC van der Wal; G van 't Hof, EJ Kuipers, Rijnstate Hospital, Arnhem (23): EJ Spillenaar Bilgen, P van Embden; Medical Center Rijnmond Zuid, Rotterdam (29): E van der Harst, JF Lange, NA Wijffels, LA van Walraven; Vrije Universiteit Medical Center, Amsterdam (15): CJ Mulder.

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Part B

Intervention Strategies

Chapter 6

Surgical intervention in patients with necrotizing pancreatitis

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Abstract

Background

This study evaluated the various surgical strategies for treatment of (suspected) infected necrotizing pancreatitis (INP) and patient referrals for this condition in the Netherlands.

Methods

This retrospective study included all 106 consecutive patients who had surgical treatment for INP in the period 2000–2003 in one of eight Dutch university medical centres including three teaching hospitals. Surgical approaches included an open abdomen strategy, laparotomy with continuous postoperative lavage, minimally invasive procedures or laparotomy with primary abdominal closure. The National Hospital Registration System was searched to identify patients with acute pancreatitis who were admitted to the 90 Dutch hospitals that did not participate in the present study.

Results

The overall mortality rate was 34%, 70% (16 of 23) for the open abdomen strategy, 25% (13 of 53) for continuous peritoneal lavage, 11% (two of 18) for minimally invasive procedures and 42% (five of 12) for primary abdominal closure ($p<0.001$). During the study interval, 44 (12%) of 362 patients with acute pancreatitis who were likely to require surgical intervention had been referred to university medical centres.

Conclusion

Laparotomy with continuous postoperative lavage is the surgical strategy most often used in the Netherlands. The results of the open abdomen strategy are poor whereas a minimally invasive approach seems promising.

Introduction

There is international consensus that surgical intervention in acute pancreatitis is indicated only in the case of (suspected) infected necrotizing pancreatitis (INP).[1-5] However, there is no agreement bout the optimal surgical strategy in INP.[4-6] Several specialist centres have reported the outcome of various surgical approaches for INP with mortality rates varying from 6 to 47%.[4-20] The different strategies have never been compared in randomised controlled trials (RCTs).[6] Retrospective studies usually span quite an extensive time period making it difficult to interpret their outcome. The major reasons for the lack of RCTs are probably the low surgical volume%re, as well as the complexity and heterogeneity of the disease. Due to the low surgical volume centralisation of patients with INP, as advocated by the UK guidelines[1], is considered important in order to improve results of surgical treatment. Data on referral patterns are however essentially lacking. In the last two decades an increase in the incidence of acute pancreatitis of 5% annually in the Netherlands has been observed.[21,22] Despite many achievements in intensive care medicine, mortality of INP remained high. In the Netherlands, the Dutch Acute Pancreatitis Study Group decided to undertake the present multicentre retrospective study to obtain baseline results for an optimal design of a future multicentre RCT. A second aim of this study was to determine the referral patterns of INP patients.

Patients and methods

In 2002 the Dutch Acute Pancreatitis Study Group with participation of all eight university medical centres (UMCs) and three teaching hospitals was founded. It was agreed that a retrospective national multicentre audit would be conducted, in combination with other nationwide projects.[23] Progress of activity was monitored during regular central meetings of the group and feedback was given by E-mail and through an open access website (www.pancreatitis.nl).

Identification of patients

For the current study, it was decided to retrieve case notes of all consecutive patients undergoing surgical treatment for INP in the period between 1 October 1 2000 and 1 October 2003, to obtain results on a relatively large number of patients over a short time span. In the 11 participating hospitals, 106 patients were identified by a computer database search for acute pancreatitis operation codes to allow for complete list of

consecutive patients. In order to describe prevalence and mortality of acute pancreatitis in all 101 Dutch hospitals in the same time span a search was confined in the National Hospital Registration System (Prismant, NHRS) to patients in the 90 Dutch hospitals not participating in this study with the International Classification of Disease-9 (ICD-9) code for acute pancreatitis (577.0). Patients younger than 18 years of age, patients with an acute flare-up of chronic pancreatitis and patients undergoing elective surgery for pancreatic pseudocysts were excluded. The diagnosis of necrotizing pancreatitis was accepted, when confirmed by contrast-enhanced computer tomographic (CECT) scan or during surgery.

Data collection

Two of the authors (MGHB and MTdB) visited the participating centres and collected data from computerized medical records, patient charts, radiology reports and operation records. Referring hospitals (n=32) were contacted if data were lacking. All data were entered into a database on a pre-established digital case-record-form, agreed upon by the study group. From all participating centres and referring hospitals, preoperative CT scans were collected and digitalized. Data on the following variables were collected: hospital, referring hospital, gender, age, date of hospital admission, aetiology of disease, CT-guided percutaneous drainage or fine needle aspiration (FNA) procedures including bacteriology, type of first surgical intervention (date, indication, bacteriology), type of re-intervention (date, indication), outcome and complications leading to re-intervention (bleeding or bowel perforation), duration of hospital and ICU admission, date of death or hospital discharge. To minimize inter-observer bias, computer tomography severity index (CTSI) scores (0-10) were calculated post-hoc from the digitalized preoperative CECT-scans by a single radiologist (TLB, see acknowledgments), blinded for the surgical treatment installed. Finally, hospitals were compared for hospital volume, surgical volume, timing of surgical intervention, post-intervention mortality and total acute pancreatitis mortality, using the NHRS.

Indication for intervention

The indications for intervention were: persistent sepsis despite maximal conservative therapy or clinical deterioration after initial clinical improvement (suspected infection), documented infection of (peri-) pancreatic necrosis by FNA, air collections in (peri-) pancreatic necrosis on CECT-scan, suspected bowel perforation or active bleeding. A positive fine needle aspiration (FNA) was not considered mandatory, in all participating hospitals, if infected INP was suspected.

Surgical strategies

Patients were grouped retrospectively into one of four surgical approaches according to the decision made at the first surgical intervention (intention-to-treat principle). With the open abdomen strategy (OAS) the abdomen was left open following the first laparotomy for debridement; planned relaparotomy or relaparotomy on demand were both possible after the first laparotomy. Primary closure with continuous postoperative lavage (CPL) involved rinsing the necrosectomy areas after debridement for INP, followed by closure of the abdomen and continuous postoperative local or locoregional lavage with liberal amounts of fluid. Minimally invasive procedures (MIP) comprised open or videoscopically assisted retroperitoneal debridement, followed by closure of the abdomen and continuous local or locoregional lavage with liberal amounts of fluid. The preferred route was straight into the retroperitoneum through a small left-sided lumbar incision. If this was not possible, an anterior transabdominal laparoscopic approach was used. Laparotomy with primary abdominal closure (PAC) comprised laparotomy and blunt debridement of necrotic tissue, followed by abdominal closure with no postoperative lavage system in place.

Statistical analysis

The different surgical strategies were compared with mortality as the primary outcome. For categorical outcomes, the Fisher's exact test was used and for continuous outcomes the Kruskal Wallis test. Results are expressed as median with range (continuous data) or count with percentage (categorical data). Model building by means of logistic regression was used to determine which factors contributed to mortality. Confounders were gender and age. Odds ratios (OR) are given with the respective 95% confidence interval (CI). A two-tailed $p<0.05$ was considered statistically significant in comparing outcome for the four groups. In case two of the four groups were compared (subgroup analysis), according to the Bonferroni correction, $p<0.008$ was considered significant.

Results

The patient characteristics are summarized in *Table 1*. Despite the retrospective design 99.7% of the desired data were retrieved. Aetiology of pancreatitis was biliary in 34, ERCP in 13, alcoholic in 11, other (such as trauma, hypercholesterolemia) in 19 and idiopathic in 29 patients. In the three-year study period a total of 1238 patients were treated for acute pancreatitis in the 11 participating hospitals. The total percentage of patients with acute pancreatitis that was operated upon was 8.6% (106/1238). Forty-

four (42%) of the 106 patients who underwent surgical intervention had been referred from elsewhere to the UMCs for surgical intervention. If the referred patients are not taken into account, the (true) percentage of surgical intervention in acute pancreatitis in the 11 participating hospitals was 5.2% (62/1194).

Table 1 Preoperative characteristics of 106 patients operated upon for acute necrotizing pancreatitis.

Strategy	OAS (n=23)	CPL (n=53)	MIP (n=18)	PAC (n=12)	Total (n=106)	p value
Male (%)	20 (87)	38 (72)	13 (72)	5 (42)	76 (72)	0.053
Age (range)	67 (24-79)	60 (20-80)	53 (29-68)	58 (39-81)	59 (20-81)	0.176
CTSI score (range)	6 (4-10)	6 (2-10)	8 (4-10)	6 (4-10)	6 (2-10)	0.02
Referred patients (%)	12 (52)	22 (42)	7 (41)	4 (33)	45 (42)	0.728
Preop. percutan.drainage (%)	3 (13)	16 (30)	12 (67)	3 (25)	34 (32)	0.006
Preop. ICU admission (%)	13 (57)	27 (51)	10 (56)	5 (42)	55 (52)	0.835
Preop. ICU stay (range)	4 (1-38)	8 (1-77)	7 (1-105)	10 (2-19)	8 (1-105)	0.40
Preop. hospital stay (range)#	11 (1-66)	20 (0-117)	48 (0-181)	11 (0-50)	20 (0-181)	0.001

OAS = open abdomen strategy, CPL = laparotomy and continuous postoperative lavage,
MIP=minimally invasive procedures, PAC = laparotomy and primary abdominal closure.
All time periods are represented in median and range in days.
#Timing of surgical intervention

CT-guided percutaneous drainage

In 34 of 106 patients, a preoperative CT-guided percutaneous drainage of the collection(s) with necrosis and fluid was performed (median 1 procedure; range 1-6; usually a (regular) 14 french drain). No drainage-related complications were reported. About one third of the drains had been placed directly in the left or right retroperitoneum as a 'guidance drain' (see *Table 1)*.

Timing of intervention

Median time between hospital admission and first surgical intervention was approximately three weeks. OAS patients were operated upon earlier than CPL patients. In MIP patients the time interval between admission and interventions was the largest, see *Table 1*.

Indication for intervention

Table 2 shows the indications for intervention per surgical strategy. Persistent sepsis despite maximal conservative therapy (suspected infection) of (peri-) pancreatic necrosis was the main indication for surgery.

Table 2 Indication for intervention in 106 patients operated upon for acute necrotizing pancreatitis

Strategy	OAS (n=23)	CPL (n=53)	MIP (n=18)	PAC (n=12)	Total (n=106)	p value
Sepsis/deterioration (n)	14 (61%)	34 (64%)	10 (56%)	6 (50%)	64 (60%)	0.793
Positive FNA* (n)	3 (13%)	9 (16%)	7 (39%)	2 (17%)	21 (20%)	0.163
Suspect bowel perforation (n)	3 (13%)	5 (9%)	0 (0%)	3 (25%)	11 (10%)	0.119
Air on CECT (n)	3 (13%)	4 (8%)	1 (6%)	1 (8%)	9 (9%)	0.832
Bleeding (n)	0 (0%)	1 (2%)	0 (0%)	0 (0%)	1 (1%)	0.799

*FNA= fine needle aspiration

Surgical intervention

Necrosectomy by laparotomy followed by CPL was the strategy of overall preference. Four hospitals used OAS as 'strategy of preference' in INP and they treated 74% of all OAS patients. Of all patients undergoing CPL, 94% was reported from the seven other hospitals; 100% of MIP patients were treated in four of the seven latter hospitals using CPL. PAC patients were equally divided over all hospitals.

Bacteriology

Peroperative cultures from (peri-)pancreatic necrosis were positive in 76 patients. Gram positive and gram negative bacteria were cultured in equal amounts. The remaining patients with negative bacteriology had been operated upon earlier as compared to patients with positive bacteriology (7 days, range 0-140 days *vs* 27 days, range 0-181 *vs*, p=0.0001). See *Table 3* for results.

Table 3 Peroperative bacteriology in 106 patients operated upon for acute necrotizing pancreatitis

Negative culture (%)	30 (28)
Positive culture (%)	76 (72)
Gram pos (%)	16 (15)
Gram neg (%)	20 (19)
Gram pos and neg (%)	23 (22)
Candida and bacteria (%)	14 (13)
Candida (%)	3 (3)
Total (%)	**106 (100)**

Complications

The frequency of postoperative bleeding (p=0.13) and bowel perforation (p=0.18) did not differ between the different strategies, see *Table 4*. A large proportion of patients (72%) had a re-intervention either for further necrosectomy, to remove surgical gauzes or merely for local inspection.

Table 4 Outcome in 106 patients operated upon for acute necrotizing pancreatitis

Strategy	OAS (n=23)	CPL (n=53)	MIP (n=18)	PAC (n=12)	Total (n=106)	p value
Re-intervention (%)	23 (100)	39 (74)	12 (67)	2 (17)	76 (72)	0.001
Number of re-interventions (range)	5 (2-63)	2 (1-14)	2 (1-11)	1 (1-4)	2 (1-63)	0.001
Bowel perforation (%)	7 (30)	11 (21)	3 (18)	0 (0)	21 (20)	0.180
Bleeding (transfusion) (%)	11 (48)	17 (32)	3 (18)	2 (17)	33 (31)	0.130
Postop. ICU stay survivors (range)	16 (0-68)	10 (0-206)	2 (0-83)	2 (0-17)	9 (0-206)	0.046
Postop. stay survivors (range)	70 (45-139)	87 (8-236)	35 (18-162)	13 (1-62)	58 (1-236)	0.001
Total stay survivors (range)	111 (30-112)	109 (60-253)	100 (43-240)	21 (11-63)	98 (11-253)	0.001
In-hospital deaths (%)	16 (70)	13 (25)	2 (11)	5 (42)	36 (34)	0.001

Outcome

The overall in hospital mortality in this series was 34% (36/106). Mortality was highest (70%) in the OAS-group. In seven patients, OAS was used as a 'rescue-strategy' after failure of CPL and three died. Results of these seven patients were included in the CPL-group according to the 'intention-to-treat' principle. There was no difference in mortality for the different indications (p=0.613) and for the presence or absence of infection (21/76, 28% *vs* 14/30, 47%, p=0.070). End model building with forward stepwise regression analysis showed that preoperative drainage and timing of surgery had no influence on mortality, but age, preoperative ICU admission, bleeding and type of surgical strategy (both CPL and MIP *vs* OAS) had (see *Table 5*).

OAS vs PAC

In order to further explore the poor results of OAS, subgroup analysis was performed. In order to exclude bias due to early timing of intervention, OAS was compared with PAC since surgery in both groups was performed within 14 days (11 *vs* 11 days, p=0.780). Incidences of both postoperative bleeding (p=0.014) and postoperative bowel perforation (p=0.014) were lower in PAC than in OAS patients.

Table 5 End model building identifying risk factors for mortality

Factor	Odds ratio	95% CI	p value
Gender*	0.764	0.273-2.138	0.609
Patient age*	0.929	0.886-0.975	0.003
Type of surgical intervention OAS	1.000		
Type of surgical intervention OAS *vs* CPL	0.296	0.050-1.769	0.001
Type of surgical intervention OAS *vs* MIP	0.281	0.484-12.150	0.007
Preoperative intensive care admission	3.057	1.058-8.832	0.033
Bleeding requiring reintervention	5.193	1.543-17.478	0.005

*Entered as confounder in the model

Hospital characterization

In order to further analyze the cause of poor outcome for OAS, the four hospitals using OAS was compared with the seven hospitals using CPL and MIP. In the four hospitals using OAS, surgeons generally operated earlier on INP patients (p=0.001) which is reflected by a higher mortality (p=0.025) than in hospitals using CPL and MIP. Patient volume did not differ between the two groups of hospitals (p=0.648) whereas the surgical volume was smaller in the hospitals using OAS (p=0.073). The results are shown in *Table 6*.

Table 6 Hospital characterization based on the preferred surgical strategy for necrotizing pancreatitis per hospital.

	Hospitals using OAS, and no MIP (n=4)	Hospitals using CPL and MIP (n=7)	Total (n=11)	p value
University hospital	2	6	8	
Acute pancreatitis (n)	434	804	1238	0.648
Acute pancreatitis%re*	76 (65-198)	105 (90-164)	84 (15-213)	0.648
In-hospital mortality* (%)	32 (7.4)	44 (5.5)	76 (6.1)	0.412
Surgery for INP, any strategy	25	81	106	
Surgery for INP%re†	6 (5-9)	11 (5-23)	10 (4-23)	0.073
Timing of intervention# (range)	7 (0-44)	25 (0-181)	20 (0-181)	0.001
Post-intervention mortality# (%)	13 (52)	22 (27)	35 (33)	0.025

All figures represent results per hospital during the total three year study period.
*National Hospital Registration System (Prismant) based on ICD-code 577.0
†Number of patients with surgical intervention for INP per hospital, all types of surgery are included (for instance CPL in hospitals using OAS)
#Based on data per patient, rather than average data per hospital.

Analysis of patient referral patterns

In order to obtain data about patient referral characteristics, a search was conducted in 7233 patients admitted with acute pancreatitis to the 90 Dutch hospitals not participating in this study (NHRS). In-hospital mortality of all acute pancreatitis patients was 4.8%. Since at least 5% of all acute pancreatitis patients will require surgical intervention, the number of patients that 'might have been referred' for INP can be calculated. During the three year study period, in the 90 hospitals (5% x 7233 patients) 361 INP patients were calculated to have been eligible for referral to dedicated centres. In the present series 44 of the 106 (42%) operatively treated patients had been referred to the UMCs. These 44 referred patients comprise 12% of the total (calculated) group of 361 patients eligible for referral. Therefore, approximately 88% of all acute pancreatitis patients requiring surgical intervention had not been referred. Outcome for these patients was not registered in the NHRS.

Discussion

This multicentre study showed that, in the Netherlands, CPL is the preferred technique for surgical treatment of INP and that MIP is a promising alternative. The results of OAS are discouraging and this technique should be considered obsolete.

The mortality rate in this series (34%) is slightly higher than the rate of 28% reported in a single-centre series of 88 patients from Liverpool.[14] In a recent systematic review a mortality rate of 27% for OAS was reported.[6] Early intervention (median 11 days) may have contributed to the poor outcome of OAS-patients in this series. This suggestion is supported by the RCT by Mier *et al.*, showing that delayed surgery, after at least 12 days, may reduce mortality.[24] Postoperative bleeding and bowel perforation were less often detected in the PAC group than in the OAS group, with similar baseline characteristics and timing of intervention. Therefore, it is likely that the repeated interventions in OAS, harbouring the risk of iatrogenic complications, contributed to the discouraging results.

The use of CPL after primary necrosectomy, first described by Beger *et al.*[8], has been advocated by the groups from Ulm and Bern with a reported mortality rate 15-25%.[2,8,25] The mortality of CPL in the present series (25%) is therefore comparable with the upper range of numbers reported.

The mortality rate associated with MIP (11%) is comparable with rates of 13% for endoscopic and 27% for open retroperitoneal approach reported in a recent systematic review.[6] New techniques like MIP[12-14,17,26] have to be developed further in centres well equipped for all aspects of analysis and treatment of necrotizing pancreatitis.[1] In this series, the hospitals using CPL and MIP had a larger surgical volume with better outcome underlining that referral of patients with INP to experienced centres is likely to positively influence outcome.

The lowest rate of surgical intervention in acute pancreatitis (3.2%) was reported from Boston.[7] In the present series 5.2% of patients admitted with acute pancreatitis were treated surgically. Preoperative FNA was performed in 19.8% of patients. This low percentage reflects the worry about false-negative results of FNA and the risk of introducing infection, especially after multiple aspirations.

The present study highlights the use of preoperative CT-guided percutaneous drainage, performed in about a third of the patients treated. Although drainage as such will not drain necrotic tissue, it may be beneficial to relief 'infected fluid under pressure' (source control), with the potential to prevent or postpone surgical intervention.[27] In series with percutaneous drainage, success-rates (success defined as obviating the need for surgery) varying from 30-100% have been reported.[28-31]
This series certainly has the drawbacks of a retrospective study and a multicentre design. In general, results of a multicentre study are more likely to reflect 'daily practice' as compared to 'optimal uniform approach'. Compared to other retrospective series, however, this study comprises a large series in a relatively short time frame, with a minimum of confounding by changes in treatment strategies with time. Selection-bias may have led to the favourable results reported for MIP and the unfavourable results reported for OAS. However, since the vast majority of OAS patients were reported from four hospitals that apparently used OAS as 'strategy of preference' it is unlikely that this group of patients had a more severe form of pancreatitis. This can also not be argued for MIP patients, since both preoperative ICU admission and preoperative CTSI scores were comparable with OAS and CPL patients, indicating similar rates of organ failure and similar extent of (peri-)pancreatic collections and necrosis. The favourable results for MIP can therefore only in part be explained by selection bias.

This study indicates that the rate of referral for surgical intervention in acute pancreatitis is low in the Netherlands. This should be improved to increase surgical volume and most likely improve outcome. Prospective randomised studies are required to determine the optimal strategy for INP. In the design of such studies CPL may be regarded the current 'gold standard' as it is well standardized and reproducible with consistently good results. On the basis of the results of this nationwide study, a multicentre RCT comparing CPL with a less invasive strategy was deemed feasible, and recruitment of patients has started.[32]

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Authors' contributions

MGB drafted the manuscript.
MGB and MTdB performed the data analysis.
HGG supervised the study.
All authors participated in the design of the study during meetings of the study group.
All authors edited the manuscript.
All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests. All authors are members of the Dutch Acute Pancreatitis Study Group.

Dutch Acute Pancreatitis Study Group

The following study group members included patients; in parentheses the number of included patients are depicted University Medical Center Groningen (22): HS Hofker, RJ Ploeg; University Hospital Maastricht (14): JP Rutten, CH Dejong; Academic Medical Center Amsterdam (11): DJ Gouma, MA Boermeester; St. Antonius Hospital Nieuwegein

(11): B van Ramshorst; Leiden University Medical Center (10): AF Schaapherder; University Medical Center Utrecht (9): MG Besselink, E Buskens, VB Nieuwenhuijs, HG Gooszen; Erasmus Medical Center Rotterdam (8): CH van Eijck; Vrije Universiteit Medical Center Amsterdam (6): MA Cuesta; Radboud University Nijmegen Medical Centre, Nijmegen (5): H van Goor; Medical Center Rijnmond Zuid Rotterdam (5): N Wijffels, L van Walraven, JF Lange; Medical Center Leeuwarden (5): JPEN Pierie

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Chapter 7

Describing computed tomography findings in acute necrotizing pancreatitis with the Atlanta classification: an interobserver agreement study

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Abstract

Background

The 1992 Atlanta classification is a clinically based classification system that defines the severity and complications of acute pancreatitis. A study was undertaken to assess the interobserver agreement of categorizing peripancreatic collections on CT using the Atlanta classification

.

Methods

Preoperative contrast-enhanced CT's from 70 consecutive patients (49 male, median age 59 years, range 29-79) operated for acute necrotizing pancreatitis (2000-2003) in 11 hospitals were reviewed. Five abdominal radiologists independently categorized the peripancreatic collections according to the Atlanta classification. Radiologists were aware of the timing of the CT and the clinical condition of the patient. Interobserver agreement was determined.

Results

Interobserver agreement amongst the radiologists was poor (Kappa 0.144, SD 0.095). In 3/70 cases (4%) the same Atlanta definition was chosen. In 13/70 cases (19%) 4 radiologists agreed and in 42/70 cases (60%) 3 radiologists agreed on the definition. In 21 cases (30%), one or more of the radiologists classified a collection as 'pancreatic abscess' whereas one or more of the radiologists used another Atlanta definition.

Conclusion

The interobserver agreement of the Atlanta classification for categorising peripancreatic collections in acute pancreatitis on CT is poor. The Atlanta classification should not be used to describe complications of acute pancreatitis on CT.

Introduction

Treatment of acute necrotizing pancreatitis (ANP) is a challenge, and consultation with or referral to specialised institutions is advised on several occasions;[1-3] therefore adequate communication regarding both the severity and complications of ANP is of utmost importance. In 1992 an international symposium on acute pancreatitis was held in Atlanta to resolve lingering disputes regarding the definitions of various complications in acute pancreatitis. Agreement was reached on a clinically based classification system to define the disease and its complications: the so-called Atlanta classification.[4]
Contrast-enhanced computed tomography (CECT) is the primary radiological diagnostic modality to assess the various complications of acute pancreatitis.[1-3] The CT severity index (CTSI)[5], was specifically designed to assess the severity of acute pancreatitis. Recently, a 'modified CTSI' was presented.[6] However, the CTSI and modified CTSI are not designed to characterise peripancreatic collections, and consequently the Atlanta classification is frequently used to describe (peri-)pancreatic collections on CT.
To our knowledge, no study has determined the interobserver agreement when using the Atlanta classification for this purpose. In the current study, to determine the interobserver agreement, five abdominal radiologists re-evaluated preoperative CECT's from 70 consecutive patients operated for ANP.

Patients and methods

Patient identification

In 11 hospitals of the Dutch Acute Pancreatitis Study Group, including all eight Dutch university medical centres, 106 consecutive patients (age >18) who underwent surgical intervention for proven or suspected infection of (peri-)pancreatic necrosis between October 1, 2000, and October 1, 2003, were identified by a hospital computer database search. Surgical outcome of these patients has been described elsewhere.[7] Patients were included in the present study if a preoperative CECT had been performed and was available for analysis.

Data collection

Date of hospital admission, date of CT, the original CT reports and the preoperative CT's were retrieved from the participating and referring centres. In the CT reports the use of the exact terms 'pancreatic necrosis', 'pseudocyst' and 'pancreatic abscess' was scored. Terms such as 'non-enhancement' or 'fluid collection' were not scored as they

are not used in the Atlanta classification. All preoperative CT's were digitalised by high resolution scanning (Diagnostic Pro, Vidar Systems Corporation, Herndon, Virginia, United States).

Study protocol

From the (last) preoperative CT scan, 2-3 slices at different anatomic levels were selected; including the slice depicting the maximum diameter of the fluid-containing collection in or around the pancreas. These images were inserted in a computer slide presentation. The individual slides were coded and the dates of the following events were added: date of hospital admission, date of (preoperative) CT and date of surgical intervention. Five experienced abdominal radiologists from five different hospitals (TLB, MSVL, JSL, EJVDJ, SPS) independently reviewed the selected images or the entire CT. The radiologists were aware of the fact that all patients were operated upon for severe acute pancreatitis. The radiologists were, however, blinded for the results of the original report and were blinded for the reports of the other radiologists. If required by a radiologist, the complete digitalised CT was presented. The radiologists were asked to characterise the (peri-)pancreatic collections as acute fluid collection, pseudocyst, pancreatic abscess or pancreatic necrosis according to the Atlanta classification, (*Table 1*). A fifth option 'mixture' was added for cases in which the radiologist felt the morphological changes depicted by CT had features of several definitions and thus did not fit within the confines of the Atlanta classification. The option 'no collection' was added as it was anticipated that in some cases no peripancreatic collection was apparent on the CT scan. The radiologists were familiar with the Atlanta classification and all read the appropriate definitions prior to review. The definitions remained available during the entire review process.

Table 1 The complications of acute pancreatitis as described by the 1992 Atlanta classification.

Acute fluid collection	Occur early in the course of acute pancreatitis, are located in or near the pancreas, and always lack a wall of granulation or fibrous tissue.
Acute pseudocyst	A collection of pancreatic juice enclosed by a wall of fibrous or granulation tissue which arises as a consequence of acute pancreatitis or pancreatic trauma, or chronic pancreatitis not containing necrotic material. Formation of a pseudocyst requires 4 or more weeks from the onset of acute pancreatitis.
Pancreatic abscess	A circumscribed intra-abdominal collection of pus, usually in proximity to the pancreas, containing little or no pancreatic necrosis which arises as a consequence of acute pancreatitis or pancreatic trauma.
Pancreatic necrosis	A diffuse or focal area(s) of nonviable pancreatic parenchyma, which is typically associated with peripancreatic fat necrosis.

Statistical analysis

The interobserver agreement was calculated using Kappa statistics. A Kappa level <0.00 represents no agreement, 0.00-0.20 slight, 0.21-0.40 fair, 0.41-0.60 moderate, 0.61-0.80 substantial and 0.81-1.00 almost perfect agreement.[8] Mean Kappa with standard deviation (SD) was calculated for all 10 radiologist-pairs within the five radiologists. Categorical data were compared using Fisher's exact test. Results of continuous data were expressed as median (range). Comparison of continuous variables was performed using Mann-Whitney-U test or Kruskal Wallis test for multiple groups. A two-tailed p< 0.05 was considered statistically significant.

Results

Patients

Of 80 scans available, 10 were non-contrast-enhanced and were excluded. Therefore, preoperative contrast-enhanced CT scans were available for 70/106 patients (75%). The patient baseline characteristics are shown in *Table 2*. Indications for surgical intervention were persistent sepsis despite maximal conservative therapy (61%), positive fine needle aspiration culture (23%), (peri-)pancreatic air on CT scan (10%) or suspected perforation of the gastrointestinal tract (6%). The diagnosis of infected pancreatic necrosis was confirmed in 63 patients (79%) by means of a positive culture from a preoperative aspirate.

Table 2 Patient characteristics.

Male (%)	49 (70)
Age (range)	59 (29-79)
Preop. ICU admission (%)	38 (54)
Preop. ICU stay (range)*	7 (1-55)
Preop. hospital stay (range)	24 (1-140)
Mortality (%)	24 (34)

*Of patients admitted to ICU.

Original CT reports

Sixty-four original CT-reports (91%) were retrieved. In 48 reports (75%) one of the following terms was used 'pancreatic necrosis' (n=28), 'pseudocyst' (n=6) or 'pancreatic abscess' (n=14).

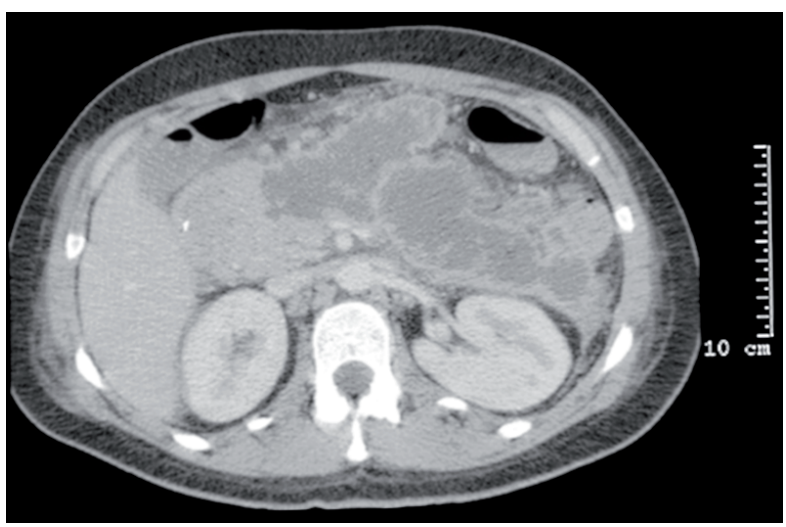
Interobserver agreement

Amongst the five abdominal radiologists, there was slight interobserver agreement for categorising collections according to the Atlanta classification (Kappa 0.144, SD 0.095), see *Table 3*. In 3/70 cases (4%) the radiologists chose the same definition (mixture (n=2) and pancreatic necrosis (n=1)). Four out of five radiologists agreed in 15 (19%) cases, and three out of five agreed in 49 (61%) cases. In 24 (30%) cases, one or more of the radiologists classified a collection as 'pancreatic abscess' whereas one or more of the other radiologists classified the collection as 'acute fluid collection', 'pseudocyst' or 'pancreatic necrosis'. See *Figure 1* for examples.

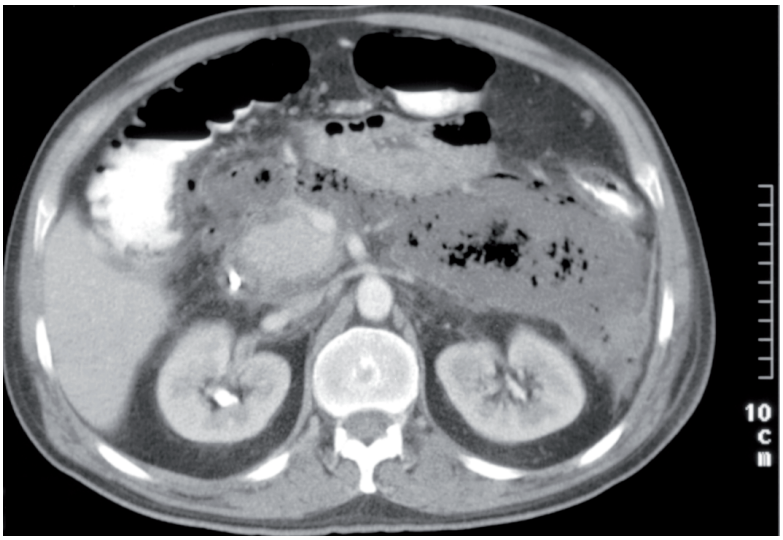
Table 3 Atlanta classification used for defining (peri-)pancreatic collections in 70 necrotizing pancreatitis patients.

HPB radiologist	Ac. fluid collection N (%)	Pancreatic abscess N (%)	Pseudocyst N (%)	Pancreatic necrosis N (%)	Mixture N (%)	No collection N (%)	Total N (%)
1	10 (14)	22 (31)	0 (0)	4 (6)	32 (46)	2 (3)	70 (100)
2	1 (1)	1 (1)	0 (0)	14 (20)	53 (76)	1 (1)	70 (100)
3	8 (11)	4 (6)	0 (0)	7 (10)	51 (73)	0 (0)	70 (100)
4	14 (20)	16 (23)	2 (3)	24 (34)	14 (20)	0 (0)	70 (100)
5	15 (21)	21 (30)	20 (29)	6 (9)	3 (4)	5 (7)	70 (100)
Mean	10 (14)	13 (18)	4 (6)	11 (16)	31 (44)	2 (2)	70 (100)

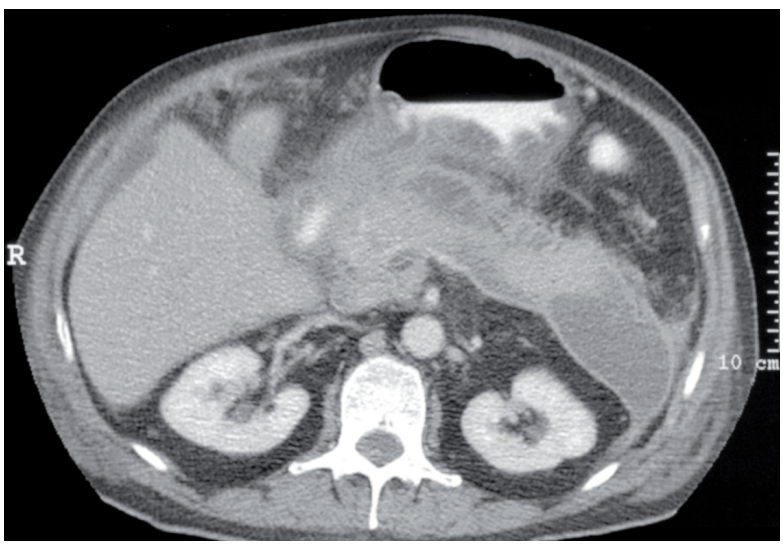
Figure 1 The use of the Atlanta classification on CT in necrotizing pancreatitis



a. CT scan 12 days after onset of disease. The definitions chosen for this collection were 'pseudocyst' (n=1), 'pancreatic abscess' (n=1), 'pancreatic necrosis' (n=1) and 'mixture' (n=2).



b. CT scan 27 days after onset of disease. The definitions chosen for this collection were ‘pancreatic abscess’ (n=3), and ‘mixture’ (n=2).



c. CT scan 31 days after onset of disease. The definitions chosen were ‘pancreatic necrosis’ (n=1), ‘pancreatic abscess’ (n=1), ‘pseudocyst’ (n=1) and ‘mixture’ (n=2).

Discussion

This study shows that the interobserver agreement for using the Atlanta classification to categorise (peri-)pancreatic collections on CT is very poor, even when experienced abdominal radiologists are asked to judge CECT's.

Surgeons and gastroenterologists tend to rely heavily on the radiologist's CT report of a patient with ANP to decide upon further treatment. The impact of a report describing a 'pseudocyst' is completely different from that of 'infected pancreatic necrosis' or a 'pancreatic abscess'.[1-3] As shown by the original CT reports in this study, radiologists often use the terms of the Atlanta classification, even though it is known that the Atlanta classification was not designed originally for this specific purpose.

The major finding in this study was that the interobserver agreement for describing (peri-) pancreatic collections on CT scan is very poor even when abdominal radiologists were supplied with the most relevant clinical data in these patients in whom the decision to operate had been made. These findings may be explained partially by the fact that the Atlanta classification does not describe all manifestations of the disease with respect to (peri-)pancreatic collections.[9] For example, a collection containing both necrosis and fluid does not fit with any of the definitions in the Atlanta classification (see *Table 1*). Collections containing both impacted air and necrosis are often called 'pancreatic abscess' when according to the Atlanta classification, they are not. This may force the radiologist to decide between two definitions, feeling uncomfortable with both. This is a relevant problem as illustrated by the fact that the added option 'mixture' was used by the radiologists in up to three quarters of cases. Furthermore, it has been noted that CT is often not capable of detecting solid debris in pancreatic collections, especially when a significant fluid component is present.[10] This leads to problems in the diagnosis of a 'pseudocyst'. It is an extremely common error that collections containing a mixture of necrosis debris and fluid are termed 'pseudocyst'.[9] In 9% of the original scan reports and in a mean 6% of the 'expert' reports this term was used, when in fact none of the patients had a pseudocyst as pancreatic necrosis was detected during surgery in all cases. The recently revised UK guidelines on acute pancreatitis acknowledges this fact as it states that an ultrasound or magnetic resonance (MR) should always be performed before the diagnosis of pseudocyst is established. Furthermore, the guideline recommends considering all localised collections following necrotizing pancreatitis to be localised necrosis until proven otherwise.[1]

Most guidelines advice the use of the CTSI developed by Balthazar *et al.* to quantify the extent of morphological changes on CT.[1-3] The CTSI has been reported to have a good interobserver agreement.[11] However, the CTSI is not concordant with the definitions of the Atlanta classification.

Interobserver-agreement studies have never been reported for the Atlanta classification, so the present study can not be compared with previous studies. However, our study design is not likely to have introduced much observer bias, as the review was performed in a blinded, controlled manner. One might argue that the inclusion of only CT scans of patients operated upon for suspected infected ANP caused selection bias. However, in daily practice, it is in these patients, in whom the decision for intervention is about to be taken, that the characterisation of CT findings is most relevant. Different complications require different treatment strategies, ranging from conservative management to invasive percutaneous or surgical intervention. Inter-observer variability in characterisation of (peri-)pancreatic collections will potentially mislead the clinician in his choice for the appropriate therapy. This fact is illustrated in the present series by the 30% of cases in which the diagnosis of pancreatic abscess (treatment: percutaneous drainage) was used where other radiologists used the definitions pseudocyst (treatment: initially conservative), acute fluid collection (treatment: conservative) or pancreatic necrosis (treatment: surgery when infected).

The poor inter-observer agreement of the Atlanta classification for characterising (peri-) pancreatic collections on CT has several major implications. It should especially be taken into account in inter-hospital communication on acute pancreatitis patients, and it may even change our view of previously published clinical studies on intervention strategies in ANP. The Atlanta classification should not be used to describe complications of acute pancreatitis on CT scan and a new descriptive radiological classification system for acute pancreatitis should be designed. Obviously, both interobserver and clinical studies will have to show the clinical relevance of such a classification. CT reports should be descriptive and mention the presence or absence of pancreatic necrosis, fluid collections, encapsulation and/or air. Finally, CT-images should be reviewed by the radiologist and the clinician in unison as this is likely to be the best safeguard against miscommunication and poor interpretation of peripancreatic collections in ANP.

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Authors' contributions

MGHB drafted the manuscript.
MGHB, HCVS, TLB, MSVL and HGG participated in the design of the study.
All authors edited the manuscript.
All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Dutch Acute Pancreatitis Study Group

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Chapter 8

Feasibility of minimally invasive approaches in patients with infected necrotizing pancreatitis

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Abstract

Background

Minimally invasive procedures to treat infected necrotizing pancreatitis (INP) are gaining popularity. The proportion of patients suitable for this approach remains unknown.

Methods

Preoperative computed tomography (CT) scans were reviewed from 106 consecutive patients who had surgery for INP between 2000 and 2003 in 11 Dutch hospitals. Collections related to the pancreas were classified according to their distance from the left abdominal wall. Five radiologists judged 'accessibility' for drain placement and the likelihood that there was a fluid component that would drain ('drainability'). Agreement between radiologists was determined.

Results

CT scans of 80 (75%) patients were available (59 men; age range 29-80 years). The median interval between hospital admission and preoperative CT scan was 20 days. In 55 (69 %) patients, the lateral border of the collection was less than 5 cm from the left abdominal wall. Placement of a drain was deemed feasible in 67 (84%, range 77-89) patients; mean (s.d.) kappa 0.428(0.096). In 45 (56%) patients, a drain could be placed through the left retroperitoneum. In 43 (54%, range 49-82) patients, collections were judged to contain a drainable fluid component. Interobserver agreement on 'drainability' was poor, mean (s.d.) kappa 0.289(0.101).

Conclusion

Most peripancreatic collections in INP were considered accessible to a minimally invasive approach.

Introduction

Surgical intervention for (suspected) infected necrotizing pancreatitis (INP) is accompanied by a high morbidity and mortality.[1-4] In the past decade, surgery for INP has increasingly been delayed to allow for demarcation and encapsulation of the infected necrotic material.[3-5] This has been accompanied by a shift from open necrosectomy to minimally invasive procedures involving radiological and endoscopic approaches to drainage and surgery.[6-8]

In 1998, Freeny *et al.* described computed tomography (CT) - guided percutaneous catheter drainage of INP.[9] Drainage of 'infected fluid under pressure' may improve the patient's clinical condition and postpone or even obviate the need for surgical intervention. If drainage does not lead to clinical improvement, the percutaneous drain can be used as a 'guidance drain' for minimally invasive (retroperitoneal) surgery. Several strategies for drain-guided surgery have been reported.[6-8,10,11] All the minimally invasive procedures (radiological, endoscopic or surgical) have a common first step, with the placement of a drain in the peripancreatic collection. The collections must therefore be accessible for drain placement if minimally invasive approaches are to be widely implemented. So far, results have been based on small series from expert centres open to criticisms of selection bias (only patients with amenable collections having been chosen, along with the least sick). Information on the distribution of peripancreatic collections in INP is also sparse. The location of these collections is important, as those extending close to the left flank are easily accessible for (retroperitoneal) drain placement, in contrast to those near the pancreatic head.

The present pilot study was undertaken for a Dutch nationwide trial comparing minimally invasive techniques for necrosectomy with laparotomy in patients thought to have INP. The aim was to evaluate the intra-abdominal distribution, 'accessibility' and 'drainability' of peripancreatic collections in a large series of consecutive patients who had surgery for INP, to see what proportion of patients might be suitable for such a trial and what stratification might be necessary.

Methods

Between October 2000 and October 2003, 106 consecutive patients (older than 18 years) who had surgical intervention for suspected INP were identified by a database

search in 11 Dutch hospitals, including all the university medical centres and three large teaching hospitals. Patients were included if a CT scan was available for review (*Figure 1*). They were excluded if the indication for surgery had been acute bleeding or perforation of a visceral organ. The results of surgical treatment of these patients have been previously published.[12]

Pre- and postoperative clinical and radiological records as well as preoperative CT scans were retrieved. The following variables were collected: patient age, sex, date of admission and date of preoperative CT scan. All preoperative CT scans were digitalized by high-resolution scanning (Diagnostic Pro™; Vidar Systems, Herndon, Virginia, USA). From each scan, two or three slices at different levels were selected, including the slice depicting the maximum diameter of the collection. These images were transferred to a computer slide presentation.
Scans were reviewed in consensus to classify peripancreatic collections by intra-abdominal location. The distance between the left lateral border of the collection and the left abdominal wall ('inner' abdominal wall, not the skin) was measured using the original metric scale on the CT scan. Interposition of intra-abdominal organs was taken into account. If possible, the shortest retroperitoneal route was chosen; if not, then the shortest route was measured from the lateral border of the collection to the abdominal wall. The collections were classified as follows: left (left lateral border of the collection 5 cm or less from the left abdominal wall), intermediate (left lateral border of the collection more than 5 cm from the left abdominal wall and 5 cm or less from the midline) or central (left lateral border of the collection less than 5 cm from the midline). When multiple collections were present, the most prominent was chosen.

Five experienced hepatopancreatobiliary radiologists from five Dutch tertiary referral centres independently reviewed the preoperative scans using the computer slide presentation and, if desired, the complete CT scan. They were unaware of the original radiological report but were given the dates of admission, CT scan and first surgical intervention. Each radiologist individually judged the accessibility of the peripancreatic collections for placement of a percutaneous or endoscopic transgastric drain in the collection. They were asked the following question, with the possible answers ranked in order on the basis that a left retroperitoneal drain is preferable for performing minimally invasive drain-guided surgery: 'Which route is most feasible and safe for the placement of a 14-French drain in the collection: (a) through the left retroperitoneal space, (b) through the right retroperitoneal space, (c) through the transperitoneal space, (d) through an endoscopic transgastric entrance or (e) no route possible?'. If drain

placement was considered *not* possible, one of the following reasons had to be given: ‘(a) no safe access route present’ or ‘(b) no physical collection present’.
Each radiologist then judged whether the peripancreatic collection was ‘drainable’. A collection was defined as ‘drainable’ if it was expected to contain at least 50 ml of aspirate immediately after first drain placement.

Statistical analysis

Statistical analysis was performed using SPSS® software (SPSS®, Chicago, Illinois, USA). $p<0.050$ for a two-tailed test was considered statistically significant. Unless indicated otherwise, results are given as means. The interobserver agreement was calculated using kappa statistics. The mean(s.d.) kappa coefficient was calculated for all ten possible radiologist pairs. A kappa level less than 0.000 represented no agreement, 0.000-0.200 slight, 0.210-0.400 fair, 0.410-0.600 moderate, 0.610-0.800 substantial and 0.810-1.000 almost perfect agreement.[13]

Results

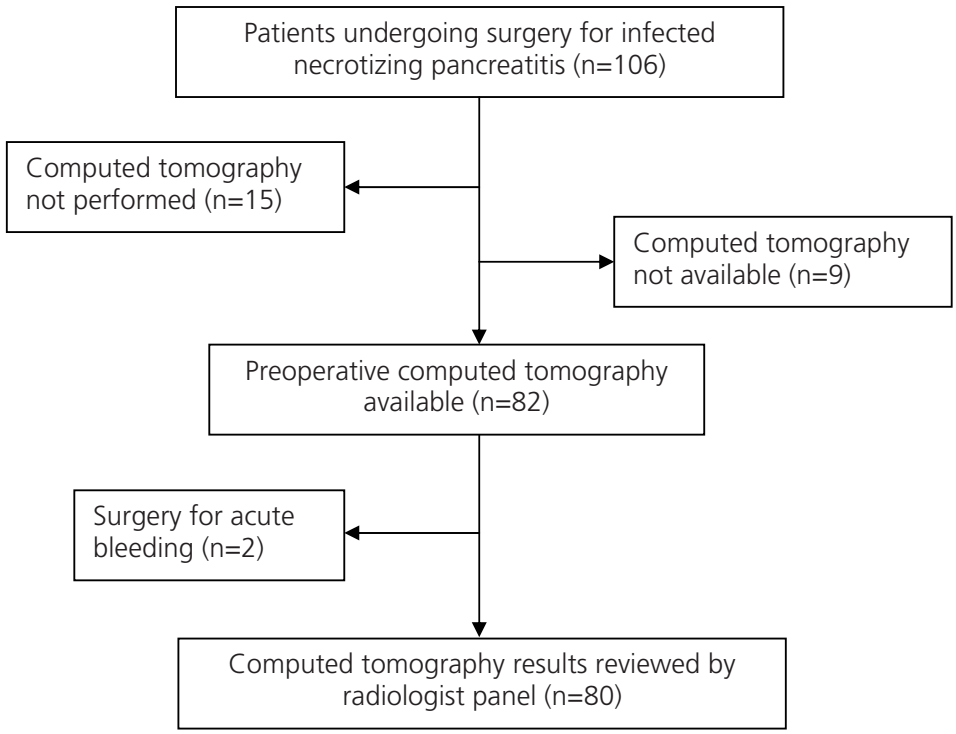
Preoperative CT scans were available from 80 (75%) of 106 patients. *Figure 1* shows the patient inclusion flow chart, and baseline characteristics are shown in *Table 1*. Of the peripancreatic collections, 55 (69%) of 80 were classified as left, 19 (24%) as intermediate and six (8%) as central. Seven (9%) patients had multiple collections.

Table 1 Characteristics of 80 patients with necrotizing pancreatitis

Age (years)	57 (29-80)
Sex ratio (M : F)*	59:21 (74:26)
Documented infected necrosis*	63 (79)
Preoperative ICU admission*	38 (48)
Preoperative ICU stay (days)	8 (1-105)
Preoperative hospital stay (days)	20 (1-179)
Timing of computed tomography (days after admission)	20 (1-179)
Computed tomography severity index	6 (2-10)

Values are median (range) unless otherwise indicated.
*Values are number of patients, with percentages in parentheses. Hospital and ICU stay includes time spent in referring hospitals. ICU, intensive care unit.

Figure 1 Study flow chart



Drain placement was considered feasible in 67 (84%, range 77-89) of 80 patients. The interobserver agreement for accessibility was moderate (kappa 0.428; s.d. 0.094). In 45 (67 %) of these 67, it was deemed feasible to place a retroperitoneal drain from the left flank (*Table 2*). Drain placement was more often feasible in left (50 of 55) than intermediate (12 of 19) and central collections (two of six) ($p<0.001$). In the 13 patients in whom drainage was considered not possible, the reason was 'no safe access route' in 50% (range 25-77) and 'no physical collection' in 50% (range 23-75). All radiologists agreed that it would not be possible to place a drain in only two (2.5 %) of 80 patients. In one of these, no safe percutaneous or transgastric access route could be found, and in the other the scan showed pancreatic necrosis but no physical collection.

In 43 (54%, range 49-82) of 80 patients, the radiologists designated the collection as 'drainable'. The interobserver agreement for 'drainability' was fair (kappa 0.289; s.d. 0.101).

Table 2 Preferred route of drain placement based on appearance by computed tomography

Radiologist	Not possible	Left retroperitoneum	Right retroperitoneum	Anterior transperitoneal	Transgastric endoscopic procedure	Total
1	12 (15)	50 (63)	2 (3)	11 (14)	5 (6)	80 (100)
2	9 (11)	57 (71)	0 (0)	9 (11)	5 (6)	80 (100)
3	16 (20)	43 (54)	0 (0)	16 (20)	5 (6)	80 (100)
4	11 (14)	38 (48)	3 (4)	27 (34)	1 (1)	80 (100)
5	18 (23)	35 (44)	1 (1)	19 (24)	7 (9)	80 (100)
Mean	13 (16)	45 (56)	1 (1)	16 (20)	5 (6)	80 (100)

Values in parentheses are percentages.

Discussion

The present pilot study for a trial comparing minimally invasive procedures with laparotomy in INP demonstrated that most (84%) peripancreatic collections in INP are accessible from a minimally invasive approach and that more than two-thirds are within 5 cm of the left abdominal wall.

Success rates of percutaneous catheter drainage in INP (defined as obviating the need for surgery) vary from 30 to 100%.[9,14-16] Several variations of minimally invasive 'drain-guided' surgery have been reported with mortality rates of 0 to 27%[6-8,10] and it has been suggested that minimally invasive procedures are possible only in a subgroup of patients. The present results contradict this, with drainage deemed feasible in 84 % of patients.

The present study discriminated between 'accessibility' and 'drainability'. The interobserver agreement for 'accessibility' was moderate, probably representing different levels of experience with placement of percutaneous drains. 'Drainability' was arbitrarily defined as anticipated aspiration of more than 50 ml at the time of first drain placement. Agreement on drainability of the peripancreatic collections among radiologists was poor. This probably reflects the fact that CT cannot always discriminate between fluid and necrotic content in INP.[17,18] This finding should be kept in mind when considering the indication and expected outcome (drain production and clinical improvement) of percutaneous drainage.

In the present series, retroperitoneal drain placement from the left flank and hence minimally invasive 'drain-guided' retroperitoneal surgery was deemed feasible in 56% of unselected patients with INP. These findings are in line with the largest published case series, in which drain-guided retroperitoneal necrosectomy was achieved in 47 of 88 patients.[7] Future studies will need to allow for the fact that not all collections are accessible from a retroperitoneal approach and that necrosectomy from a transperitoneal approach (laparotomy or laparoscopy) will remain useful.

The wider implementation of minimally invasive procedures for INP should be based on prospective, controlled studies undertaken by dedicated multidisciplinary teams.[1,4] To that end, the Dutch Acute Pancreatitis Study Group has recently started a prospective, randomised, multicentre trial to compare the minimally invasive approach with laparotomy in INP.[19]

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Chapter 9

Timing of surgical intervention in necrotizing pancreatitis

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Abstract

Background

To determine the impact of timing of surgical intervention for necrotizing pancreatitis on outcome.

Method

Retrospective study of 53 patients in a tertiary referral centre and a systematic review. Mean outcome measure: mortality

Results

Median timing of intervention was 28 days, 83% had infected necrosis and 55% preoperative organ failure. The mortality rate was 36%. Sixteen patients were operated within 14 days, 11 patients from day 15-29 and 26 patients on day 30 or later (30+ subgroup). The 30+ subgroup received preoperative antibiotics for a longer period of time (p=0.0001) and *Candida* and antibiotic resistant organisms were more often cultured from the (peri-)pancreatic necrosis (p=0.022). Mortality was the lowest in the 30+ subgroup (75, 45 and 8%, p<0.0001), this difference persisted when outcome was stratified for preoperative (multi-)organ failure. During the second half of the study, necrosectomy was further postponed (20 *vs* 43 days, p=0.062) and mortality decreased (47 *vs* 22%, p=0.085). To compare our results with previous work, 11 studies with a total of 1136 patients were included in a systematic review. Median surgical patient volume was 8.3 patients per year (5.3-15.6), timing of surgical intervention 26 days (3-31 days) and mortality 25% (6-56%). Also in the review a significant correlation was observed between timing of intervention and mortality (R=-0.603, p=0.050, 95% CI -2.10 to -0.02)

Conclusions

Postponing necrosectomy until day 30 is associated with decreased mortality, prolonged use of antibiotics and increased incidence of *Candida* and antibiotic resistant organisms.

Introduction

Timing of surgical intervention in acute necrotizing pancreatitis (ANP) has changed substantially over the last decade, from early necrosectomy, without respect to the state of infection, to delayed surgery in case of documented or suspected infection of pancreatic necrosis. It has been hypothesised that postponing surgical intervention allows the immune system to encapsulate the necrotic tissue, thus technically facilitating necrosectomy, potentially reducing mortality.[1] The beneficial effect of this strategy was confirmed in one randomised controlled trial comparing intervention within 72 hours with surgery after 12 days.[2] A reduction in mortality from 56% to 27% in favor of surgery after 12 days was demonstrated. Consequently, recent surgical guidelines of the *International Association of Pancreatology* (IAP) state that surgical intervention should preferably be performed between day 15 and day 28 (in the third or fourth week) after admission.[3] A recent European survey demonstrated that there still is no consensus on this subject as 43% of surgeons preferred intervention in the first 14 days, whereas 29% preferred to wait for at least 21 days.[4] Furthermore, it was recently suggested that the 'delay' in surgical intervention in ANP may lead to prolonged use of (prophylactic) antibiotics, leading to an increased incidence of *Candida* infections and antibiotic resistant organisms.[5]

This study describes our increasing experience in postponing surgical intervention in necrotizing pancreatitis. This strategy may lead to necrosectomy being performed after the upper end of the interval suggested by the IAP guidelines, that is after day 28. Furthermore, we assessed the impact of postponing surgical intervention on the use of antibiotics, fungal infections and antibiotic resistance. Finally, we performed a systematic literature review in order to further explore potential associations between timing of surgical intervention and outcome.

Methods

A search was performed in the patient database of our tertiary referral centre for the International Classification of Disease-9 (ICD-9) code for acute pancreatitis 577.0 in patients admitted from January 1995 to December 2004.

Treatment protocol

Acute pancreatitis was diagnosed in 445 patients with clinical signs of acute abdominal pain and a serum amylase above 1000 U/L. Pancreatic necrosis was detected using

contrast-enhanced computed tomography (CT). FNA was only used if the outcome would influence the clinical strategy, i.e. in a patient in which the clinical signs of infected necrosis were not convincing. Antibiotic prophylaxis was used in the majority of patients in whom (peri-)pancreatic necrosis was detected. Antibiotics were used in all patients with documented or clinical suspicion of infection of (peri-) pancreatic necrosis. Indication for necrosectomy was either persisting organ failure despite maximum medical management and/or documented infected necrosis. The surgical treatment results were previously described elsewhere.[6] In the first year of the current series our preferred strategy changed from an 'open abdomen approach'[7] to closed lavage as described by Beger *et al*.[8] Patients were managed either on the nursing ward, the medium care unit or on the intensive care unit (ICU).

Surgical strategy

During laparotomy the abdomen was inspected and blunt debridement of necrotic tissue performed. Two double-lumen drainage tubes were inserted through separate incisions with their tips in the lesser sac and necrotic cavities. The abdomen was closed afterwards and local continuous lavage was started. Re-intervention was performed when 'packing gauzes' had been left in-situ, but mainly because of clinical deterioration.

Variables

Of the 53 consecutive adult patients that underwent necrosectomy the following variables were collected: age, gender, APACHE II score during first 24 hours, Ranson score in first 48 hours, co-morbidity, etiology of pancreatitis, time of first surgical intervention, (multi-)organ failure at the time of first surgical intervention, results of microbiological cultures for bacteria and *Candida*, exposure to broad spectrum antibiotics defined as: amoxicillin/clavulanate, piperacillin/tazobactam and imipenem/cilastatin, exposure to all antibiotics, duration of stay in the ICU, duration of hospital stay and mortality during hospital stay. If patients were referred from other hospitals, data from the initial admission were used to calculate preoperative hospital stay (timing of surgery), APACHE II and Ranson scores, ICU and hospital stay and antibiotic use. Antibiotic prophylaxis was defined as all antibiotics prior to the first positive intra-abdominal culture by means FNA or per-operative culture during necrosectomy. Groups were analyzed on the basis of the timing of the first surgical intervention after initial admission: within 14 days, from day 15 to 29 or day 30 or later. These cut-off points were chosen in accordance with the IAP guidelines.[3]

Organ failure

Organ failure was defined as: 'PaO2 <60mm Hg despite 4 L O2/min via nasal tube or need for mechanical ventilation' (*pulmonary insufficiency*), 'serum creatinine >2.0 mg/dL (>177 mmol/L) or need for hemofiltration or hemodialysis' (*renal failure*), 'systolic blood pressure <90mm Hg or need for catecholamine support' (*cardiocirculatory insufficiency*) and serum calcium <1.87 mmol/L or thrombocytes <100 x 10^9/L (*metabolic disorder*), adapted from the Atlanta symposium.[9] Multi-organ failure was defined as failure of two or more organ systems.

Microbiology

Intra-abdominal cultures were available from all 53 patients operated upon. Culture results were derived from the hospital's central microbiology database and were checked by a single microbiologist (BUR). The following species were considered resistant to antibiotics since they are intrinsically resistant against or have a high risk of becoming resistant during therapy with third generation cephalosporins: *Acinetobacter, Enterobacter cloacae, Enterobacter aerogenes, Pseudomonas, Stenothrophomonas maltophililia*, Methicillin resistant *Staphylococcus aureus* (MRSA) and Vancomycine resistant *Enterococci* (VRE). All *Candida* species cultured from intra-abdominal specimens (i.e. FNA material, lavage fluid, and tissue obtained at operation) were documented. A primary positive *Candida* culture was defined as a positive *Candida* culture during FNA before the first necrosectomy or from material obtained during the first surgical intervention. All cultures for bacteria and *Candida* were performed using standard microbiological methods.

Trends in time

To detect differences in treatment strategy over time, the 10-year period was divided into two periods of 5 years (1995-1999 and 2000-2004). The following variables were compared: prevalence of acute pancreatitis, timing of intervention since first hospital admission, antibiotic prophylaxis, patient referral, culturing of *Candida* and antibiotic resistant organisms, hospital and ICU stay and mortality.

Systematic review

The Medline database was searched using a search strategy comprising the following terms: *acute pancreatitis*, *surgery, necrosectomy.* Cross reference search was performed in the studies found. The search was limited to studies published in the previous ten years (1 Jan 1996 to 1 Jan 2006). Only papers published in the English language were included. In order to diminish the influence of selection bias only studies which

comprised at least 25 consecutive patients that underwent surgical intervention for ANP were included. Furthermore, the studies had to contain data on time of surgical intervention for the entire study population and mortality and subgroups of patients were not allowed. Data of a single arm of a randomised controlled trial were allowed since the randomization process essentially excludes selection bias. Finally, since patient series are frequently described in multiple publications, only the last publication of a single institution was included.[6] Outcome parameters were: patient volume (number of patients operated upon for ANP per year), documented infection of (peri-)pancreatic necrosis, timing of surgical intervention and mortality.

Statistical analysis

Categorical data were compared using Fisher's exact test. Results of continuous data are presented as median (range). Comparison of continuous variables with a skewed distribution was performed using Mann-Whitney-U or Kruskal-Wallis tests. Logistic regression was used to determine which factors contributed to mortality. Odds ratios (OR) are given with the respective 95% confidence interval (CI). Correlations between continuous outcomes were explored by linear regression. A two-tailed $p \leq 0.050$ was considered statistically significant.

Results

During the 10-year period, 445 patients with acute pancreatitis were admitted (55% male, median age 53, range 18-86). Fifty-three ANP patients underwent necrosectomy (34 men, median age 57, range 29-75). Forty-two patients (79%) had been referred from other hospitals, all for surgical intervention. In total 403 patients were primarily admitted; 11 of these patients underwent surgical intervention. The percentage 'surgical intervention in acute pancreatitis' of the patients primarily admitted to our hospital was therefore 2.7% (11/403).

First surgical intervention

Median timing of surgery was 28 days (mean 35 days, range 1-105). Sixteen patients (30%) were operated from 1-14 days, 11 patients (21%) from 15-29 days and 26 patients (49%) from day 30 on. The median APACHE II score on admission was 9 (range 2-21). Baseline characteristics, etiology and co-morbidity did not differ between the three subgroups. See *Table 1* for baseline characteristics at admission.

Table 1 Baseline characteristics on admission

		Day 1-14 (n=16)	Day 15-29 (n=11)	Day 30+ (n=26)	p value
Timing of surgery (range)		7 (1-13)	22 (15-28)	48 (30-164)	
Male sex (%)		8 (50)	7 (64)	19 (73)	0.317
Age (range)		57 (30-72)	57 (38-74)	58 (29-73)	0.889
Referral (%)		11 (69)	10 (91)	21 (81)	0.365
Ranson score* (range)		5 (2-8)	4 (2-7)	3 (2-7)	0.134
APACHE II score# (range)		9 (3-21)	11 (4-19)	9 (2-14)	0.569
Co-morbidity (%)	cardiovascular	6 (38)	1 (9)	5 (19)	0.188
	renal	5 (31)	2 (18)	5 (19)	0.614
	pulmonal	5 (31)	2 (18)	2 (8)	0.141
	diabetes	0 (0)	0 (0)	2 (8)	0.340
Etiology (%)	biliary	4 (25)	3 (27)	15 (57)	0.063
	alcohol	2 (13)	2 (18)	3 (12)	0.857
	ERCP	1 (6)	2 (18)	3 (12)	0.629
	other/ unknown	*9 (56)*	*4 (37)*	*5 (19)*	0.048

All numbers are in median, timing of surgery is in days. *Initial 48 hours, #initial 24 hours.

Indication for intervention

At the time of first surgical intervention 29 patients (55%) had organ failure, 12 in 1 organ, 7 in 2 organs, 10 in 3 or more organs. At time of first surgical intervention 30 patients (57%) had been admitted or were admitted to the ICU. Both preoperative organ failure (p=0.198) and ICU admission (p=0.407) did not differ between the three subgroups. See *Table 2* for the patient characteristics at the first surgical intervention. Thirty of 53 (57%) patients had a preoperative FNA, which was positive in 22 (73%) patients. The 8 patients with negative FNA were all operated upon because of their deteriorating clinical condition. In 4 of these 8 patients intra-operative cultures were positive, representing a negative predictive value for FNA of 50%. Of the 23 patients without a preoperative FNA, in 18 patients (78%) per-operative cultures were positive. Overall, in 44 of 53 patients (83%) infected ANP was documented. Infected ANP was most often documented in the 30+ subgroup (p=0.019), see *Table 2*. Four patients were operated upon without both organ failure and infected necrosis, 3 of these patients were initially operated upon in referring hospital for unclear reasons and one patient, primarily admitted, was operated upon for persisting high fevers.

Table 2 Characteristics at time of first surgical intervention

		Day 1-14 (n=16)	Day 15-29 (n=11)	Day 30+ (n=26)	p value
Infected necrosis (%)		10 (63)	9 (82)	25 (96)	0.019
Preoperative organ failure (%)		11 (69)	7 (64)	11 (42)	0.198
Preoperative multi-organ failure (%)		6 (38)	5 (45)	6 (23)	0.352
Absence of both infected necrosis and organ failure (%)		3 (19)	0 (0)	1 (4)	0.117
Intensive care admission (%)		9 (56)	8 (73)	13 (50)	0.407
Intensive care stay	1-7 days	5	3	6	0.180
	8-14 days	4	0	2	
	15 or more days	0	5	5	
Antibiotic prophylaxis >30 days (%)		0 (0)	1 (9)	10 (38)	0.037
Antibiotic days (range)		4 (0-11)	17 (0-29)	25 (0-77)	0.0001
Number of antibiotics used (range)		1 (0-3)	2 (0-6)	2 (0-9)	0.138
Primary *Candida* or resistant m.o.* (%)		1 (6)	1 (6)	9 (35)	0.050
Primary *Candida* (%)		0 (0)	0 (0)	4 (15)	0.106
Primary resistant m.o. (%)		1 (6)	1 (6)	6 (23)	0.275

*m.o. = micro-organisms

Antibiotics

Forty-five (85%) patients received antibiotic prophylaxis. The median duration of antibiotic treatment prior to first intervention was 14 days (range 0-77), with a median of 2 antibiotics used (range 0-9). Patients in the 30+ subgroup more often received antibiotic prophylaxis for longer than 30 days (p=0.037) and received antibiotics for a longer period of time (p=0.0001), whereas the number of antibiotics used prior to the first necrosectomy did not differ (p=0.138), see *Table 2*. During the entire admission the median total length of antibiotic treatment was 38 days (range 1-200), length of broad spectrum antibiotic treatment was 22 days (range 0-82) and median 4 antibiotics were used (1-12). During the admission patients in the 30+ subgroup received antibiotics (p=0.001) and broad spectrum antibiotics (p=0.002) for a longer period of time. The number of antibiotics used did not differ between the groups (p=0.075), see *Table 3*.

Candida

Candida-species were isolated in 25 patients (47%). In 19 patients, one *Candida*-species was found, mostly *Candida albicans* (n=15). Combinations of *Candida albicans, glabrata, parapsilosis* and *tropicalis* were found in six patients. In one patient, *Candida* was cultured from a blood culture after one month hospital admission. By that time

the patient had received 11 different antibiotics; he died one month later from multiple organ failure. The incidence of positive fungal cultures during admission did not differ between the groups (p=0.455), see *Table 3*.

Table 3 Clinical course and outcome

	Day 1-14 (n=16)	Day 15-29 (n=11)	Day 30+ (n=26)	p value
Antibiotic days (range)	15 (2-200)	46 (16-78)	42 (1-102)	0.001
Broad spectrum antibiotic days (range)	12 (0-38)	28 (0-66)	26 (0-82)	0.002
Number of antibiotics (range)	1 (1-6)	6 (2-12)	5 (1-10)	0.075
Candida during admission (%)	6 (38)	7 (64)	12 (46)	0.455
Resistant m.o during admission (%)	6 (38)	5 (45)	19 (73)	0.055
Number of interventions (range)	4 (1-15)	3 (1-14)	3 (1-19)	0.441
Re-intervention (%)	12 (75)	9 (82)	19 (73)	0.275
Total ICU stay (range)	16 (0-61)	31 (0-82)	19 (0-88)	0.485
Hospital stay (range)*	29 (4-146)	85 (41-131)	108 (6-237)	0.0001

*Including both deceased and survivors.

Hospital stay is in days.

Antibiotic resistant micro-organisms

In 30 patients (57%) antibiotic resistant micro-organisms were cultured. In 24 patients (45%) one resistant micro-organism was cultured and in 6 patients multiple resistant species were cultured. Only in one patient MRSA was cultured. *Candida* or resistant organisms was more often cultured during FNA or during the first surgical intervention in the 30+ subgroup (p=0.050), see *Table 2*. During admission, more resistant micro-organisms were cultured in the 30+ subgroup (p=0.055), see *Table 3*.

Organ failure and outcome

Overall mortality was 36% (19/53). Mortality in patients without organ failure at time of first surgery was 21% (5 of 24), with preoperative mono-organ failure 33% (4 of 12) and with multi-organ failure 59% (10/17) (p=0.016). Of the 4 patients operated upon with failure of 4 organ systems, all within 14 days, mortality was 100%. See *Table 4* for comparison of the three groups with stratification for organ failure. In all strata (no organ failure, organ failure, multi-organ failure) outcome was better in the 30+ subgroup (p=0.045, 0.0001 and 0.001). There was a trend towards decreased mortality in patients operated upon from 15-29 days as compared to patients operated upon in the first 14 days (5/11 (45%) *vs* 12/16 (75%), p=0.079). In the 30+ subgroup, mortality was further improved as compared to patients operated on from 15-29 days (2/26 (8%)

vs 5/11 (45%), p=0.016). Total hospital stay was 89 days; 109 days for survivors and 35 days for non-survivors.

Table 4 Hospital mortality stratified for the presence of organ failure at time of first intervention

Hospital mortality	Day 1-14 (n=16)	Day 15-29 (n=11)	Day 30+ (n=26)	p value
In patients without organ failure (%)	3/5* (60)	0/4 (0)	2/15 (13)	0.045
In patients with organ failure (%)	9/11 (82)	5/7 (71)	0/11 (0)	0.0001
In patients with multi-organ failure (%)	6/6 (100)	4/5 (80)	0/6 (0)	0.001
Total	12/16 (75)	5/11 (45)	2/26 (8)	0.0001

*Explanation: of the 16 patients operated upon in the first 14 days of hospital admission, 5 patients had no signs of organ failure at first surgery, 3 of whom died (60%) during their hospital stay.

Table 5 Changes in the treatment of acute pancreatitis over a 10-year period

		1995-1999	2000-2004	p value
Acute pancreatitis	Acute pancreatitis (n)*	210	235	
	Male (%)	105 (50)	145 (62)	0.017
	Age (range)	53 (21-80)	53 (18-86)	0.359
	Hospital stay (range)	16 (1-181)	14 (1-266)	0.003
	Mortality (%)	28 (13)	21 (9)	0.920
Surgical intervention	Necrosectomy (n)	30	23	
	Male (%)	18 (60)	16 (70)	0.569
	Age (range)	57 (29-75)	58 (38-67)	0.865
	Referral (%)	26 (87)	16 (70)	0.177
	Antibiotic prophylaxis (%)	24 (80)	20 (87)	0.715
	Preop. organ failure (%)	17 (57)	12 (52)	0.786
	Preop. multi-organ failure (%)	10 (33)	7 (30)	1.000
	Infected necrosis (%)	24 (80)	20 (87)	0.715
	Timing of surgery (range)	20 (1-80)	43 (1-164)	0.062
	1-14 days	11 (37)	5 (22)	
	15-29 days	8 (27)	3 (13)	
	30 or more days	11 (37)	15 (65)	
	Postop. stay survivors (range)	84 (11-138)	45 (9-159)	0.078
	Mortality (%)	14 (47)	5 (22)	0.085

All numbers are in median.

*All acute pancreatitis patients admitted, including those treated surgically.

Hospital stay, timing of surgery and postoperative hospital stay are depicted in days

Risk factors

Patients with preoperative organ failure had higher mortality (48% *vs* 21%, p=0.038). There was no significant difference in mortality between, a) infected and non-infected pancreatic necrosis groups (32% *vs* 56%, p=0.255), b) positive *vs* negative *Candida* cultures (36% in both groups), c) presence of antibiotic resistant organisms *vs* no resistant organisms (27% *vs* 48%, p=0.151), d) referred and primarily admitted patients (38% *vs* 27%, p=0.726). There was no mortality in the 5 patients with false negative FNA. *Table 3* gives an overview of the clinical course in relation to timing of surgery. Model building by means of logistic regression demonstrated that, with age and gender entered as confounders, surgery within 30 days and (OR 28.2, 95% CI 4.72-168.0, p=0.0001) and the presence of preoperative multi-organ failure (OR 0.151, 95% CI 0.023-0.967, p=0.030) were the only factors with an effect on mortality.

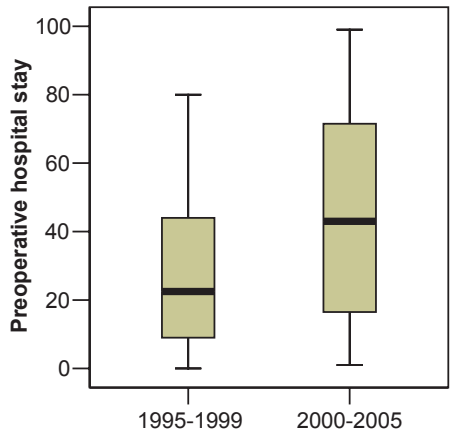
Trend in time

Figure 1 demonstrates that in the second five-year period pancreatic necrosectomy was performed at a later stage of disease (p=0.062). This was accompanied by a trend towards lower mortality (p=0.085), see *Table 5*. For all acute pancreatitis patients (including the conservatively treated patients) a slight but significant reduction in median hospital stay was observed during the second five-year period (p=0.003).

Systematic review

Of 123 manuscripts reviewed only 10 studies contained the required data on timing and outcome, see *Table 6*.[2,10-18] Several studies, although fulfilling all other inclusion criteria, did not mention timing of surgical intervention for the entire population and consequently were excluded.[19-26] The results of the 'delayed surgery' arm from the Mier trial was excluded as this group only consisted of 11 patients.[2] A review of 1136 patients from 11 series (including the present series) was performed. The median number of patients operated for necrotizing pancreatitis was 8.3 per centre per year, median timing of first surgical intervention was 27 days and mortality 25%. An association was observed between (postponed) timing and (decreased) mortality (R=-0.603, p=0.050, 95% CI -2.10 to -0.02), see *Figure 2*. No association between patient volume (number of acute pancreatitis patients operated upon per year) and mortality (p=0.930) and between infection of pancreatic necrosis and mortality (p=0.321) could be demonstrated.

Figure 1 Timing of surgical intervention in necrotizing pancreatitis over a 10-year period



The boxes represent the 25%-75% intervals, the black line represents the mean, the error bars represent the range. In the period 2000-2005 for graphical reasons one 'outlier' (x = 164) is not depicted

Figure 2 Association between time of surgical intervention (from initial admission) for necrotizing pancreatitis and mortality.

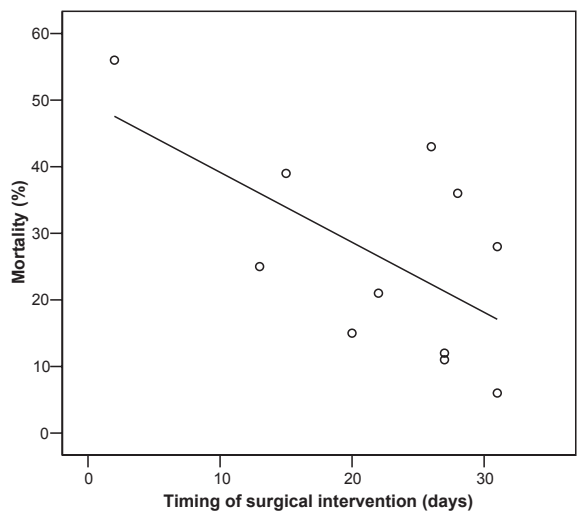


Table 6 Systematic review of series with at least 25 patients operated upon for necrotizing pancreatitis

First author	Year	Design	Patients	Pts/year	Infection (%)	Timing (days)	Mortality (%)
Mier	1997	RCT*	25	8.3	60	2	56
Fernandez-del Cast.	1998	Retro	64	9.1	56	31	6
Branum	1998	Retro	50	8.3	84	27	12
Farkas	1998	Retro	203	11.3	100	20	15
Buchler	2000	Pro	28	5.6	96	22	21
Ashley	2001	Retro	36	7.2	92	27	11
Beattie	2002	Retro	54	6.8	68	26	43
Gotzinger	2003	Pro#	250	15.6	74	15	39
Connor	2005	Pro#	88	14.7	77	31	28
Rau	2005	Retro/pro$	285	15	49	13	25
Present study	2005	Retro	53	5.3	83	28	36
Total†			**54**	**8.3**	**77**	**26**	**25**

*One arm of randomised controlled trial, intervention <72 hours.

#Retrospective analysis of a prospective database

$Partially prospective.

†All data are in median

Discussion

Current surgical guidelines recommend performing necrosectomy in infected necrotizing pancreatitis between day 15 and 28 after onset of disease.[3] In contrast, the present series demonstrates that surgical intervention performed after day 29 is associated with a lower mortality rate. This 'postponed intervention' does lead to prolonged use of antibiotics with concomitant increase in the incidence of fungal and antibiotic-resistant micro-organisms infections of pancreatic necrosis. Also in the systematic review a significant association between 'postponed' timing of surgical intervention and lower mortality was observed. Interestingly, the median time of first surgical intervention in the review was 26 days, implicating that nearly 50% of ANP patients in tertiary referral centres are operated upon after the interval as indicated by the IAP Guidelines.

Mier *et al* showed, in a randomised controlled trial, that delaying intervention in ANP beyond the first 12 days is beneficial as compared to surgery within 72 hours.[2] These results are reflected in the IAP guidelines that state that early surgery within 14 days after onset of the disease is not recommended in patients with necrotizing pancreatitis.[3]

Our results do indeed show a trend towards decreased mortality for intervention in the third and fourth weeks as compared to the first 14 days. However, mortality was significantly further improved if surgical intervention was performed after day 29.

Although APACHE II scores on admission were similar, the mortality in this series (36%) is higher than reported in three large series from the United States (10% mortality in 150 patients).[10,12,16] However, the present mortality, and especially the 22% mortality in the last five years, does not differ much from mortality reported from six European tertiary referral centres (29% mortality in 908 patients).[11,13-15,17,18] Since in the review 'inter-continental' timing of intervention did not differ, the explanation for this difference in mortality has yet to be found.

During the time period of this study, the worldwide trend to postpone surgery influenced our management of ANP patients. This strategy has been more and more adopted in our hospital as of the turn of the century. This is reflected by the delayed intervention in the second study period. There is no data to suggest that patients treated in the second five years of the study period differed in the severity of pancreatitis from the patients treated in the first five years. All patients were treated at our surgical medium care and ICU, at which the treatment for acute pancreatitis patients did not essentially change during the 10 years study period. Therefore, it is apparently possible to delay surgical intervention in a subgroup of ANP patients.

Strikingly, there was no mortality in patients operated upon with multi-organ failure in the 30+ subgroup. One should realise that these patients had similar preoperative ICU stay as compared to the two other subgroups. Apparently, the patients in the 30+ subgroup developed multi-organ failure at a later stage of their disease. The stratification further indicates that surgical intervention in patients with multi-organ failure within the first 14 days carries a mortality of 100% and may therefore be omitted. Since it has been reported that with the improved intensive care treatment it is possible to essentially avoid mortality in the first two weeks[27] it would seem possible to essentially withhold surgical intervention in the first 14 days.

In the literature there is only one study that reports on outcome of surgical intervention in ANP after the first four weeks of admission. Fernandez-del Castillo *et al.* reported on 64 consecutive ANP patients requiring surgical intervention in a 7-year period.[16] From the data of this study it also appears that intervention in the weeks preceding day 27 has the likelihood of a worse outcome compared to intervention in the weeks after day

27 [*Fernandez-Del Castillo, personal communication*]. Due to the impressive low overall mortality of 6.2% in their series the difference in mortality between both groups did not reach significance.

The incidence of positive *Candida* and antibiotic resistant organism cultures in this series is relatively high; fortunately neither influenced mortality. In the literature there is controversy regarding the relevance of fungal infection. Three retrospective studies demonstrated a higher mortality in cases of fungal infection,[28-30] whereas two prospective studies failed to demonstrate such an effect.[31,32] No study has yet demonstrated an effect of antibiotic resistance on mortality in acute pancreatitis. It seems likely that restrictive use of broad spectrum (prophylactic) antibiotics will decrease selection of *Candida* and antibiotic resistant organisms. Since recently two placebo-controlled trials[33,34] did not show any evidence for the use of antibiotic prophylaxis we are currently tailoring our antibiotic treatment on the basis of blood, fine needle aspiration and per-operative culture results. Some investigators also advocate the use of percutaneous drainage of infected (peri-)pancreatic collections as a means of temporizing, or even averting, surgery until day 30.[35]

The results of the systematic review are in line with the findings from our series. Although the demonstrated association between timing and mortality may not be considered proof of a causal relationship, the findings are very suggestive indeed. The fact that an association between patient volume and outcome could not be detected may be due to the fact that only large (>25 patients) series from tertiary referral centres were included. As demonstrated by median timing of intervention in the review, apparently many tertiary referral centres worldwide postpone surgical intervention when possible.

Since this study is not a randomised controlled trial, selection bias may have played a role in the positive outcome of patients operated upon after the first 29 days. However, both the APACHE II score and Ranson scores were similar for the three subgroups. Furthermore, the stratification for the presence of organ failure also does not indicate the presence of any selection bias. The systematic review may be subject to some extent of information bias as several studies did not report data on timing of intervention.

In conclusion, the current study demonstrates that necrosectomy for documented or suspected infected ANP performed after 29 days is associated with lower mortality. However, an increase in fungal colonization and resistant microorganisms is to be expected due to the increased use of antibiotics. Tailored use of antibiotics and critical

review of the patients' clinical condition in order to select cases in which surgery may be delayed seem indicated. We feel the present study provides strong arguments to withhold surgical intervention in the first 14 days, even in the presence of multi-organ failure. Whenever possible, surgical intervention should be postponed until day 30. Based on the findings in this study, this strategy is currently being practiced in a Dutch randomised controlled multicentre trial on surgical intervention in infected necrotizing pancreatitis that recently started including patients.[36,37] To compare interinstitutional data, future studies on surgical intervention in ANP should report on the median timing of first surgical intervention.

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Authors' contributions

MGHB drafted the manuscript.
MGHB, VBN and HGG participated in the design of the study.
MGHB, TJV and EJPS collected and analyzed the data.
All authors edited, read and approved the final manuscript.

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Chapter 10

Early endoscopic retrograde cholangiopancreatography *vs* conservative management in acute biliary pancreatitis without cholangitis: a meta-analysis of randomised trials

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Abstract

Background

Early endoscopic retrograde cholangiopancreatography (ERCP) should be performed in all patients with acute biliary pancreatitis (ABP) and co-existing acute cholangitis. In patients without cholangitis and predicted mild ABP it is generally accepted that early ERCP should not be performed. Nevertheless, there is a controversy regarding the role of early ERCP in the treatment of patients with predicted severe ABP without cholangitis. We reviewed randomised trials on early ERCP *vs* conservative management in patients with ABP without acute cholangitis.

Methods

Relevant publications in three electronic databases (MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials) were systematically reviewed and meta-analysed.

Results

Seven randomised trials on ERCP in acute pancreatitis were found, of which 3 including a total of 450 patients (230 in the invasive arm and 220 in the control arm) qualified for a meta-analysis according to predefined criteria. In all patients with ABP (predicted mild and severe), early ERCP was associated with a non-significant reduction in complications (relative risk (RR) 0.76; 95% confidence interval (CI) 0.41-1.40; p=0.38) and a non-significant increase in mortality (RR 1.13; 95% CI 0.23-5.63; p=0.88). Subgroup analysis based on predicted severity did not affect these outcomes (complications: predicted mild: RR 0.86; 95% CI 0.62-1.19; p=0.36; predicted severe: RR 0.82; 95% CI 0.32-2.10; p=0.68; mortality: predicted mild: RR 1.90; 95% CI 0.25-14.55; p=0.53; predicted severe: RR 1.28; 95% CI 0.20-8.06; p=0.80).

Conclusion

In this meta-analysis, early ERCP in patients with predicted mild and predicted severe ABP without acute cholangitis did not lead to a significant reduction in the risk of complications and mortality.

Introduction

Acute biliary pancreatitis (ABP) is the most frequent form of acute pancreatitis in Western countries.[1,2] There are two mechanisms generally accepted regarding the pathogenesis of ABP: reflux of bile into the pancreatic duct and transient ampullary obstruction caused by sludge or an impacted stone in the ampulla. Patients with small gallstones and sludge are particularly at risk for acute pancreatitis.[5] By early endoscopic retrograde cholangiopancreatography (ERCP) and endoscopic sphincterotomy (ES) bile-duct stones and sludge can be removed, and obstruction released with potentially improved outcome. Nevertheless, despite technical improvements shown in recent years and an increased experience of endoscopists, there is a documented risk of procedure-related complications.[6-8] In addition, it is well recognized that most small gallstones pass spontaneously without causing further harm.[4,9]

Indisputable benefits of endoscopic biliary drainage in patients with acute cholangitis have been stressed in the recent Tokyo Guidelines.[10] It is generally accepted that patients with predicted mild ABP without signs of acute cholangitis do not benefit from early ERCP.[1,11] Controversy persists, however, whether patients with predicted severe ABP in absence of acute cholangitis should undergo early ERCP.[11-13] The 2005 UK guidelines on acute pancreatitis state that all patients with predicted severe ABP (irrespective of the presence of acute cholangitis) should undergo early ERCP,[14] while the recent guidelines of the American College of Gastroenterology recommend that early ERCP is performed only in patients with acute cholangitis and severe acute pancreatitis (organ failure).[1] The 2007 guidelines of the American Gastroenterology Association state that early ERCP in patients with predicted severe ABP without signs of acute cholangitis is controversial and the available data are not uniform in support of this practice.[15]

Indeed, several randomised controlled trials (RCTs) that compared early ERCP, with or without ES, to conservative treatment with selective ERCP, with or without ES, have shown conflicting results.[16-19] The first meta-analysis on this subject did not provide a definite answer.[20] The second meta-analysis aimed to control for a possible modifying effect of acute cholangitis and showed that early ERCP decreased complications in all patients with predicted severe ABP, regardless of the presence of cholangitis.[21] However, this meta-analysis included a RCT in which 35% of patients suffered from acute pancreatitis of a non-biliary cause.[17] Finally, one new RCT has been published since that time.[19] Therefore, the present meta-analysis aims to compare early ERCP, with or without ES, with conservative management in patients with ABP without signs of cholangitis. A

predefined subgroup analysis on patients with predicted severe and predicted mild ABP will be performed.

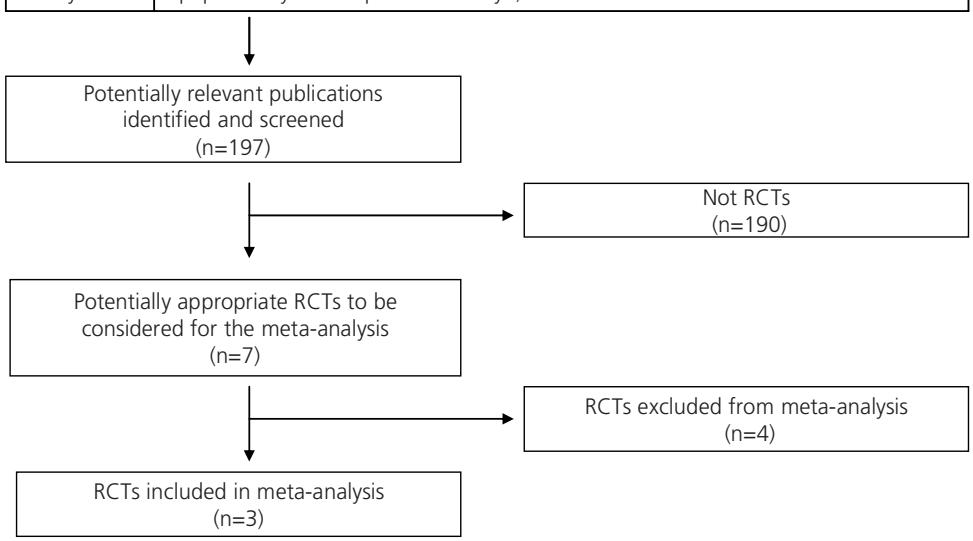
Methods

Search strategy and selection criteria

A systematic literature search with predefined search terms was carried out in the MEDLINE, EMBASE and Cochrane databases for articles published until March 1, 2007 (*Figure 1*). All identified articles and review papers were screened for cross references of articles that included information on ERCP in acute pancreatitis. Language restrictions were not applied.

Figure 1 Flow-chart illustrating the details of the search and study selection process

Database	Search strategy
MEDLINE	("acute pancreatitis" [Title/Abstract] OR "pancreatitis" [Title/Abstract]) AND ("gallstone"[Title/Abstract] OR "gallstones"[Title/Abstract] OR "biliary" [Title/Abstract]) AND ("ERCP" [Title/Abstract] OR "endoscopic retrograde cholangiopancreatography" [Title/Abstract] OR "sphincterotomy" [Title/Abstract] OR "papillotomy" [Title/Abstract]) AND ("conservative" [Title/Abstract])
EMBASE	"pancreatitis" AND ("ERCP" OR "endoscopic retrograde cholangiopancreatography" OR "Vater papillotomy" OR "sphincterotomy") AND [humans]/lim
Cochrane library	pancreatitis AND ("endoscopic retrograde cholangiopancreatography" OR "papillotomy" OR "sphincterotomy")



The title and abstract of all identified papers were screened for the following inclusion criteria:
1) Study population: patients with ABP without signs of acute cholangitis. Acute cholangitis should be either an exclusion criterion or separate data on patients without acute cholangitis should be presented.
2) Intervention: early ERCP (i.e. within 72 hours after admission) with or without ES.
3) Comparison: conservative treatment with selective ERCP with or without ES.
4) Outcomes: mortality and overall complications.
5) Study design: participants were assigned to either ERCP or comparator by random allocation.

Data extraction and quality assessment

Titles and abstracts of all retrieved records and subsequently full-text articles were examined independently by two authors (MSP, HCvS) to identify trials that satisfied the inclusion criteria. Discrepancies in selection were resolved by discussion between the authors of this meta-analysis.
The Jadad scale was used to grade the methodological quality of the trials included.[22] This scale consists of three items regarding (1) random allocation, (2) masking of patients, (3) dropouts and withdrawals. The quality scale ranges from 0 to 5 points, with 2 or less indicating low-quality and 3 or higher indicating high-quality. In addition, 3 other criteria were applied regarding: (4) allocation concealment (yes or no), (5) blinding of endpoint assessment (yes or no, irrespective of blinding of treatment for patient and physician), and (6) missing data (at least 90% of the data reported).
Data with regard to the reported group size, baseline characteristics and numbers of events for each endpoint were extracted and documented independently by two authors (MSP, HCvS).

Statistical analysis

The data analysis was performed with the meta-analysis software Comprehensive Meta-Analysis (Borenstein M, Hedges L, Higgins J, Rothstein H. Comprehensive Meta-analysis Version 2, Biostat, Englewood NJ 2005). From the pooled data, the risk ratio (RR) and risk difference (RD) with the 95% confidence interval (CI) were calculated for the following endpoints: mortality and overall complications. The presence of heterogeneity was assessed using I^2 measure, with I^2>0.2 indicating marked heterogeneity. Since absence of statistical proof of heterogeneity is not equal to statistical proof for the absence of heterogeneity (especially if the number of included studies is small) a random effects model was used, irrespective of the degree of heterogeneity of effect among

the included trials. The Mantel-Haenszel method was used for the pooled analysis of included trials. When no events were observed in both treatment groups of a particular trial, we added an event fraction (0.001) to the ERCP group in order to allow inclusion of such trials in the pooled data-analysis. Funnel plots were created to explore possible biases (i.e. reporting, publication and reviewer bias).

Results

The literature search yielded 197 publications. The details of the literature search and selection of studies are shown in *Figure 1*. Seven potentially eligible RCTs on ERCP in acute pancreatitis were identified and four studies were excluded. The first excluded trial[17] studied patients with AP, irrespective of the cause, instead of only ABP. Moreover, neither patients with acute cholangitis were excluded nor was data for this subgroup presented separately. Patients in the second excluded RCT[23] were randomised to early ERCP, with or without ES, only in the case of persisting ampullary obstruction (based on clinical, biochemical, and imaging criteria) during more than 24 h. Consequently, ERCP was performed only in 47% of patients in the intervention arm. The third excluded RCT[24] aimed to study exclusively patients with severe non-biliary pancreatitis. The fourth trial[25] was excluded because patients undergoing duodenoscopy for suspected ABP were subsequently randomised to ES or no ES (i.e. a RCT with different intervention and comparison than the current meta-analysis). Moreover, the last 2 studies[24,25] were only published in abstract form.

In the 3 RCTs satisfying the inclusion criteria, patients with acute cholangitis were either excluded specifically,[18,19] or outcome of patients without acute cholangitis was presented separately.[16] With funnel plots publication bias for the different outcomes could not be detected (data not shown). The study characteristics for the 3 trials, including their definition of acute cholangitis, are shown in *Table 1*. A total of 450 patients were included: 230 were allocated to early ERCP with or without ES; 220 were allocated to conservative treatment with elective ERCP with or without ES. In the treatment groups of the 3 trials altogether, ERCP was successful in 214 out of 230 patients (93%). In these 214 patients, ES was performed in 114 patients (53%) and a common bile duct stones were removed in 111 patients (52%). ERCP procedure-related complications occurred in 5 patients (2%). In the control group of the 3 trials altogether, 33 of the 220 patients (15%) underwent ERCP, of which 30 (91%) were successful. In addition, 14 patients underwent ES (47%), common bile duct stones were removed in 33 patients (43%) and ERCP procedure-related complications did not occur.

Table 1 Summary of study characteristics for the included trials

Study	Number of patients		Time to ERCP	Definition of cholangitis	Criteria of predicted severe ABP	Predicted severe ABP		Criteria of ABP
	Intervention group	Control group				Intervention group	Control group	
Neoptolemos *et al.*[16]	53	57	< 72 h of admission	Not stated	Glasgow ≥3	20 (38%)	25 (44%)	Gallstones on US or cholestatic laboratory abnormalities
Fölsch *et al.*[18]	126	112	< 72 h of onset	Bilirubin >5 mg/dl (90 µmol/L)‡	Glasgow ≥3	26 (23%)†	20 (18%)†	Gallstones on US or CT or cholestatic laboratory abnormalities
Oria *et al.*[19]	51	51	< 48 h of onset	Charcot's triad	APACHE II ≥6	17 (33%)	21 (41%)	Gallstones on US or CT
Total	230	220				63 (27%)	66 (30%)	

† Patients were assigned severity score post hoc.

‡ 12 patients had serum bilirubin concentrations higher 5 mg/dl (90 µmol/L) but were analyzed on an intention-to-treat basis.

ERCP= endoscopic retrograde cholangiopancreatography

ABP= acute biliary pancreatitis, US= ultrasound, CT= computed tomography

Table 2 Quality assessment of trials included in the meta-analysis

Study	Primary endpoint	Double blinding	Randomization	Withdrawals*	Jadad score	Allocation concealment	Blinded endpoint assessment	Missing data *
Neoptolemos *et al.*[16]	Mortality	No	Not stated	6/4	2	Unclear	No	0/0
Fölsch *et al.*[18]	Mortality	No	Not stated	0/0	2	Unclear	Yes	16/16†
Oria *et al.*[19]	Organ failure	No	Sealed envelope	0/1	3	Potentially manipulable	No	0/0

* Intervention group/ Control group

† Patients with undefined severity

Table 3 Baseline characteristics of patients

Study	Setting	Centres	Female		Age in years	
			Intervention group	Control group	Intervention group	Control group
Neoptolemos *et al*.[16]	UK	1	34 (58%)*	35 (56%)*	55 (20-86)/ 74 (38-85)*†#	67.5 (30-87)/ 76.5 (37-96)*†#
Fölsch *et al*.[18]	Germany	22	66 (52%)	76 (68%)	63 (24-90)#	63 (15-93)#
Oria *et al*.[19]	Argentina	1	35 (69%)	38 (75%)	49.9 ± 17.4‡	44 ± 17.7‡

* Including patients with acute cholangitis
† Predicted mild/ predicted severe groups
# Values are median (range)
‡ Values are mean ± standard deviation

Table 4 Meta-analysis for complications and mortality comparing Early ERCP with conservative treatment

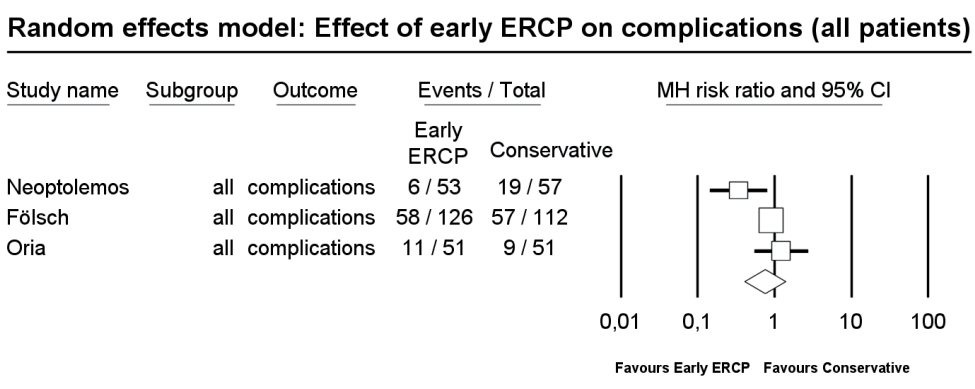
Complications	Relative risk (95% confidence intervals)	p value	Heterogeneity, I^2	Risk difference (95% confidence intervals)	p value	Heterogeneity, I^2
Predicted mild and severe ABP	0.76 (0.41-1.40)	0.38	0.65	-0.08 (-0.22-0.07)	0.29	0.66
Predicted mild ABP	0.86 (0.62-1.19)	0.36	0	-0.05 (-0.13-0.04)	0.32	0
Predicted severe ABP	0.82 (0.32-2.10)	0.68	0.77	-0.09 (-0.49-0.30)	0.64	0.84
Mortality	**Relative risk (95% confidence intervals)**	**P-value**	**Heterogeneity, I^2**	**Risk difference (95% confidence intervals)**	**P-value**	**Heterogeneity, I^2**
Predicted mild and severe ABP	1.13 (0.23-5.63)	0.88	0.54	0.001 (-0.08-0.09)	0.97	0.74
Predicted mild ABP	1.90 (0.25-14.55)	0.53	0	0.01 (-0.02-0.04)	0.40	0
Predicted severe ABP	1.28 (0.20-8.06)	0.80	0.56	0.01 (-0.22-0.24)	0.91	0.77

ABP= acute biliary pancreatitis

Two of 3 RCTs had a Jadad quality score[22] grade 2 (*Table 2*). The demographic data of patients are summarized in *Table 3*. Complications and mortality were reported in all 3 trials. The results of the meta-analyses of the included trials for complications and mortality are presented in *Table 4*.

With marked heterogeneity across studies, early ERCP reduced the risk for complications (pooled RR for all ABP patients: 0.76; 95% CI 0.41-1.40) (*Figure 2*) while it increased the risk of mortality (pooled RR for all ABP patients: 1.13; 95% CI 0.23-5.63) (*Figure 3*). These results did, however, not reach statistical significance. Because of the low absolute risks for mortality and complications, these RRs translate in a very small reduction in the absolute risk for complications (pooled RD for all ABP patients: -0.08; 95% CI -0.22-0.07) and a very small increase in the absolute risk for mortality (pooled RD for all ABP patients 0.001; 95% CI -0.08-0.09) (*Table 4*). These results did, however, not reach statistical significance.

Figure 2 Random effects model of relative risk of complications associated with early ERCP with or without ES compared with conservative management in all patients with acute biliary pancreatitis.



Based on the reported data the meta-analysis was stratified for predicted severe and predicted mild ABP. For this analysis additional unpublished data were provided by Oria *et al*.[19] It should be noted that Fölsch *et al*[18] failed to report the disease severity for 32 patients due to post-hoc classification of their data. Consequently, the data on these 32 patients could not be analyzed. The stratification for severity did not result in significant differences for the risk of complications (*Figure 4 and 5*) and mortality (*Figure 6 and 7*) between the ERCP group and the conservative treatment group in both patients with predicted mild and predicted severe ABP.

Figure 3 Random effects model of relative risk of mortality associated with early ERCP with or without ES compared with conservative management in all patients with acute biliary pancreatitis.

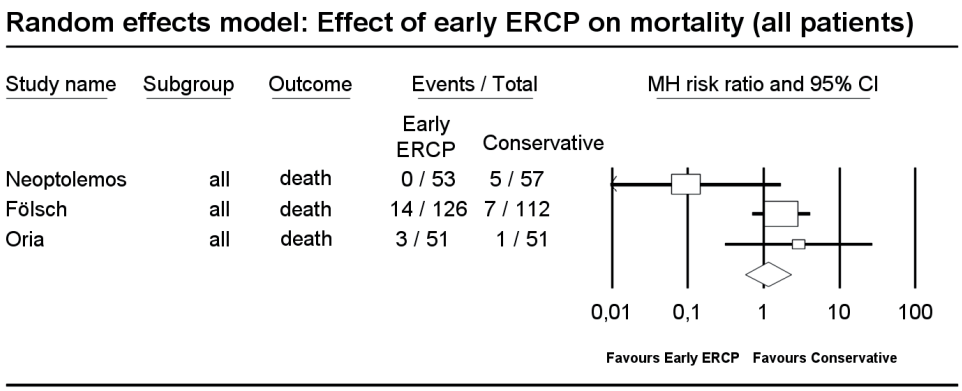


Figure 4 Random effects model of relative risk of complications associated with early ERCP with or without ES compared with conservative management in patients with predicted mild acute biliary pancreatitis.

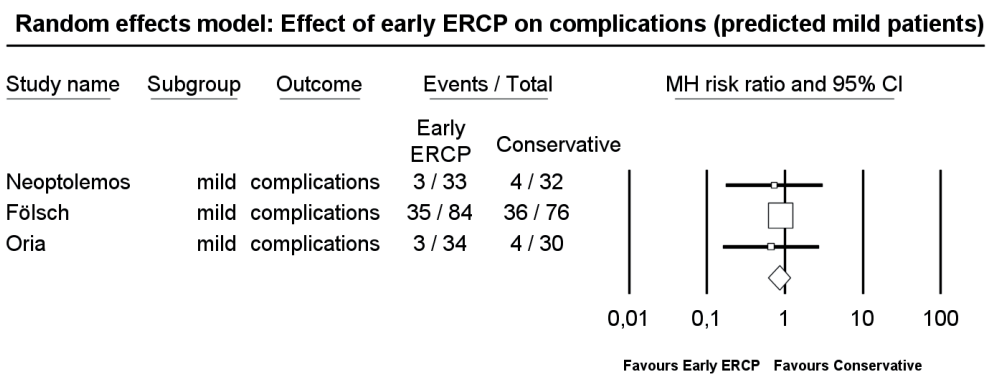


Figure 5 Random effects model of relative risk of complications associated with early ERCP with or without ES compared with conservative management in patients with predicted severe acute biliary pancreatitis.

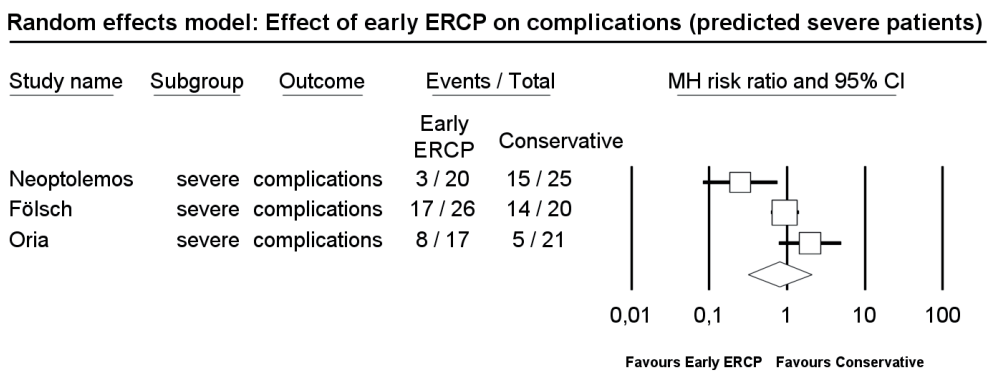


Figure 6 Random effects model of relative risk of mortality associated with early ERCP with or without ES compared with conservative management in patients with predicted mild acute biliary pancreatitis.

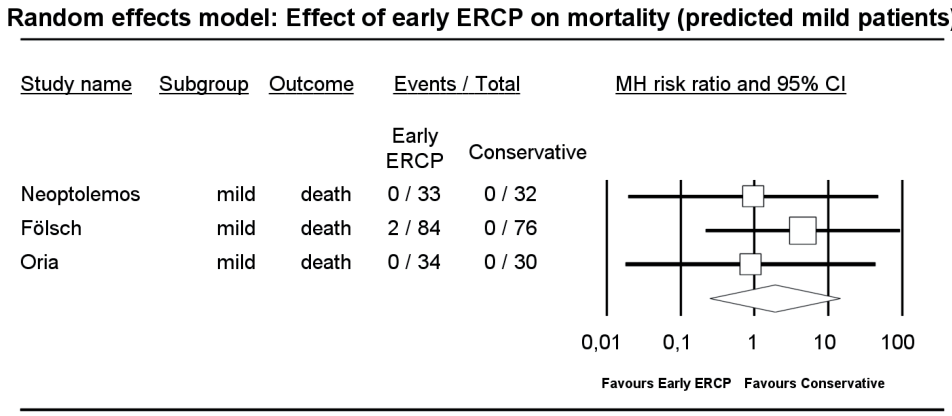
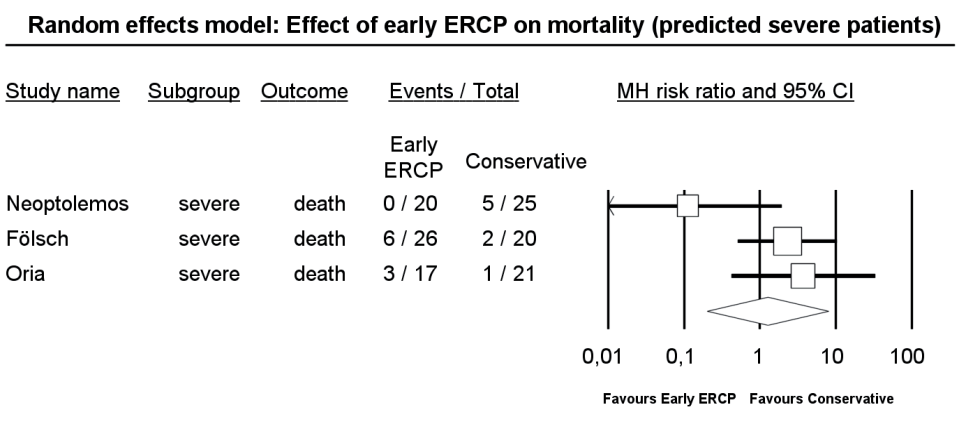


Figure 7 Random effects model of relative risk of mortality associated with early ERCP with or without ES compared with conservative management in patients with predicted severe acute biliary pancreatitis.



Discussion

In this meta-analysis of RCTs comparing early ERCP, with or without ES, with conservative treatment in patients with ABP without signs of acute cholangitis, no beneficial effect of early ERCP on mortality and overall complications was observed both in patients with predicted mild and patients with predicted severe ABP.

These results suggest that early ERCP in patients with ABP without co-existing cholangitis is an unnecessary invasive procedure. Notably, in the included RCTs, only about half the patients that underwent a successful ERCP were found to have common bile duct stones. This finding is in accordance with the recent study by Acosta *et al.*,[23] in which 71% of patients with ABP and ampullary obstruction (defined in this study as severe and continuous epigastric pain, bile-free gastric aspirate, and elevated serum bilirubin level) showed spontaneous relief of obstruction within 48 hours from the onset of symptoms. In the RCTs included in this meta-analysis, ES was performed mainly when common bile duct stones were visualized during ERCP. In daily clinical practice, however, ES is often also performed in the absence of common bile duct stones because of a potential false-negative ERCP in case of sludge, microlithiasis or missed common bile duct stones.

The design of an optimal strategy in biliary pancreatitis is frustrated by a low sensitivity of pre-ERCP diagnostic tools to confirm the presence of common bile duct stones. In order to increase on this sensitivity several studies with new imaging modalities have been performed. Magnetic resonance cholangiopancreatography (MRCP) and endoscopic ultrasonography (EUS) have been proposed as minimal invasive diagnostic techniques to identify common bile duct stones, to further reduce the number of unnecessary ERCPs.[26-30] A recent RCT comparing EUS (with selective ERCP and ES in case of common bile duct stones) with ERCP and selective ES in 140 patients with ABP showed a higher success rate for examination rate of the biliary tree with a comparable morbidity and mortality risk in patients undergoing EUS with selective ERCP.[31] However, MRCP is known to miss small gallstones (<6 mm),[32] while these are associated with the risk for acute pancreatitis.[5] Moreover, both MRCP and EUS are not yet widely available and because experience is scant, EUS may be technically difficult to perform in the early stage of ABP.

In the interpretation of the present meta-analysis the following aspects deserve attention. Firstly, the methodological quality of the included trials was relatively low (i.e. Jadad score[22] below 3 for two of the three included trials). However, these data are still the best available.

Secondly, the included trials used different definitions with respect to acute cholangitis, and included different subgroups of patients with ABP (*Table 1*). Neoptolemos *et al.*[16] included all patients with ABP and presented separate data on patients without acute cholangitis. Oria *et al.*[19] included only patients with ABP and clinical evidence of biliopancreatic obstruction without acute cholangitis. Fölsch *et al.*[18] excluded all

patients with a bilirubin >5 mg per decilitre (90 µmol per liter), thereby excluding a proportion of patients with acute cholangitis, but also likely excluding some patients with biliopancreatic obstruction without acute cholangitis. Furthermore, the incidence of cholestasis varied among the three included RCTs as a consequence of the different eligibility criteria of these trials. Although focus has historically been on acute cholangitis rather than cholestasis (without cholangitis), the presence of cholestasis alone might also be of influence on the clinical impact of early ERCP.

Thirdly, the three studies used different definitions for 'early' ERCP. Neoptolemos *et al.*[16] considered 'early' ERCP as within 72 hours after admission, regardless of duration of symptoms at time of admission. The two other trials[18,19] defined 'early' as within 48-72 h after onset of symptoms.

Fourthly, there was considerable variation among the three trials in the definition of 'overall complications' as outcome (e.g. gallbladder empyema, recurrent pancreatitis, respiratory insufficiency, ascites, lumbar osteitis, infected pancreatic necrosis). As a likely result of this variation, the incidence of complications in the patients treated conservatively varied from 19%[19] to 51%.[18] The abovementioned differences in patient populations and definitions on intervention and outcome might explain the different outcomes of various trials.

Fifthly, based on the individual findings of the performed RCTs[16-19] and previous meta-analyses,[20,21] the role of early ERCP is most controversial in the subgroup of patients suffering from a predicted severe attack of ABP without signs of acute cholangitis. Although in the current meta-analysis patients with predicted severe ABP did not benefit from early ERCP, it should be noted that the number of patients with predicted severe APB included was relatively small (n=129). Moreover, the accuracy of current clinical scores for predicting severity is known to be quite poor. Oria *et al.*[19] used quite a low cut-off level (APACHE II ≥6) for 'predicted severe' ABP. As a result, few patients identified as 'predicted severe' eventually did suffer from clinically severe pancreatitis, as shown by low rates of organ failure and limited pancreatic necrosis (a low computed tomography severity index).[19] Fölsch *et al.*[18] defined severity *post hoc* which resulted in a failure to define severity in 13% of randomised patients.

Finally, the results of this meta-analysis conflict with those of a previous Cochrane meta-analysis,[21] which, unlike this study, included the trial by Fan *et al.*[17] We excluded the trial of Fan *et al.* because this study included patients with a non-biliary cause of acute

pancreatitis and included patients with acute cholangitis, without presenting separate data for patients without acute cholangitis. However, when we included trial by Fan *et al*. the results of this meta-analysis did not principally change (data not shown).

In conclusion, the present meta-analysis does not show a beneficial effect of early ERCP, with or without ES, in both patients with predicted mild and severe ABP without cholangitis. There is, however, a lack of data on the subgroup of patients with predicted severe ABP. Therefore, a new adequately powered RCT in this setting is needed. In this future study, patients with acute cholangitis should be excluded, timing after onset of the disease should be clearly defined and stratification for the presence or absence of cholestasis (biochemical and radiological) seems appropriate.

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Contributors

MSP was the principal investigator, coordinated the study, and had overall responsibility for the final version. MSP, HCvS and MGHB participated in the study design. MSP and HCvS undertook the literature search, selected reports for analysis, undertook data analysis. MGHB contributed to data extraction, data checking. GJMGvdH contributed as the epidemiological and statistical adviser, and answered the statistical questions of the reviewers. KJvE and HGG edited the paper. All investigators contributed to the interpretation of results and preparation of the manuscript.

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Chapter 11

General discussion and summary

Acute pancreatitis is a common, potentially lethal, costly and poorly understood disease. In the past decade, the incidence of acute pancreatitis in the Netherlands increased by 50% to 3413 admissions for acute pancreatitis in 2006 (Prismant; National Hospital Registration System). In that same year, the overall mortality rate of acute pancreatitis was 4.0%. About 20% of patients will develop severe acute pancreatitis, characterized by organ failure and/or pancreatic necrosis. Although an average Dutch hospital admits 35 patients with acute pancreatitis per year, only 6-8 of these patients will develop severe acute pancreatitis.[1] Furthermore, only 2-3 patients per hospital, per year will develop infected necrotizing pancreatitis. Because of this low patient volume, it has traditionally been extremely difficult to perform large-scale randomised trials in patients with severe acute pancreatitis. To that end, in 2002 the nationwide, multidisciplinary Dutch Acute Pancreatitis Study Group was founded. The majority of studies described in this thesis have been performed by 15 centres participating in this study group, including all Dutch university medical centres.

Since the incidence of acute pancreatitis is increasing rapidly and it is estimated that about 80% of deaths are associated with infectious complications, the main aim of this thesis was to develop and test the following strategies:

A – Prevention strategies: aimed at preventing acute pancreatitis and related infections, especially infected (peri-)pancreatic necrosis

B – Intervention strategies: aimed at improving outcome of intervention in patients with infected (peri-)pancreatic necrosis.

Part A – Prevention Strategies

Previous retrospective studies have suggested that ursodeoxocholic acid is capable of preventing biliary colics and complications, such as biliary pancreatitis, in patients with symptomatic gallstone disease.[2,3] In **chapter 2**, we studied this suggestion in a multicentre randomised, double-blind, placebo-controlled trial. In three Dutch centres, 177 patients with highly symptomatic gallstone disease (median 13 colics in the preceding year), scheduled to undergo cholecystectomy were randomised between ursodeoxocholic acid or placebo. The primary endpoint was the occurrence of a biliary colic. At baseline, groups were highly comparable. We found that 74% of patients had recurrent colics in the ursodeoxycholic acid group *vs* 67% in the placebo group (p=0.30). Furthermore, none of the secondary endpoints pointed to an advantage of the use of ursodeoxocholic acid. It was concluded that ursodeoxycholic acid does

not reduce biliary symptoms in highly symptomatic patients. Early cholecystectomy is warranted in patients with symptomatic gallstones.

Two small double-blind, placebo-controlled randomised trials demonstrated a beneficial effect of probiotic prophylaxis in acute pancreatitis. Both trials were performed by the same Hungarian group[4,5]; the first trial used a mono-species preparation and the second used a multispecies preparation. In **chapter 3**, we analysed whether a multispecies probiotic preparation (Ecologic 641) was capable of preventing infectious complications in acute pancreatitis. We performed a multicentre randomised, double-blind, placebo-controlled trial (PROPATRIA: probiotics in pancreatitis trial) on probiotic prophylaxis in 296 patients with predicted severe acute pancreatitis enrolled in 15 Dutch centres. At baseline, groups were highly comparable. After follow up, the rate of infectious complications was similar in both groups (30% probiotics vs 28% placebo) whereas mortality was 2.5 times higher in the probiotics group (16% vs 6%, relative risk 2.5, 95% confidence interval 1.2-5.3). Nine patients in the probiotics group developed bowel ischaemia (eight with fatal outcome), compared with none in the placebo group. It was concluded that probiotic prophylaxis with the combination of strains used should not be administered in patients with predicted severe acute pancreatitis.

Although frequently claimed in experimental studies, no clinical study has reported an association between intestinal barrier dysfunction and infectious complications. Furthermore, no clinical study has confirmed the suggestion from animal studies that probiotic prophylaxis is capable of restoring intestinal barrier function and hereby preventing infectious complications. In **chapter 4**, we analysed the relationship between intestinal barrier dysfunction and infectious complications in a prospective cohort of patients randomised in PROPATRIA. We performed a polyethylene glycol test for gastrointestinal permeability in 101 patients. Four polyethylene glycol molecules were enterally administered and recovered in 24-hours urine. Furthermore, intestinal mucosal damage was assessed by determining the concentration of intestinal fatty acid binding protein (IFABP) levels in the 24-hour urine collected for the permeability test. IFABP is a protein that is specifically located in the top of small bowel villi: it is released into the circulation in cases of enterocyte death. We found that IFABP levels in the first 72 hours of acute pancreatitis were higher in patients who developed bacteraemia, infected pancreatic necrosis and organ failure. Permeability was increased in the first 72 hours in patients who developed bacteraemia, organ failure or who died. Probiotic prophylaxis did not influence intestinal permeability but was associated with an increase in intestinal mucosal damage, but only in patients suffering from organ failure.

If any prophylactic strategy aiming to prevent infections is to be successful it should be instituted prior to the onset of infections. In chapter 5, we analysed the time of onset and clinical relevance of infections in a prospective cohort of 732 patients with a first episode of acute pancreatitis. We found that the initial infectious complication in 173 patients was diagnosed at a median of 8 days after admission (interquartile range 3-20 days). Of these initial infections 20% were diagnosed within the first 2 days of admission. Eighty percent% of the 61 deceased patients had been diagnosed with an infection. In 154 patients with pancreatic necrosis, bacteraemia was associated with increased risk of developing infected necrosis (65 *vs* 38%, p=0.002). In 98 patients with infected necrosis, bacteraemia was associated with higher mortality (40 vs 16%, p=0.014). The only infectious complication that was associated with mortality after multivariate analysis was bacteraemia (OR 3.4). It was concluded that infectious complications occur very early in the course of acute pancreatitis, and have a significant impact on mortality, especially in cases of bacteraemia. The focus of prophylactic strategies should be shifted to very early intervention.

Part B – Intervention Strategies

In chapter 6, we evaluated the various surgical strategies for (suspected) infected necrotizing pancreatitis and patient referrals for this condition in the Netherlands. We included all 106 consecutive patients who had surgical treatment in the period 2000–2003 in 11 Dutch hospitals, including all university medical centres. We searched the National Hospital Registration System to identify patients who were admitted to the 90 Dutch hospitals that did not participate in the present study. The overall mortality rate after surgical intervention for (suspected) infected necrotizing pancreatitis was 34%. For the open abdomen strategy, mortality was 70% (16 of 23), for continuous postoperative lavage 25% (13 of 53), for minimally invasive procedures 11% (two of 18) and for primary abdominal closure 42% (five of 12) (mortality between groups: p<0.001). During the study interval, only 12% of 362 patients with acute pancreatitis who were likely to require surgical intervention had been referred to university medical centres. It was concluded that laparotomy with continuous postoperative lavage is the surgical strategy most often used in the Netherlands. The results of the open abdomen strategy are poor whereas a minimally invasive approach seems promising.

The 1992 Atlanta classification is the worldwide standard for classifying peripancreatic collections in acute pancreatitis (acute fluid collection, pancreatic abscess, pseudocyst,

pancreatic necrosis).[6] Although frequently criticised in recent years, no study has formally addressed the potential drawbacks of this classification. In **chapter 7**, we analysed the interobserver agreement among radiologists when using the Atlanta classification. Preoperative contrast-enhanced CTs from 70 consecutive patients operated upon for necrotizing pancreatitis were reviewed by five abdominal radiologists who were aware of the timing of the CT. We found the interobserver agreement to be very poor among the radiologists. In 3 of 70 cases (4%), the same Atlanta definition was chosen. It was concluded that the Atlanta classification should no longer be used to describe complications of acute pancreatitis on CT.

Although minimally invasive procedures are gaining popularity, their general applicability in patients with infected necrotizing pancreatitis is unknown.[7] In **chapter 8,** we analysed the feasibility of minimally invasive procedures to treat infected necrotizing pancreatitis. Eighty preoperative CTs of patients operated on for (suspected) infected necrotizing pancreatitis in 11 Dutch hospitals were reviewed by five abdominal radiologists. Agreement between radiologists was determined. In 55 (69%) patients, the lateral border of the collection was less than 5 cm from the left abdominal wall. Placement of a drain was deemed feasible in 84% of patients. In 56% of patients, it was deemed possible to place a drain through the left retroperitoneum. It was concluded that most peripancreatic collections in infected necrotizing pancreatitis were considered accessible to a minimally invasive approach.

In recent years, timing of surgical intervention in infected necrotizing pancreatitis has greatly shifted from early, immediate intervention in cases of sterile necrosis to delayed intervention in cases of encapsulated infected necrosis. Current guidelines advocate intervention between day 14 and 30 of admission.[8] In **chapter 9**, we analysed the effect of timing of surgical intervention in necrotizing pancreatitis. In a retrospective series of 53 patients from the University Medical Center Utrecht the median timing of intervention was 28 days and mortality 36%. The 26 patients operated upon after day 29 had received antibiotics for a longer period (p=0.001), and *Candida* species and antibiotic-resistant organisms were more often cultured (p=0.02). This group also had the lowest mortality (8% vs 75% in the 1-14 days group and 45% in the 15-29 days group, p<0.001); this difference persisted when the outcome was stratified for the presence of preoperative organ failure. During the second half of the 10-year study, necrosectomy was further postponed (43 vs 20 days, p=0.06) and mortality decreased (22% vs 47%, p=0.09). In a review of 11 studies with a total of 1136 patients operated upon for necrotizing pancreatitis, the median mortality was 25% (range 6%-56%). A

correlation between timing of intervention and mortality was observed (*R*=–0.603; 95% confidence interval, –2.10 to –0.02; p=0.05). It was concluded that, whenever possible, necrosectomy should be postponed until 30 days after initial hospital admission as this is associated with decreased mortality.

There is an ongoing discussion about whether ERCP should be performed in patients with biliary pancreatitis who do not show signs of cholangitis.[9] In **chapter 10**, we performed a meta-analysis of all randomised controlled trials performed in patients with biliary pancreatitis without cholangitis. Three of seven retrieved trials, including a total of 450 patients, qualified for a meta-analysis according to the predefined criteria. In all patients with biliary pancreatitis (both predicted mild and severe), early ERCP was associated with a non-significant reduction in overall risk of complications (risk ratio (RR) 0.76; 95% confidence interval 0.41–1.04; p=0.38) and a non-significant increase in mortality (RR 1.13; 95% confidence interval 0.23–5.63; p=0.88). Subgroup analysis based on predicted severity did not affect these outcomes. It was concluded that early ERCP in patients with biliary pancreatitis without acute cholangitis does not lead to a significant reduction in the risk of overall complications and mortality.

Future perspectives

Good research raises more questions than it answers. This thesis is no exception to that rule. Here, we summarise key areas of future research in prevention and intervention strategies in acute pancreatitis.

Prevention Strategies

As identified in **chapters 2** and **4**, probiotics may be associated with intestinal damage in patients who suffer from organ failure due to severe acute pancreatitis. Experimental studies should analyse the mechanism by which probiotics exert this deleterious effect. Further factors needing to be addressed are the impact of the type of probiotics, dose of probiotics, type of enteral nutrition, type of administration (bolus/continuously), and the place of administration (stomach, small bowel) and the potential adverse effects associated with them.

As identified in **chapters 4** and **5**, infectious complications occur very early in the course of acute pancreatitis. Future randomised studies should therefore focus on very early onset (first 12-24 hours of admission) of prophylaxis. A promising strategy could be the

very early onset of enteral nutrition in patients with predicted severe acute pancreatitis. In the intensive care setting this has proven to be effective in reducing the incidence of infectious complications.[10] The PYTHON trial of the Dutch Acute Pancreatitis Study Group will indeed focus on this strategy (Controlled Trials registration: ISRCTN18170985). Very early onset of antibiotic prophylaxis is also a new promising strategy[11] that should be studied further. However, issues regarding antibiotic resistance will need to be considered.

Future studies should also analyse whether 'intensive resuscitation bundles'[12], incorporating strategies such as epidural pain control and tailored, aggressive fluid replacement therapy, aiming at optimal organ function, can minimise or even prevent SIRS related organ failure and infectious complications in acute pancreatitis.

Intervention Strategies

As identified in chapters 6 and 8, minimally invasive intervention is both feasible and promising in the treatment of patients with infected necrotizing pancreatitis in whom conservative treatment has failed. No randomised trial has ever compared a minimally invasive strategy with laparotomy in this condition. Therefore, the results of the PANTER trial, randomising between minimally invasive 'step-up' approach and laparotomy, are eagerly awaited.[13] Future studies will need to be based on the PANTER results.

Future randomised studies will also need to address the potential benefits of NOTES (natural orifice tract endoscopic surgery) in patients with infected necrotizing pancreatitis or patients that are 'persistently unwell' for several months due to bulky collections containing sterile necrosis. A promising strategy in this respect is endoscopic transgastric necrosectomy.[14-16]

Future studies should determine what the added value is of postponing intervention in a patient with documented infected pancreatic necrosis until such time that the collection has become fully organized/ encapsulated. The presumed benefits of postponing intervention are safe and easier necrosectomy, reduced risk of peri-operative severe bleeding and the potential resolution of organ failure with antibiotic therapy. The presumed disadvantages include the risk of new onset or aggravation of organ failure.[17] Obviously, the design of such a trial should include balancing for the presence of organ failure at the time of randomisation.

As pointed out in **chapter 7**, future studies on intervention strategies should not be based on the Atlanta classification. The new international classification of acute pancreatitis that is nearly finished at the time of the writing of this thesis, will be helpful. New interobserver and large prospective cohort studies should be performed to validate this classification. The role of magnetic resonance imaging should be taken into account since it was shown that computed tomography is very poor at detecting solid debris in a collection predominantly containing fluid.[18]

Given the low patient numbers and wide confidence intervals reported in **chapter 10**, a new randomised trial is still needed to decide on the matter of the efficacy of ERCP in patients with biliary pancreatitis without cholangitis. This study should stratify for the presence of cholestasis as patients with cholestasis are more likely to benefit from desobstruction of the biliary duct.

It should be no surprise to the reader that the Dutch Acute Pancreatitis Study Group is currently working in all the fields described in this 'future perspectives' paragraph. It is the sincere hope and belief of the author that this highly successful study group will continue to guide clinicians throughout the world in making evidence-based decisions when treating their patients with acute pancreatitis. It is only through collaborative research that we will be able to improve our understanding and the outcome of this common, potentially lethal, costly, and poorly understood disease.

Table 1 Answers to study questions addressed in this thesis

Chapter	Study question and answers
2.	*Can ursodeoxycholic acid prevent biliary colics and complications in patients with symptomatic gallstone disease awaiting cholecystectomy?* Ursodeoxycholic acid does not reduce biliary symptoms in highly symptomatic patients. Early cholecystectomy is warranted in patients with symptomatic gallstones.
3.	*Can a multispecies probotic (Ecologic 641) prevent infectious complications in patients with predicted severe acute pancreatitis?* In patients with predicted severe acute pancreatitis, probiotic prophylaxis with this combination of probiotic strains does not reduce the risk of infectious complications and is associated with an increased risk of mortality. Probiotic prophylaxis should therefore not be administered in this category of patients.
4	*What is the relationship between intestinal barrier dysfunction in acute pancreatitis, infectious complications and probiotic prophylaxis?* Intestinal barrier dysfunction early in the course of acute pancreatitis is associated with bacteraemia, infected necrosis, organ failure, and mortality. The probiotics administered do not alter intestinal permeability but increase mucosal damage in patients with organ failure.
5.	*What is the time of onset and impact of infections in acute pancreatitis?* Infectious complications occur very early in the course of acute pancreatitis and have a significant impact on mortality, especially in cases of bacteraemia. The focus of prophylactic strategies should be shifted to very early intervention.
6.	*What are the results of the various surgical strategies using for infected necrotizing pancreatitis in the Netherlands?* Laparotomy with continuous postoperative lavage is the surgical strategy most often used in the Netherlands. The results of the open abdomen strategy are poor whereas a minimally invasive approach seems promising.
7.	*What is the interobserver agreement when using the Atlanta classification to describe computed tomography findings in acute pancreatitis?* The interobserver agreement of the Atlanta classification for categorising peripancreatic collections in acute pancreatitis on CT is poor. The Atlanta classification should not be used to describe complications of acute pancreatitis on CT.
8.	*What is the feasibility of minimally invasive approaches in patients with infected necrotizing pancreatitis?* Most peripancreatic collections in infected necrotizing pancreatitis are considered accessible to a minimally invasive approach.
9.	*What is the impact of the timing of surgical intervention in necrotizing pancreatitis?* Postponing necrosectomy until 30 days after initial hospital admission is associated with decreased mortality, prolonged use of antibiotics, and increased incidence of *Candida* species and antibiotic resistant organisms.
10.	*What is the role of early endoscopic retrograde cholangiopancreatography in acute biliary pancreatitis without cholangitis?* Early endoscopic retrograde cholangiopancreatography in patients with biliary pancreatitis without acute cholangitis does not lead to a significant reduction in the risk of overall complications and mortality.

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Chapter 12

Summary in Dutch (Nederlandse samenvatting)

Acknowledgements (Dankwoord)

Curriculum Vitae

List of publications

Review committee

Summary in Dutch (Nederlandse samenvatting)

De alvleesklier, het pancreas, is een 12-15 cm langwerpig orgaan welke is gelegen in een ruimte achter de maag, het retroperitoneum (zie figuur 1, hoofdstuk 1). Het pancreas produceert hormonen die in de bloedbaan de glucosehuishouding regelen (insuline en glucagon) en spijsverteringssappen die helpen bij de vertering van voedsel.

Acute pancreatitis is de acute ontsteking van de alvleesklier, gekarakteriseerd door hevige acute pijn boven in de buik. Acute pancreatitis is een veel voorkomende, dure, potentieel dodelijke, en slecht begrepen ziekte. De voornaamste oorzaak (ca. 50%) van acute pancreatitis in de Westerse wereld is galsteenlijden, de tweede meest voorkomende oorzaak (ca. 20-25%) is alcoholmisbruik.[1] In 2006 bedroeg de sterftekans van acute pancreatitis 4,0%; 137 van 3413 patiënten opgenomen vanwege acute pancreatitis in dat jaar overleden (bron: Prismant, landelijke ziekenhuisregistratie). Ongeveer 20% van alle patiënten met acute pancreatitis ontwikkelen ernstige acute pancreatitis, een ziektebeeld gekarakteriseerd door orgaanfalen (falen van de functie van hart, longen of nieren) en/of pancreasnecrose (afgestorven pancreasweefsel).[2] Pancreasnecrose is in eerste instantie steriel maar kan na enkele weken secundair infecteren met darmbacteriën.[3] Infectie van pancreasnecrose is de meest ernstige complicatie van acute pancreatitis; zonder operatie zullen bijna alle patiënten met deze complicatie komen te overlijden. De sterftekans van ernstige acute pancreatitis bedraagt maar liefst 17%, terwijl de sterftekans van milde acute pancreatitis slechts 1% bedraagt.[4] Men schat in dat bij 80% van de overledenen de doodsoorzaak samenhangt met infectieus (zoals bloedvergiftiging, longontsteking, geïnfecteerde pancreasnecrose).

In de afgelopen 10 jaar is de incidentie van acute pancreatitis in Nederland gestegen met 50%.[5] In 2006 werden in een gemiddeld Nederlands ziekenhuis 35 patiënten met acute pancreatitis opgenomen. Een gemiddeld ziekenhuis ziet slechts 6-8 patiënten met ernstige acute pancreatitis per jaar waarvan 2-3 patiënten geïnfecteerde pancreasnecrose ontwikkelen. Vanwege dit lage aantal patiënten per ziekenhuis is het bijzonder moeilijk gebleken om goede, grote studies uit te voeren bij patiënten met ernstige acute pancreatitis. Om hier verandering in te brengen werd op initiatief van de afdeling Heelkunde van het UMC Utrecht in 2002 een landelijke studiegroep, de Acute Pancreatitis Werkgroep Nederland, opgericht. Deze studiegroep, momenteel bestaande uit meer dan 80 chirurgen, maag-darm-lever-artsen, radiologen, microbiologen, epidemiologen en arts-onderzoekers, ontwikkelt gezamenlijk studies. De meerderheid van de studies beschreven in dit proefschrift zijn uitgevoerd in 15 van de ziekenhuizen van de werkgroep, inclusief alle Nederlandse universitair medische centra.

Vanwege de hoge sterftekans geassocieerd met infecties bij acute pancreatitis zijn de voornaamste doelen in het onderzoek naar acute pancreatitis het voorkomen en behandelen van deze infecties, en vooral geïnfecteerde pancreasnecrose. Dit proefschrift beschrijft dan ook de ontwikkeling en het testen van de volgende strategieën:

A - Preventiestrategieën: bedoeld om acute pancreatitis en de hiermee samengaande infecties, vooral geïnfecteerde pancreasnecrose, te voorkomen.

B - Interventiestrategieën: bedoeld om de behandeling van geïnfecteerde pancreasnecrose te verbeteren

Deel A – Preventiestrategieën

De meest voorkomende vorm van acute pancreatitis is galsteenpancreatitis. Veel patiënten worden 'gewaarschuwd' voor galsteenpancreatitis door galsteenkolieken, hevige buikpijnaanvallen. Een cholecystectomie (galblaasverwijdering) kan dan voorkomen dat acute pancreatitis optreedt. In sommige ziekenhuizen is er een lange wachttijd voordat deze operatie kan plaatsvinden. Eerdere studies hadden gesuggereerd dat het medicijn ursodeoxycholzuur in staat was galsteenkolieken en bijbehorende complicaties, zoals acute pancreatitis te voorkomen.[6,7] In **hoofdstuk 2** beschreven wij een gerandomiseerde, dubbelblinde, placebogecontroleerde trial. In drie ziekenhuizen werden 177 patiënten met galsteenkolieken (mediaan 13 kolieken in het voorafgaande jaar) die op de wachtlijst stonden voor cholecystectomie gerandomiseerd tussen ursodeoxycholzuur en placebo. Het primaire doel van de studie was het voorkomen van galsteenkolieken in de wachttijd tot operatie. Na afloop van de studie bleek er een niet significante stijging te zijn van kolieken in de behandelde groep (74 *vs* 67%). Ook in de andere eindpunten was er geen verschil. Er werd geconcludeerd dat ursodeoxchycholzuur galsteenkolieken niet voorkomt. Patiënten met galsteenkolieken dienen op korte termijn een cholecystectomie te ondergaan.

Twee kleine gerandomiseerde trials, beiden uit Hongarije, suggereerden dat probiotica (levende bacteriën met en gezondheidsbevorderend effect, vooral melkzuurbacteriën (lactobacillen)), toegediend vanaf opname, in staat zouden zijn om infecties te voorkomen.[8,9] In **hoofdstuk 3** beschreven wij een gerandomiseerde, dubbelblinde, placebogecontroleerde trial waarin bestudeerd werd of het multispecies probioticum Ecologic 641 in staat was om infectieuze complicaties te voorkomen. In totaal werden 296 patiënten met voorspeld ernstige pancreatitis ('hoog-risico patiënten') in 15 ziekenhuizen in de studie ingesloten. Na afloop van de studie bleken infecties in gelijke mate voor te

komen in beide groepen (30% probiotica vs 28% placebo) terwijl de sterftekans 2,5 keer hoger was in de probiotica-groep (16% probiotica vs 6% placebo, relatieve risico 2,5; 95% betrouwbaarheidsinterval 1,2-5,3). Negen patiënten in de probioticagroep ontwikkelden darmischemie (zuurstoftekort in de darmen, acht patiënten overleden hieraan), terwijl darmischemie niet optrad in de placebogroep. Er werd geconcludeerd dat deze combinatie van probiotica niet gegeven moet worden aan patiënten met acute pancreatitis.

Het wordt verondersteld dat een verstoorde darmbarrièrefunctie mede verantwoordelijk is voor de translocatie van darmbacteriën naar pancreasnecrose en andere organen, maar het bewijs voor deze hypothese uit klinische studies ontbreekt. Het wordt tevens verondersteld dat probiotica in staat zijn om darmbarrièrefunctie in geval van ziekte te herstellen en hierdoor infectieuze complicaties kunnen voorkomen. Ook voor deze hypothese ontbreekt echter het bewijs. In **hoofdstuk 4** beschreven wij een studie die werd uitgevoerd tijdens de probioticastudie beschreven in hoofdstuk 3. In totaal 101 patiënten ondergingen een test voor darmpermeabiliteit (doorlaatbaarheid van de darm) en 141 patiënten ondergingen een test voor schade aan de darmmucosa (darmslijmvlies). Deze testen werden uitgevoerd in de eerste 72 uur van de opname voor acute pancreatitis, maar altijd 24-48 uur na de start van probiotica/placebo behandeling. De schade aan de darmmucosa was hoger in patiënten die uiteindelijk bacteriëmie (levende bacteriën in de bloedbaan), geïnfecteerde pancreasnecrose en orgaanfalen ontwikkelden. De darmdoorlaatbaarheid was verhoogd bij patiënten die uiteindelijk bacteriëmie of orgaanfalen ontwikkelden en/of overleden. Probiotica hadden geen gunstig effect op de darmpermeabiliteit, maar bleken geassocieerd met meer schade aan de darmmucosa. Het negatieve effect van probiotica bleek beperkt te zijn tot patiënten met orgaanfalen tijdens de opname, de ziekste patiënten. In patiënten zonder orgaanfalen tijdens de opname hadden probiotica geen invloed op de darmmucosa.

Algemeen wordt aangenomen dat infectieuze complicaties in acute pancreatitis pas na enkele weken optreden. Als een strategie infecties wil voorkomen dan moet deze strategie starten voordat de infecties optreden. In **hoofdstuk 5** bestudeerden wij de tijd van diagnose van infecties in 732 patiënten met acute pancreatitis. Infecties bleken mediaan gediagnosticeerd te worden op dag 8 na opname en bij 20% van de patiënten al in de eerste 2 dagen. Tachtig procent van 61 overleden patiënten had een infectie ontwikkeld tijdens opname. In de groep van 154 patiënten met pancreasnecrose was de kans op geïnfecteerde necrose hoger indien deze patiënten ook gediagnosticeerd waren met bacteriëmie (65 vs 38%, p=0,002). In de groep van 98 patiënten met geïnfecteerde

necrose was de sterftekans ruim tweemaal verhoogd indien deze patiënten ook gediagnosticeerd waren met bacteriëmie (40 vs 16%, p=0,014). Na statistische analyse bleek bacteriëmie de enige infectieuze complicatie te zijn (onafhankelijk van andere diagnoses) die de kans op sterfte voorspelde. Er werd geconcludeerd dat infecties zeer snel in het beloop van acute pancreatitis optreden en een sterk effect op de sterftekans hebben vooral in geval van bacteriëmie. De focus van preventiestrategieën moet verplaatst worden naar zeer vroege interventie.

Deel B – Interventiestrategieën

De sterftekans na chirurgische behandeling van geïnfecteerde pancreasnecrose is ca 30%.[4] Door de lage patiëntenaantallen per ziekenhuis zijn er wereldwijd alleen studies gepubliceerd uit enkele gespecialiseerde ziekenhuizen. In **hoofdstuk 6** evalueerden wij de uitkomst van de diverse chirurgische technieken voor geïnfecteerde pancreasnecrose. Wij analyseerden tevens hoe vaak patiënten werden verwezen naar de Nederlandse universitair medische centra voor een operatie. We includeerden 106 opeenvolgende patiënten die waren geopereerd in de jaren 2000-2003 in 11 ziekenhuizen waaronder alle Nederlandse universitair medische centra. Via Prismant werd het aantal patiënten met acute pancreatitis verkregen die tijdens de studie werden opgenomen in de 90 overige ziekenhuizen. De totale sterftekans bedroeg 34%. Voor de 'openbuikbehandeling' (buikoperatie waarna de buik niet meer gesloten wordt zodat om de buik frequent te kunnen spoelen) was deze 70% (16 van 23), voor 'laparotomie en continue postoperatieve lavage' (buikoperatie waarna de buik wel gesloten wordt en gespoeld wordt via achtergelaten drains) 25% (13 of 53), voor 'minimale invasieve technieken' 11% (twee uit 18) en voor 'laparotomie zonder lavage' 42% (vijf van 12) (mortaliteit verschil tussen de groepen: $p<0.001$). Tijdens de studie werden slechts 12% van de 361 patiënten met acute pancreatitis die een operatie nodig hadden verwezen naar de universitaire centra. Geconcludeerd werd dat 'laparotomie en continue postoperatieve lavage' de meeste gebruikte chirurgische behandeling van geïnfecteerde necrose in Nederland is. De uitkomst van de 'openbuikbehandeling' waren zeer teleurstellend terwijl de 'minimale invasieve technieken' veelbelovend zijn.

De Atlanta classificatie uit 1992 is de wereldwijde standaard voor de classificatie van de afwijkingen die tijdens acute pancreatitis in, rond en naast het pancreas op kunnen treden. Deze afwijkingen (acute vochtcollectie, abces, pseudocyste, pancreasnecrose) worden doorgaans gediagnosticeerd met een CT scan. De Atlanta classificatie is

vaak bekritiseerd maar de bruikbaarheid van de classificatie werd nog nooit formeel bestudeerd. In **hoofdstuk 7** analyseerden wij de overeenkomsten in diagnose tussen 5 radiologen die de Atlanta classificatie gebruikten om CT scans van 70 patiënten die geopereerd waren voor acute pancreatitis te beoordelen. Wij vonden een zeer matige interbeoordelaar-overeenkomst. Slechts in 3 van de 70 CT scans kozen de radiologen dezelfde diagnose (4%). Er werd geconcludeerd dat de Atlanta classificatie niet gebruikt moet worden om afwijkingen tijdens acute pancreatitis op CT te beschrijven.

Hoewel het gebruik van minimaal invasieve procedures toeneemt, is toepasbaarheid van deze technieken bij alle patiënten met geïnfecteerde necrose onbekend.[10] Alle minimaal invasieve technieken hebben gemeen dat het mogelijk moet zijn om een (percutane of transgastrische) drain in de collectie te plaatsen. In **hoofdstuk 8** analyseerden wij de mening van vijf radiologen met betrekking tot de mogelijkheid van het plaatsen van een percutane drain in de geïnfecteerde vochtcollecties in 80 CT scans van patiënten die waren geopereerd voor geïnfecteerde necrose. In 55 (69%) patiënten was de rand van de collectie gelegen binnen 5 cm van de buikwand. In 84% van de patiënten werd het mogelijk geacht om een drain in de collectie te plaatsen. In 56% van de patiënten werd het mogelijk geacht om deze drain via de linker flank te plaatsen. Er werd geconcludeerd dat in de meeste patiënten met geïnfecteerde necrose het gebruik van minimaal invasieve technieken, hetzij radiologisch, hetzij chirurgisch, mogelijk is.

In de laatste jaren wordt chirurgische interventie bij acute pancreatitis in toenemende mate uitgesteld totdat het moment dat de geïnfecteerde necrose is afgekapseld zodat de kans op bloeding tijdens operatie kleiner is.[11] De huidige internationale richtlijnen adviseren te opereren tussen dag 14 en 30 na opname.[12] In **hoofdstuk 9** analyseerden wij de relatie tussen timing van operatie en de sterftekans na operatie. In een retrospectieve serie van 53 patiënten geopereerd in het Universitair Medisch Centrum Utrecht gedurende 10 jaren (1995-2005) werd mediaan na 28 dagen geopereerd met een sterfte van 36%. In de tweede helft van de studie (2001-2005) bleek er later geopereerd te zijn (43 vs 20 dagen, p=0,06) en daalde de sterftekans (22 vs 47%, p=0,09). Dit verschil in sterfte bleek niet te berusten op een verschil in ernst van de aandoening. In een systematisch literatuuroverzicht van 11 studies (1136 patiënten) was de sterftekans mediaan 25%. Er bleek een significante correlatie te bestaan tussen timing van operatie en sterftekans. Er werd geconcludeerd dat, indien mogelijk, operatie bij geïnfecteerde necrose uitgesteld moet worden tot 30 dagen na de initiële ziekenhuisopname.

Het is onduidelijk of desobstructie van galstenen en/of galgruis uit de galwegen middels endoscopische retrograde cholangiopancreaticografie (ERCP) verricht moet worden in geval van galsteenpancreatitis als er geen tekenen zijn van cholangitis (geïnfecteerde gal). Galstenen passeren mogelijk spontaan en bovendien gaat ERCP gepaard met (lage) risico's op bloeding en darmperforatie. In **hoofdstuk 10** beschreven wij een meta-analyse van alle gerandomiseerde, gecontroleerde trial uitgevoerd in patiënten met galsteenpancreatitis zonder cholangitis. Drie van zeven studies (450 patiënten) voldeden aan de gestelde criteria. In patiënten met galsteenpancreatitis (zowel voorspeld milde als ernstige pancreatitis) zonder cholangitis bleek ERCP niet in staat om complicaties (risico ratio (RR) 0.76; 95% confidence interval 0.41–1.04; p=0,38), of sterfte (p=0,88) te voorkomen. Er werd geconcludeerd dat vroege ERCP in patiënten met biliaire pancreatitis zonder cholangitis niet leidt tot een betere uitkomst.

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Goed onderzoek leidt tot meer vragen dan het beantwoordt. Dit proefschrift is geen uitzondering op deze regel. Er is nog veel onderzoek te verrichten op het gebied van preventie- en interventiestrategieën in acute pancreatitis. Het onderzoek beschreven in dit proefschrift heeft een bijdrage geleverd aan de wereldwijde zorg voor patiënten met acute pancreatitis. Het zal de lezer van dit proefschrift niet verbazen dat de Acute Pancreatitis Werkgroep Nederland ook in de komende jaren zich zal blijven inzetten voor patiënten met acute pancreatitis door een combinatie van consultatie, centralisatie en gerandomiseerd multicentrisch onderzoek. Alleen door grootschalig, multidisciplinair onderzoek kan de behandeling van deze veel voorkomende, dure en potentieel dodelijke ziekte verbeterd worden.

Tabel 1 Studievragen en antwoorden op deze vragen.

Hfdst.	Studievragen en antwoorden
2.	*Kan Ursodeoxycholzuur galsteenkolieken en complicaties voorkomen tijdens de wachttijd voor cholecystectomie bij patiënten met symptomatisch galsteenlijden?* Ursodeoxycholzuur is niet in staat om galsteenkolieken te voorkomen tijdens de wachttijd voor cholecystectomie. Patiënten met galsteenkolieken dienen op korte termijn een cholecystectomie te ondergaan.
3.	*Kan een multispecies probioticum (Ecologic 641) bij patiënten met voorspeld ernstige acute pancreatitis infecties voorkómen?* Bij patiënten met voorspeld ernstige acute pancreatitis zijn de gebruikte probiotica niet in staat om infectieuze complicaties te voorkómen maar zijn wel geassocieerd met een hogere sterfte. Probioticaprofylaxe moet niet worden toegediend aan patiënten met voorspeld ernstige acute pancreatitis.
4.	*Wat is het verband tussen verstoorde darmbarrièrefunctie in acute pancreatitis, infecties en gebruik van probiotica?* Verstoorde darmbarrièrefunctie treedt vroeg op tijdens acute pancreatitis en is geassocieerd met bacteriëmie, geïnfecteerde necrose, orgaanfalen en sterfte. Probioticaprofylaxe leidt niet tot een verandering van darmdoorlaatbaarheid maar is wel geassocieerd met een toename van schade aan de darmmucosa in patiënten met orgaanfalen.
5.	*Wanneer worden infecties gediagnosticeerd tijdens acute pancreatitis en wat is de impact van deze infecties?* Infectieuze complicaties treden zeer vroeg op in het beloop van acute pancreatitis en hebben een sterke invloed op de sterftekans, vooral in het geval van bacteriëmie. De focus van preventiestrategieën moet verschoven worden naar zeer vroege interventie.
6.	*Wat zijn de resultaten van chirurgische behandeling van geïnfecteerde necrose in Nederland?* Laparotomie met continue postoperatieve lavage is de meeste gebruikte strategie voor geïnfecteerde necrose in Nederland. De resultaten voor 'openbuikbehandeling' zijn teleurstellend terwijl de resultaten van 'minimaal invasieve technieken' veelbelovend zijn.
7.	*Wat is de interbeoordelaar-overeenkomst voor de Atlanta classificatie (acute vochtcollectie, abces, pseudocyste, pancreasnecrose) in het beoordelen van collecties in, rond en naast het pancreas tijdens acute pancreatitis?* De interbeoordelaar-overeenkomst voor de Atlanta classificatie in het beoordelen van collecties bij acute pancreatitis is zeer matig. De Atlanta classificatie moet niet worden gebruikt bij het beschrijven van CT scans van patiënten met acute pancreatitis.
8.	*Wat is de haalbaarheid van minimaal invasieve technieken bij patiënten met geïnfecteerde necrotiserende pancreatitis?* De meeste peripancreatische collecties in patiënten met geïnfecteerde pancreasnecrose zijn bereikbaar voor minimaal invasieve benadering.
9.	*Wat is de impact van timing van chirurgische interventie bij patiënten met necrotiserende pancreatitis?* Bij patiënten met necrotiserende pancreatitis is uitstel van chirurgische interventie tot dag 30 na opname geassocieerd met lagere sterfte, verlengd gebruik van antibiotica, toename van *Candida* en antibiotica-resistente organismen.
10.	*Wat is de rol van endoscopische retrograde cholangiopancreaticografie (ERCP) bij patiënten met acute biliaire pancreatitis zonder cholangitis?* Bij patiënten met acute biliaire pancreatitis zonder cholangitis leidt het verrichten van een ERCP niet tot een lagere kans op complicaties of sterfte.

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Drs. TL Bollen, beste Thomas: 'expert-radiologist'. Via Bert van Ramshorst kwamen we in contact, nog diezelfde avond bezocht je de vergadering van de werkgroep. Inmiddels beoordeelde je al ruim tweeduizend CTs van patiënten met acute pancreatitis, schreven we meerdere artikelen samen en is je proefschrift flink op weg. Er is bijna geen artikel van de 'Dutch group' te vinden waar niet in de methods vermeld wordt dat een 'expert-radiologist' alle scans persoonlijk nogmaals beoordeelde. Zonder jouw inzet was de werkgroep nu niet waar zij is.

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Annie Bakker: secretaresse. Secretaresse? Secretaresse! Jij completeerde het team.

Hilda Rijnhart: nauwkeurig, betrokken datamanager. Onvermoeibaar verwerkte jij de hoge stapels CRF's in de database.

Studenten geneeskunde

In chronologische volgorde: Menno de Bruijn, Suzanne Flier, Rik Krebs, Lars van Bindsbergen, Annemarie de Vries, Ernst Schoenmaeckers ('de Ernst' koelt nog steeds het Datacentre!) en Mark van Baal, dank voor jullie inzet.

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Curriculum vitae

Marc GH Besselink was born on 19 April 1976 in Hengelo (Ov.), the Netherlands. He grew up on his parents' farm in the beautiful region of Twente. After graduating from the Pius X College in Almelo in 1994, prior to being admitted to medical school, he studied Bioprocestechnology at Wageningen University (1994-1996). In 1996 he started his medical training at Utrecht University. During this period he was a student-teacher at numerous courses for first to third year medical students, including epidemiology, emergency care and various anatomy courses. As a medical student he enjoyed facultative clinical electives at the Departments of Emergency Care and Orthopedics at Beaumont hospital Dublin, Ireland (Prof. P. Murray, 1999) and at the Adult Trauma Services, Foothills Medical Center, Calgary, Canada (Prof. J.B. Kortbeek, 2000). During his customary electives he worked at the Departments of Ophtalmology and Ear-Nose-Threat Surgery at Pretoria Academic Hospital, Pretoria, South-Africa. During medical school and thereafter, for seven years, he was an active rower in the 'club-eight' at the Utrecht Student Rowing Club 'Triton'. He was the treasurer of the 119th 'Varsity' Dutch national university rowing regatta.

After graduating from medical school in 2002, he joined the Department of Surgery, University Medical Center Utrecht (Prof.dr. H.G. Gooszen and Prof.dr. L.M.A. Akkermans) for the PhD project described in this thesis. From December 2002 - July 2005 and from July 2007 - January 2008 he was a full-time PhD student. He was (co-)applicant of successful grant applications to Senter, an agency of the Dutch Ministry of Economic Affairs (TSGE3109, PROPATRIA trial), The Netherlands Organization for Health Research and Development (ZonMw 945-06-910, PANTER trial) and Nutricia Netherlands (08/KR/AB/002, PYTHON trial). He successfully applied for an MD-medical research traineeship (AGIKO) for The Netherlands Organization for Health Research and Development (ZonMw 920-03-368). The probiotics trial, a major part of his PhD project, drew (inter-)national media attention as it was the first clinical study ever to report a significant negative effect of probiotics. This trial won the 'best paper award' at the Dutch Surgical Association 2008 meeting.

In July 2005 he started his residency in general surgery in the St. Antonius Hospital Nieuwegein (Dr. P.M.N.Y.H. Go). In January 2010 he is scheduled to return to the University Medical Center Utrecht (Prof.dr. I.H.M. Borel Rinkes) for the final two years of surgical residency. Marc Besselink is the (co-)author of over 45 peer-review articles, invited reviews and book chapters. He lives near Utrecht in the small town of The Bilt with his girlfriend Carlien Bennebroek.

List of relevant publications

With short abstracts of the five studies most related to the research described in this thesis.

1. Petrov MS, van Santvoort HC, Besselink MG, Windsor, van der Heijden GJ, Gooszen HG. Enteral nutrition reduced the risk of mortality and infectious complications in patients with severe acute pancreatitis. A meta-analysis of randomized trials. *Arch Surg* 2008; *in press.*

   A meta-analysis of five randomised trials, including 202 patients with (predicted) severe acute pancreatitis, that compared enteral with parenteral nutrition. Enteral nutrition reduced the risk of infectious complications (relative risk [RR], 0.47; 95% confidence interval [CI], 0.28-0.77; p<0.001), pancreatic infections (RR, 0.48; 95% CI, 0.26-0.91; p=0.02) and mortality (RR, 0.32; 95% CI, 0.11-0.98; p=0.03).

2. de Vries AC, Besselink MG, Buskens E, Ridwan BU, Schipper M, van Erpecum KJ, Gooszen HG. Randomized controlled trials of antibiotic prophylaxis in severe acute pancreatitis: relationship between methodological quality and outcome. *Pancreatology* 2007; **7**: 531-8.

   A meta-analysis of six randomised trials, including 397 patients with (predicted) severe pancreatitis, that compared antibiotic prophylaxis with no treatment/ placebo. The methodological quality of the studies was scored. Systemic antibiotic prophylaxis had no significant effect on infection of pancreatic necrosis (absolute risk reduction (ARR) 0.06; 95% CI –0.08 to 0.19) and mortality (ARR 0.06, 95% CI –0.02 to 0.13). Spearman correlation showed an inverse association between methodological quality and ARR for mortality (correlation coefficient –0.84, p=0.04); the better the study, the less effect of antibiotic prophylaxis was found.

3. van Santvoort HC, Besselink MG, Bollen TL, Buskens E, van Ramshorst B, Gooszen HG for the Dutch Acute Pancreatitis Study Group. Case-matched comparison of the retroperitoneal approach with laparotomy for necrotizing pancreatitis. *World J Surg* 2007; **31**: 1635-42.

Case-matched study, 15 patients undergoing a retroperitoneal approach for infected necrosis, out of 841 consecutive patients with acute pancreatitis, were matched with 15 of 46 patients undergoing laparotomy. In addition to all matched preoperative characteristics, there were no significant differences in any other preoperative characteristic. Postoperative new-onset multi-organ failure occurred in ten patients in the laparotomy group versus two patients in the retroperitoneal group (p=0.008). Six patients died in the laparotomy group versus one patient in the retroperitoneal group (p=0.08). It was concluded that there may be a benefit of the retroperitoneal approach over laparotomy but that a randomised trial is still warranted.

4. Venneman NG, Buskens E, Besselink MG, Stads S, Go PM, Bosscha K, vanBerge-Henegouwen GP, van Erpecum KJ. Small gallstones are associated with increased risk of acute pancreatitis: potential benefits of prophylactic cholecystectomy? *Am J Gastroenterol* 2005; **100**: 2540-50.

   A retrospective studie of 528 patients with gallstone disease, including 115 patients with biliary pancreatitis. Stone characteristics were determined in all patients by measuring sizes and numbers of stones in patients by ultrasonography and endoscopic retrograde cholangiopancreatography. Multivariate analysis identified old age and small stones as independent risk factors for biliary pancreatitis.

5. Besselink MG, Timmerman HT, van Minnen LP, Akkermans LM, Gooszen HG. Prevention of bacterial translocation and infectious complications in surgical patients: potential role of probiotics. *Dig Surg* 2005; **22**: 234-44.

   Review summarising (a) the pathophysiological processes involved with bacterial translocation, (b) the potential impact of probiotic prophylaxis on these processes and (c) the randomised trials using probiotic prophylaxis in surgical patients.

6. Van Santvoort HC, Bollen TL, Besselink MG, Banks PA, Boermeester MA, Van Eijck CH, Evans J, Freeny PC, Grenacher L, Hermans JJ, Horvath KD, Hough DM, Laméris JS, Van Leeuwen MS, Mortele KJ, Neoptolemos JP, Sarr MG, Vege SS, Werner J, Gooszen HG. Describing peripancreatic collections in severe acute pancreatitis using morphologic terms: an international interobserver agreement study. *Pancreatology* 2008; *in press.*

7. van Santvoort HC, Besselink MG, Gooszen HG for the Dutch Acute Pancreatitis Study Group. Obtaining medical ethical approval for a multicentre trial in the Netherlands: prospective evaluation. *Ned Tijdschr Geneesk* 2008; *in press*

8. Petrov MS, Besselink MG, van Santvoort HC, Gooszen HG. Acute biliary pancreatitis without cholangitis: the growing role of EUS. *Ann Surg* 2008; Epub.

9. Schrover IM, Weusten BL, Besselink MG, Bollen TL, van Ramshorst B, Timmer R. EUS-guided endoscopic transgastric necrosectomy in patients with infected necrosis in acute pancreatitis. *Pancreatology* 2008; **8**: 271-6.

10. Besselink MG, van Santvoort HC, Buskens E, Boermeester MA, van Goor H, Timmerman HM, Nieuwenhuijs VB, Bollen TL, van Ramshorst B, Witteman BJ, Rosman C, Ploeg RJ, Brink MA, Schaapherder AF, Dejong CH, Wahab PJ, van Laarhoven CJ, van der Harst E, van Eijck CH, Cuesta MA, Akkermans LM, Gooszen HG for the Dutch Acute Pancreatitis Study Group. Probiotic prophylaxis in patients with predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. *Ned Tijdschr Geneeskd* 2008*;* **152***:* 685-96.

11. Besselink MG, van Santvoort HC, Buskens E, Boermeester MA, van Goor H, Timmerman HM, Nieuwenhuijs VB, Bollen TL, van Ramshorst B, Witteman BJ, Rosman C, Ploeg RJ, Brink MA, Schaapherder AF, Dejong CH, Wahab PJ, van Laarhoven CJ, der Harst E, van Eijck CH, Cuesta MA, Akkermans LM, Gooszen HG; for the Dutch Acute Pancreatitis Study Group. Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. *Lancet* 2008; **371**: 651-9.

12. Besselink MG**,** van Santvoort HC, Bollen TL, Boermeester MA, Dejong CH, Gooszen HG. Management of patients with severe acute pancreatitis in the new millenium: prophylaxis, nutrition, imaging and intervention. *Net J Crit Care* 2008; **12**: 14-19.

13. Schiphorst AH, Besselink MG, Boerma D, Timmer R, Wiezer MJ, van Erpecum KJ, Broeders IA, van Ramshorst B. Timing of cholecystectomy after endoscopic sphincterotomy for common bile-duct stones. *Surg Endosc* 2008; Epub.

14. Petrov MS, van Santvoort HC, Besselink MG, van der Heijden GJ, van Erpecum KJ, Gooszen HG. Early endoscopic retrograde cholangiopancreatography *vs* conservative management in acute biliary pancreatitis without cholangitis: a meta-analysis of randomized trials. *Ann Surg* 2008; **247**: 250-57.

15. Bollen TL, van Santvoort HC, Besselink MG, van Leeuwen MS, Horvath KD, Freeny PC, Gooszen HG. The Atlanta classification on acute pancreatitis revisited: review of the literature. *Br J Surg* 2008; **95:** 6-21.

16. van Santvoort HC, Besselink MG, Timmerman HM, van Minnen LP, Akkermans LM, Gooszen HG. Probiotics in surgery. *Surgery 2008*; **143:** 1-7

17. Ridwan BU, Koning CJ, Besselink MG, Timmerman HM, Brouwer EC, Verhoef J, Gooszen HG, Akkermans LM. Antimicrobial activity of a multispecies probiotic (Ecologic 641) against pathogens isolated from infected pancreatic necrosis. *Lett Appl Microbiol* 2008; **46:** 61-7.

18. Besselink MG, Verwer TJ, Schoenmaeckers EJ, Buskens E, Ridwan BU, Visser MR, Nieuwenhuijs VB and Gooszen HG. Timing of surgical intervention in necrotizing pancreatitis. *Arch Surg* 2007; **142:** 1194-1201.

19. Bollen TL, Besselink MG, van Santvoort HC, Gooszen HG, van Leeuwen MS. Towards an update of the Atlanta classification on acute pancreatitis: review of new and abandoned terms. *Pancreas* 2007; **35**: 107-13.

20. Hietbrink F, Besselink MG, Leenen LP. Pitfalls in gastrointestinal permeability measurement in ICU patients. *Intensive Care Med* 2007; **33**: 2216.

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22. Besselink MG, van Santvoort HC, Carter R, Charnley R, Mole DJ, Gooszen HG. Designing an optimal study for the management of infected pancreatic necrosis. In: Imrie C, Johnson C, editors. Pancreatic Disease: The Challenges. 1st ed. New York, NY: Springer publishing; 2007

23. Bollen TL, van Santvoort HC, Besselink MG, van Ramshorst B, van Es HW, Gooszen HG for the Dutch Acute Pancreatitis Study Group. Intense adrenal enhancement in patients with acute pancreatitis and early organ failure. *Emerg Radiol* 2007; **14**: 317-22

24. Petrov MS, van Santvoort HC, Besselink MG, Cirkel GA, Brink MA, Gooszen HG. Oral Refeeding After Onset of Acute Pancreatitis: A Review of Literature. *Am J Gastroenterol*. 2007; **102**: 2079-84.

25. Besselink MG, Bollen TL, Scheffer RC, Weusten BL, Timmer R, Wiezer RM, van Ramshorst B. Rupture of infected peripancreatic necrosis to the peritoneal cavity with fatal outcome. *Pancreas* 2007; **34**: 477-9.

26. Besselink MG, van Santvoort HC, Schaapherder AF, van Ramshorst B, van Goor H, Gooszen HG for the Dutch Acute Pancreatitis Study Group. Feasibility of minimally invasive approaches in patients with infected necrotizing pancreatitis. *Br J Surg* 2007; **94**: 604-08.

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28. Schiphorst AH, Besselink MGH, Venneman NG, Go PM. Symptomatic gallstone disease: an indication for surgery. *Ned Tijdschr Geneeskd* 2006; **150**: 2405-09.

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