Acute pancreatitis; persisting issues from the PROPATRIA and PANTER studies

Baal, M.C.P.M. van

2014, Dissertation

Acute Pancreatitis

Persisting Issues from the PROPATRIA and PANTER Studies

Mark C.P.M. van Baal

Aucte Pancreatitis; Persisting Issues from the PROPATRIA and PANTER Studies
Mark C.P.M. van Baal
Thesis, Radboudumc, the Netherlands

ISBN: 978-90-9028619-8

Printed by: Proefschrift-AIO, Bilthoven
Cover design: Erik Manning, Vriezeveen
Lay-out: Proefschrift-AIO, Bilthoven

Mark C.P.M. van Baal was financially supported by a grant from the Dutch Digestive Foundation (grant number WO 80-08).
This thesis was financially supported by Radboudumc, Elisabeth-Tweesteden Ziekenhuis Tilburg, Winclove Probiotics B.V., Tramedico B.V., Abbott B.V., Olympus Nederland B.V., Nutricia Advanced Medical Nutrition, Covidien B.V., Takeda Nederland B.V.

Acute Pancreatitis
Persisting Issues from the PROPATRIA and PANTER Studies

Acute Pancreatitis
Overgebleven Vragen na de PROPATRIA and PANTER Studies

(*Met een samenvatting in het Nederlands*)

Proefschrift
ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen
op gezag van de rector magnificus prof. dr. Th.L.M. Engelen,
volgens besluit van het college van decanen
in het openbaar te verdedigen op donderdag 14 december 2014
om 12.30 uur precies

door
Mark Christiaan Paulus Marie van Baal
geboren op 10 april 1984
te Haarlemmermeer

Promotoren: Prof. dr. H.G. Gooszen
Prof. dr. ir. G.T. Rijkers (UU)

Copromotor: Dr. M.G. Besselink (AMC)

Review Committee:

Prof. dr. J.P.H. Drenth

Prof. dr. M. Ritskes-Hoitinga

Prof. dr. I.H.M. Borel Rinkes (UU)

Aan mijn ouders
Voor Rowena en Tijn

Contents

Chapter 1

General Introduction

Anatomy and function

The pancreas is a small secretory gland, about 15 centimeters in length, in the retroperitoneal cavity of the abdomen, weighing 60-150 grams. It is commonly divided in a head, body and tail region. The pancreas has two major functions: exocrine enzyme secretion and endocrine hormone secretion. Exocrine pancreatic tissue consists of lobules composed of small acinar cells that drain in the main pancreatic duct. This main duct usually empties in the common bile duct, just proximal to the ampulla of Vater and the duodenum. The most important pancreatic exocrine enzymes are trypsinogen, amylase and lipase, all necessary for an adequate food digestion. Pancreatic endocrine hormones are insulin, glucagon and somatostatin, and all of these are released into the circulation in order to facilitate in the glucose and energy metabolism.

Disease of the pancreas

The most common disorders of the pancreas are inflammatory diseases, such as acute and chronic pancreatitis, and pancreatic malignancies. Chronic pancreatitis is a progressive disorder, leading to an irreversible destruction of the pancreas. Main symptoms are pain and/or endocrine and exocrine pancreas insufficiency. This thesis, however, focus on acute pancreatitis.

Acute pancreatitis

Acute pancreatitis is the acute inflammation of the pancreas which may or may not be accompanied with a systemic inflammatory response syndrome (SIRS). The exact pathophysiology of acute pancreatitis is still unknown, however, it is generally accepted that in acute pancreatitis premature activation of trypsin occurs in the pancreas, leading to activation of digestive enzymes and autodigestion of the pancreas. Sometimes the surrounding tissue is involved as well.[1-4] In Western countries, the main causes of acute pancreatitis are gallstones (~55%) and alcohol (~20%).[5,6] In biliary pancreatitis, gallstones or gall sludge (temporarily) occlude the common bile duct proximal of the main pancreatic duct, resulting in stasis of pancreatic secretions and ultimately acute pancreatitis. Acute pancreatitis can also have several other, more rare, causes, like medication and hereditary factors. However, the exact pathophysiological mechanism of acute pancreatitis is yet unknown.

The majority (~80%) of patients with an attack of acute pancreatitis experience the mild form of the disease.[7] Clinical signs of acute pancreatitis are severe (upper) abdominal pain, usually accompanied with nausea and vomiting.[8] Biochemically, serum amylase and lipase are increased. Approximately a fifth of patients develop severe acute pancreatitis, which is associated with pancreatic and peripancreatic necrosis, persistent (multiple) organ failure and increased morbidity and mortality.[8-10] In general, the clinical course of severe acute pancreatitis is biphasic. In the early phase (i.e., first two weeks after onset of symptoms), the patient develops in varying amounts

pancreatic and/or peripancreatic necrosis (necrotizing pancreatitis) and SIRS, which may progress towards (multiple) organ failure and ultimately death. This early phase is immune-mediated, although bacterial infections may play a role.[11,12] In the second phase of acute pancreatitis (i.e., more than two weeks after onset of symptoms) the systemic inflammation declines and the influence of infectious complications become more prominent. In approximately a third of patients clinical signs of infection of the (peri-)pancreatic collections become manifest, usually 3-4 weeks after onset of symptoms. Patients with infected necrotizing pancreatitis usually need some sort of intervention to remove the infected necrosis, as without intervention mortality is almost 100%.[9]

Bacterial translocation

Bacterial translocation from the small bowel is considered to be the main route of infection of (peri-)pancreatic necrosis.[13-15] Three major pathophysiological steps are thought to precede bacterial translocation: 1) bacterial overgrowth of the small bowel, 2) dysfunction of the local and systemic immune system, resulting in mucosal barrier dysfunction, and 3) increased intestinal permeability with ultimately bacterial translocation. Since infectious complications (e.g., infected necrosis) are associated with an increased mortality and morbidity, any treatment regimen capable of lowering the infection rate in acute pancreatitis could potentially reduce both morbidity and mortality. Prophylactic administration of antibiotics and probiotics both have been tried to lower the rate of infection, assuming that administration very early in the course of the disease would effectively remove the early circulating bacteria from the bloodstream. Several meta-analyses have shown that prophylactic administration of antibiotics did not result in lower morbidity and mortality.[16.17] In 2002, Olah *et al* suggested that probiotics could interfere with the intestinal flora, thus preventing bacterial translocation and ultimately lower the number of infections in patients with acute necrotizing pancreatitis.[18] Since then, several clinical and experimental studies have been performed on the effect of probiotic prophylaxis in necrotizing pancreatitis.[19-24]

PROPATRIA

The Dutch Pancreatitis Study Group launched the PROPATRIA trial in 2004.[25] This multicentre, double-blinded, placebo-controlled trial randomized patients with predicted severe pancreatitis between placebo and probiotic prophylaxis for 28 days, with the first gift administered within the first 72 hours after hospital admission. All patients received enteral nutrition through a nasojejunal feeding tube. Primary endpoint of the study was the total number of infectious complications; secondary endpoints were mortality, hospital stay and adverse events. In 2008, totally unexpected, the results of the PROPATRIA trial showed a higher mortality in

patients with probiotic prophylaxis as compared to the placebo group (16% vs. 6%, p=0.01) although the overall mortality was not higher than expected (11%). Another unexpected finding was non-occlusive mesenteric ischemia (NOMI), observed in 9 out of 152 patients (6%) with probiotic prophylaxis vs. 0/144 patients (0%) with placebo treatment (p=0.004).[19] Since then, no new studies on probiotic prophylaxis in patients with acute pancreatitis have been performed and many questions have been left unanswered, once all data had been analyzed with scrutiny.

PANTER

In the last decade, several (minimally invasive) intervention strategies are implemented in daily care as substitution or addition to conventional open surgical necrosectomy.[26-30] Unfortunately, even after intervention, mortality in patients with infected necrotizing pancreatitis remains between 10-25% and morbidity is even higher (50-100%).[31-35] The second multicentre trial conducted by the Dutch Pancreatitis Study Group was the PANTER trial.[29] Patients with suspected infection of necrosis were randomized between open surgical necrosectomy and the minimally invasive step-up approach, consisting of primarily percutaneous catheter drainage (PCD) of (peri-) pancreatic fluid collections, if unsuccessful followed by a video-assisted retroperitoneal debridement (VARD). Furthermore, intervention was postponed for preferably 4 weeks, allowing encapsulation of the necrotic cavity to facilitate necrosectomy, assumed to lead to a reduction of per-operative complications such as bleeding and intestinal perforation. The PANTER trial showed that the minimally invasive step-up approach is superior to the open surgical necrosectomy in terms of lowering the combined endpoint of major complications or death. Furthermore, it was shown that almost a third of patients recovered with only PCD, making surgical necrosectomy unnecessary and that costs were considerably lower for the step-up approach.[29]

Thesis outline

Both these randomized controlled trials left crucial questions unanswered. This was felt more strongly for the PROPATRIA trial than for the PANTER trial. A satisfactory explanation for the excess death of 15 patients in the group that received probiotic prophylaxis had not been provided and the following questions were remaining:

1. Is unfavourable interaction between probiotics and enteral nutrition in acute pancreatitis the driving force behind the increased mortality? This question was subdivided into three questions to be addressed in further studies that were designed after the PROPATRIA trial was analyzed in depth.
   a. Under which intraluminal conditions does a mixture of six different probiotic strains show the highest metabolic activity in terms of lactate and short-chain fatty acid production?

   b. Does the administration of probiotics and enteral nutrition influence infectious complications and outcome in a rat model for severe acute pancreatitis?
   c. What is the clinical outcome of patients without early organ failure treated with probiotics and enteral nutrition?
2. Could the increased mortality in the probiotic group be explained by an incorrect statistical interpretation of the data? The PROPATRIA study was powered for showing a meaningful difference in terms of infectious complications and the study was underpowered for mortality, which was not a primary endpoint.

These questions are addressed in the first part of this thesis. In the second part, the follow-up topics arising from the PANTER trial were studied:

1. How to accurately diagnose infection and how to design further interventional strategies in acute pancreatitis?
2. What is the role of routine fine-needle aspiration (FNA) in the diagnostic work-up in patients with suspected infection in necrotizing pancreatitis?
3. What is the role of percutaneous drainage in patients with suspected or documented infected pancreatic necrosis?
4. What is the role of transpapillary stenting as opposed to conservative management in patients with fistula after necrotizing pancreatitis?
5. What is the optimal timing of cholecystectomy after mild acute pancreatitis?

Interaction between probiotics and enteral nutrition in acute pancreatitis

Since the unexpected results of the PROPATRIA trial in 2007, no clinical research on probiotic treatment in patients with acute pancreatitis has been published. While probiotics were long thought to be harmless, the PROPATRIA trial has strongly suggested that probiotics could be dangerous under certain conditions in severely ill patients.[19] In PROPATRIA, eight out of nine patients with non-occlusive mesenteric ischemia suffered from early (multiple) organ failure (i.e. within the first three days of hospital admission). These patients were enterally fed with fibre-rich enteral nutrition through a naso-jejunal feeding tube. Up to now, a causal relationship between administration of probiotics and non-occlusive bowel ischemia has not been demonstrated. Although it is suggested that the interaction of probiotics and enteral nutrition and the presence of (multiple) organ failure have played a major role in this mechanism, convincing evidence is lacking.

Chapter 2 describes the results of an *in vitro* computer-controlled gastrointestinal model in which the interaction between probiotics and enteral nutrition is investigated under several different physiological conditions. Although we do know that the metabolic activity of certain probiotic strains, especially lactic acid bacteria, is reflected by the production of lactic acid,

the exact influence of the presence of bile salts, pancreatic enzymes and composition of enteral nutrition in the bowel lumen on probiotic activity remains unclear. This study provides clues and new insights in the behavior of the probiotic strains in the bowel lumen.

Prior to the PROPATRIA trial, experimental studies have been performed studying the effect of probiotic prophylaxis in acute pancreatitis. Van Minnen *et al* described the use of a specific probiotic mixture in an experimental rat model for severe acute pancreatitis.[36] This experimental study showed that mortality was lower in the animals pretreated with probiotics and that bacterial translocation to the pancreas occurred more frequently in animals treated with placebo. A major difference with the PROPATRIA trial was that all animals had free access to water and food, while most patients received enteral nutrition directly into the jejunum. As it is suggested that the administration of enteral nutrition and the interaction with the administered probiotics played a significant role in the occurrence of NOMI in PROPATRIA, **Chapter 3** describes an experimental rat model of severe acute pancreatitis in which clinical outcome and bacterial translocation were analyzed in rats treated with enteral nutrition and probiotics.

Several studies investigating the role of probiotics in critically ill patients were preliminary terminated because of the dramatically results of the PROPATRIA trial.[20,21] Since 2008, only experimental studies to probiotic treatment in acute pancreatitis have been performed.[22-24] **Chapter 4** describes the first study since the PROPATRIA trial investigating the effect of probiotic treatment and enteral nutrition in 99 patients with predicted severe pancreatitis. In these patients, clinical outcome was assessed in terms of infectious complications, bowel ischemia and mortality.

Diagnosis of infection and interventional strategies in acute pancreatitis

For long, suspicion or proof of infected necrosis in patients with necrotizing pancreatitis was a key indication for surgical (emergency) intervention.[33,37,38] In the last decade, minimally invasive strategies have gained popularity in the interventional treatment in these critically ill patients. The newly introduced techniques decreased morbidity and mortality significantly, however, the rate of infection in the different series described vary considerably.[31-35] Since sterile necrosis is associated with a low mortality and generally does not require surgical or radiological intervention, establishment of the diagnosis of infected necrosis seems crucial. In the PANTER trial, patients with (suspected) infected necrosis were randomized between a minimally invasive step-up approach and the conventional open necrosectomy.[29] In this trial, the infection rate was high without routine use of FNA for confirmation of infection and the clinical condition was dominant in deciding on the timing of intervention. The discussion on when and how to use FNA in case of suspected infection

is still awaiting a satisfactory answer. **Chapter 5** describes the value of FNA in diagnosing infected necrosis in 208 patients who underwent an intervention due to suspected infected necrosis, as compared to the presence of gas bubbles in necrotic fluid collections and clinical signs only.

After the difficulty of correctly diagnosing infected necrosis and postponing intervention as long as possible, the next obstacle is to choose the optimal intervention strategy. Although primary open surgical necrosectomy was standard practice of care for decades, the PANTER trial showed that a third of patients could be treated with percutaneous catheter drainage only, without the need for surgical necrosectomy.[29] Patients who did not improve after PCD underwent a VARD procedure. The PANTER trial was the first randomized trial showing that PCD could act as definitive treatment for infected necrosis and obviates surgical necrosectomy in over 30% of patients. Having found that, we performed a systematic review on the use of PCD in patients with (suspected) infected necrotizing pancreatitis, described in **Chapter 6**, including the results of the PANTER trial.

A well-known complication of pancreatic surgery in patients with necrotizing pancreatitis is a long lasting pancreatico-cutaneous fistula, with a reported incidence of 17-76%.[39-42] Most of these fistulas persist after removal of a percutaneous drain left after necrosectomy or PCD. In the PANTER trial, this complication occurred in 29/88 of the randomized patients (33%), without difference in incidence between patients with open surgical necrosectomy and the minimally invasive step-up approach.[29] Pancreatic fistulas are associated with considerably morbidity, consisting of metabolic and nutritional disturbances, they prolong hospitalization and frequently mandate surgical re-interventions.[40] Conservative treatment is preferred but spontaneous resolution could take as long as weeks to months. Endoscopic transpapillary stenting (ETS) is proposed to be a proper alternative to conservative treatment in patients with pancreatico-cutaneous fistulas.[43-45] **Chapter 7** described 35 patients identified from the PROPATRIA and PANTER studies with a persisting fistula who were managed conservatively or with ETS and showed that ETS could be a safe and feasible alternative to conservative treatment.

In 2011, our group reported on the timing of cholecystectomy in patients with mild biliary pancreatitis.[46] Only 142/267 patients (53%) underwent cholecystectomy within the 3 weeks after index admission, as recommended in the Dutch guidelines. Moreover, of all 249 patients who were discharged after index admission for delayed cholecystectomy, 14% of patients (n=34) suffered from recurrent biliary events (10%, n=24, biliary pancreatitis) in the interval prior to cholecystectomy. Current international guidelines for acute pancreatitis recommend early cholecystectomy after mild biliary pancreatitis, however, consensus on the definition of “early” is lacking.[5,8,9,47] **Chapter 8** shows the results of a systematic review on the optimal timing of cholecystectomy in patients with mild biliary pancreatitis.

References

1. Whitcomb DC. Acute pancreatitis: molecular biology update. J Gastrointest Surg 2003; 7:940-942.
2. Mayerle J, Weiss FU, Halangk W, et al. Molecular biochemical, and metabolic abnormalities of acute pancreatitis. In: Beger H, Warshaw AL, Buchler MW, eds. The Pancreas: an integrated textbook of basic science, medicine and surgery. 2nd ed. Massachusetts, USA, Blackwell Publishing 2008; 214-225.
3. Pandol SJ, Saluja AK, Imrie CW, et al. Acute pancreatitis: bench tot bedside. Gastroenterology 2007; 132:1127-1151.
4. Bakker OJ, van Santvoort H, Besselink MG, et al. Extrapancreatic necrosis without pancreatic parenchymal necrosis: a separate entity in necrotising pancreatitis? Gut 2012, Epub ahead of print.
5. UK guidelines for the management of acute pancreatitis. Gut 2005; 54 Suppl 3:iii1-9.
6. Gullo L, Migliori M, Oláh A, et al. Acute pancreatitis in five European countries: etiology and mortality. Pancreas 2002; 24:223-227.
7. Banks PA, Freeman ML; Practice Parameters Committee of the American College of Gastroenterology. Practice guidelines in acute pancreatitis. Am J Gastroenterol 2006; 101: 2379-2400.
8. Forsmark CE, Baillie J. AGA institute technical review on acute pancreatitis. Gastroenterology 2007; 132:2022-2044.
9. Banks PA, Freeman ML. Practice guidelines in acute pancreatitis. Am J Gastroenterol 2006; 101:2379-2400.
10. Banks PA, Bollen TL, Dervenis C, et al. Classification of acute pancreatitis--2012: revision of the Atlanta classification and definitions by international consensus. Gut 2013; 62:102-111.
11. Mayer J, Rau B, Gansauge F, et al. Inflammatory mediators in human acute pancreatitis: clinical and pathophysiological implications. Gut 2000; 47:546-552.
12. Besselink MG, van Santvoort HC, Boermeester MA, et al. Timing and impact of infections in acute pancreatitis. Br J Surg 2009; 96:267-273.
13. Ammori BJ, Leeder PC, King RF, et al. Early increase in intestinal permeability in patients with severe acute pancreatitis: correlation with endotoxemia, organ failure, and mortality. J Gastrointest Surg 1999; 3:252-262.
14. Deitch EA. The role of intestinal barrier failure and bacterial translocation in the development of systemic infection and multiple organ failure. Arch Surg 1990; 125:403-404.
15. Van Leeuwen PA, Boermeester MA, Houdijk AP, et al. Clinical significance of translocation. Gut 1994; 35:S28-S34.
16. de Vries AC, Besselink MG, Buskens E, et al. Randomized controlled trials of antibiotic prophylaxis in severe acute pancreatitis: relationship between methodological quality and outcome. Pancreatology 2007; 7:531-538.
17. Wittau M, Mayer B, Scheele J, et al. Systematic review and meta-analysis of antibiotic prophylaxis in severe acute pancreatitis. Scand J Gastroenterol 2011; 46:261-270.

18. Olah A, Belagyi T, Issekutz A, et al. Randomized clinical trial of specific lactobacillus and fibre supplement to early enteral nutrition in patients with acute pancreatitis. Br J Surg 2002; 89:1103-1107.
19. Besselink MG, van Santvoort HC, Buskens E, et al. Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. Lancet 2008; 371:651-659.
20. Sharma B, Srivastava S, Singh N, et al. Role of probiotics on gut permeability and endotoxemia in patients with acute pancreatitis: a double-blind randomized controlled trial. J Clin Gastroenterol 2011; 45:442-448.
21. Lata J, Stiburek O. Prophylactic antibiotics and probiotics in acute pancreatitis. Vnitr Lek 2010; 56:582-584.
22. Lutgendorff F, Nijmeijer RM, Sandström PA, et al. Probiotics prevent intestinal barrier dysfunction in acute pancreatitis in rats via induction of ileal mucosal glutathione biosynthesis. PLoS One 2009; 4:e4512.
23. Rychter JW, van Minnen LP, Verheem A, et al. Pretreatment but not treatment with probiotics abolishes mouse intestinal barrier dysfunction in acute pancreatitis. Surgery 2009; 145:157-167.
24. Lutgendorff F, Trulsson LM, van Minnen LP, et al. Probiotics enhance pancreatic glutathione biosynthesis and reduce oxidative stress in experimental acute pancreatitis. Am J Physiol Gastrointest Liver Physiol 2008; 295:G1111-1121.
25. Besselink MG, Timmerman HM, Buskens E, et al. Probiotic prophylaxis in patients with predicted severe acute pancreatitis (PROPATRIA): design and rationale of a double-blind, placebo-controlled randomised multicenter trial [ISRCTN38327949]. BMC Surg 2004; 4:12.
26. Freeny PC, Hauptmann E, Althaus SJ, et al. Percutaneous CT-guided catheter drainage of infected acute necrotizing pancreatitis: techniques and results. AJR Am J Roentgenol 1998; 170:969-975.
27. Carter CR, McKay CJ, Imrie CW. Percutaneous necrosectomy and sinus tract endoscopy in the management of infected pancreatic necrosis: an initial experience. Ann Surg 2000; 232:175-180.
28. Horvath KD, Kao LS, Ali A, et al. Laparoscopic assisted percutaneous drainage of infected pancreatic necrosis. Surg Endosc 2001;15:677-682.
29. van Santvoort HC, Besselink MG, Bakker OJ, et al. A step-up approach or open necrosectomy for necrotizing pancreatitis. N Engl J Med 2010; 362:1491-1502.
30. Seifert H, Biermer M, Schmitt W, et al. Transluminal endoscopic necrosectomy after acute pancreatitis: a multicentre study with long-term follow-up (the GEPARD Study). Gut 2009; 58:1260-1266.
31. van Santvoort HC, Bakker OJ, Bollen TL, et al. A conservative and minimally invasive approach to necrotizing pancreatitis improves outcome. Gastroenterology 2011; 141:1254-1263.
32. Rau B, Bothe A, Beger HG. Surgical treatment of necrotizing pancreatitis by necrosectomy and closed lavage: changing patient characteristics and outcome in a 19-year, single-center series. Surgery 2005; 138:28-39.

33. Rodriguez JR, Razo AO, Targarona J, et al. Debridement and closed packing for sterile or infected necrotizing pancreatitis: insights into indications and outcomes in 167 patients. Ann Surg 2008; 247:294-299.
34. Bakker OJ, van Santvoort HC, van Brunschot S, et al. Endoscopic transgastric vs surgical necrosectomy for infected necrotizing pancreatitis: a randomized trial. JAMA 2012; 307:1053-1061.
35. van Baal MC, van Santvoort HC, Bollen TL, et al. Systematic review of percutaneous catheter drainage as primary treatment for necrotizing pancreatitis. Br J Surg 2011; 98:18-27.
36. van Minnen LP, Timmerman HM, Lutgendorff F, et al. Modification of intestinal flora with multispecies probiotics reduces bacterial translocation and improves clinical course in a rat model of acute pancreatitis. Surgery 2007; 141:470-80.
37. Howard TJ, Patel JB, Zyromski N, et al. Declining morbidity and mortality rates in the surgical management of pancreatic necrosis. J Gastrointest Surg 2007; 11:43-49.
38. Büchler MW, Gloor B, Müller CA, et al. Acute necrotizing pancreatitis: treatment strategy according to the status of infection. Ann Surg 2000; 232:619-626.
39. Ho HS, Frey CF. Gastrointestinal and pancreatic complications associated with severe pancreatitis. Arch Surg 1995; 130:817-822.
40. Tsiotos GG, Smith CD, Sarr MG. Incidence and management of pancreatic and enteric fistulas after surgical management of severe necrotizing pancreatitis. Arch Surg 1995; 130:48-52.
41. Fotoohi M, D'Agostino HB, Wollman B, et al. Persistent pancreatocutaneous fistula after percutaneous drainage of pancreatic fluid collections: role of cause and severity of pancreatitis. Radiology 1999; 213:573-578.
42. Connor S, Ghaneh P, Raraty M, et al. Minimally invasive retroperitoneal pancreatic necrosectomy. Dig Surg 2003; 20:270-277.
43. Boerma D, Rauws EA, van Gulik TM, et al. Endoscopic stent placement for pancreaticocutaneous fistula after surgical drainage of the pancreas. Br J Surg 2000; 87:1506-1509.
44. Telford JJ, Farrell JJ, Saltzman JR, et al. Pancreatic stent placement for duct disruption. Gastrointest Endosc 2002; 56:18-24.
45. Varadarajulu S, Noone TC, Tutuian R, et al. Predictors of outcome in pancreatic duct disruption managed by endoscopic transpapillary stent placement. Gastrointest Endosc 2005; 61:568-575.
46. Bakker OJ, van Santvoort HC, Hagenaars JC, et al. Timing of cholecystectomy after mild biliary pancreatitis. Br J Surg 2011; 98:1446-1454.
47. Uhl W, Warshaw A, Imrie C et al. IAP Guidelines for the surgical management of acute pancreatitis. Pancreatology 2002; 2:565-573.

Part I

Interaction between probiotics and enteral nutrition in acute pancreatitis

Chapter 2

Metabolic consequences of the interaction between enteral nutrition and multispecies probiotics (Ecologic® 641) in an *in vitro* model of the small intestine

(Submitted)

Mark C van Baal[1]
Marjorie E Koenen[2]
Louis M Akkermans[3]
Koen Venema[2]
Hein G Gooszen[1]
Ger T Rijkers[1,4]

[1] Dept. Operating Room/Evidence Based Surgery, Radboud University Nijmegen Medical Centre, Nijmegen
[2] TNO, Zeist
[3] Dept. of Surgery, University Medical Center Utrecht, Utrecht
[4] Dept of Sciences, University College Roosevelt, Middelburg
All: the Netherlands

ABSTRACT

Objectives: Enteral nutrition and probiotic prophylaxis in patients with predicted severe acute pancreatitis may lead to an increased mortality, possibly related to non-occlusive bowel ischemia. We investigated the interaction between enteral nutrition and probiotics in the gastro-intestinal tract in a dynamic, computer-controlled model of the small intestine (TIM-1).

Methods: The TIM-1 system mimics the gastro-intestinal tract. Several effects were tested in this system: a) the addition of pancreatic enzymes and/or, b) the addition of probiotics, and c) the administration of different types of enteral nutrition: 'fibre-rich' and 'protein-rich'. Total lactic acid and short-chain fatty acids (SCFA) production were to determine the effect of digestive enzymes and enteral nutrition on local probiotic activity.

Results: Metabolic activity of the probiotics, expressed as lactic acid and SCFA production, increased when pancreatic enzymes were added to the TIM-1 system while addition of bile salts decreased metabolic activity. Fibre-rich enteral nutrition resulted in a higher SCFA production than protein-rich enteral nutrition.

Conclusion: Pancreatic enzymes increased and bile salts decreased metabolic activity of probiotics. Fibre-rich enteral nutrition resulted in more heterofermentative bacterial activity and SCFA production. No direct clues have been found in the search for the increased mortality in patients with pancreatitis treated with probiotics.

INTRODUCTION

Bacterial translocation from the small bowel is generally considered to be the main cause of bacteriaemia and ensuing infection of pancreatic necrosis.[1-3] Early start of enteral feeding, prophylactic use of antibiotics, and prophylactic use of probiotics have been hypothesized to reduce the infection rate in acute pancreatitis (AP). Only for enteral nutrition a reduction of infections and mortality in AP has been indeed reported.[4,5] Although antibiotics are widely used, several meta-analyses show that prophylactic use does not reduce infectious complications and mortality.[6,7] Three randomized trials have evaluated the prophylactic use of probiotics in patients with predicted severe pancreatitis.[8-10] The first trial by Olah *et al* showed a reduction of systemic infection and the second trial showed no effect.[8] Both trials by Olah *et al*, although promising, had some distinct shortcomings (e.g. small sample size and poor methodological quality).[8,9] The Dutch PROPATRIA study included almost 300 patients with predicted severe pancreatitis who were randomized for prophylactic use of probiotics or placebo.[10] The study hypothesis was that prophylactic use of probiotics would reduce the rate of infectious complications, but no effect on this endpoint was shown. Unexpectedly, probiotic prophylaxis was associated with increased mortality and a high rate of non-occlusive bowel ischemia.[10] A causal relation between administration of probiotics and non-occlusive bowel ischemia has not been established yet. It has been hypothesized that, in these very sick patients, a negative interaction between enteral administration of probiotics and enteral feeding through a nasojejunal feeding tube has led to an increased oxygen demand, resulting in non-occlusive bowel ischemia. An alternative, but not mutual exclusive theory, is that probiotics had generated potentially cytotoxic fermentation products from the enteral nutrition. However, up to now, neither of these hypotheses has been tested in an experimental setting.

In 1995, Minekus *et al* developed a dynamic, computer-controlled model to allow simulation of *in vivo* conditions of the stomach and small bowel, nick-named TIM-1.[11] This *in vitro* model mimics the gradual transit of ingested compounds through the upper digestive tract and has been validated and used for many studies, including survival and metabolic activity of probiotics in the gastro-intestinal tract.[12,13]

The objective of this study was to investigate the effect of the probiotic mixture and enteral nutrition used in the PROPATRIA study in TIM-1 to identify fermentation products which are suggested to have a cytotoxic effect on intestinal mucosa in patients with systemic inflammatory syndrome (SIRS) in general and acute pancreatitis specifically. It was hypothesized that in case of severe pancreatitis, the production of pancreatic exocrine enzymes and endocrine function are severely suppressed and as a consequence, pancreatic enzyme excretion to the duodenum would be significantly decreased. In addition, obstructive jaundice is a common co-phenomenon in acute

pancreatitis leading to a decrease in bile salt secretion to the duodenal lumen. Thus, the experiments were designed to mimic these pathophysiological processes in the proximal part of the small bowel.

We were particularly interested in: 1) which intraluminal conditions increased the metabolic activity of the probiotics most and 2) whether the composition of enteral nutrition could influence the metabolic activity of the probiotics.

MATERIALS AND METHODS

Dynamic gastrointestinal system

TNO's dynamic, computer-controlled *in vitro* gastro-intestinal model of the stomach and small intestine (TIM-1) was used in the current study and is schematically shown in Figure 1. The technical details of system, including validation, were previously described by Minekus *et al*.[11] In short, the system comprises four different compartments, the stomach, duodenum, jejunum and ileum which are sequentially connected via computer-controlled valves, controlling the transit time through the compartments. A temperature of 37°C is maintained throughout the system. In the stomach compartment, water, gastric acid and stomach enzymes are added, whereas bile salts, pancreatic enzymes and electrolytes are added in the duodenum. Addition of bicarbonate takes place in the small intestinal compartments, to control and maintain the pH. Peristaltic gastrointestinal movements are simulated by alternate contractions of the flexible walls of the different compartments. Dialysis of bowel content is performed in the jejunum and ileum compartments by two hollow fiber devices with a closed dialysis system (Figure 1). For further details see Martinez *et al*.[13]

Probiotics

A probiotic mixture, identical to the product used in the clinical trial, of six viable and freeze-dried probiotic strains was used (Ecologic® 641; Winclove Probiotics b.v., Amsterdam, the Netherlands) containing three *Lactobacillus* strains (*L. aidophilus* W70, L. casei W56 and L. salivarus W24), two Bifidobacterium strains (*B. bifidum* W23 and *B. lactis* W52 (previously classified as *B. infantis* W52)) and *Lactococcus lactis* W58, together with carrier substance (corn-starch). The placebo consisted of carrier substance only. The probiotic or placebo product was reconstituted in sterile water for 15 minutes at 37°C before addition to TIM-1. The probiotic mixture was administered in a single shot of 5.0 ml (containing a total of 5 x 109 CFU bacteria) to the duodenum compartment one hour after start of the experiment, to mimic administration in the PROPATRIA patients.[10]

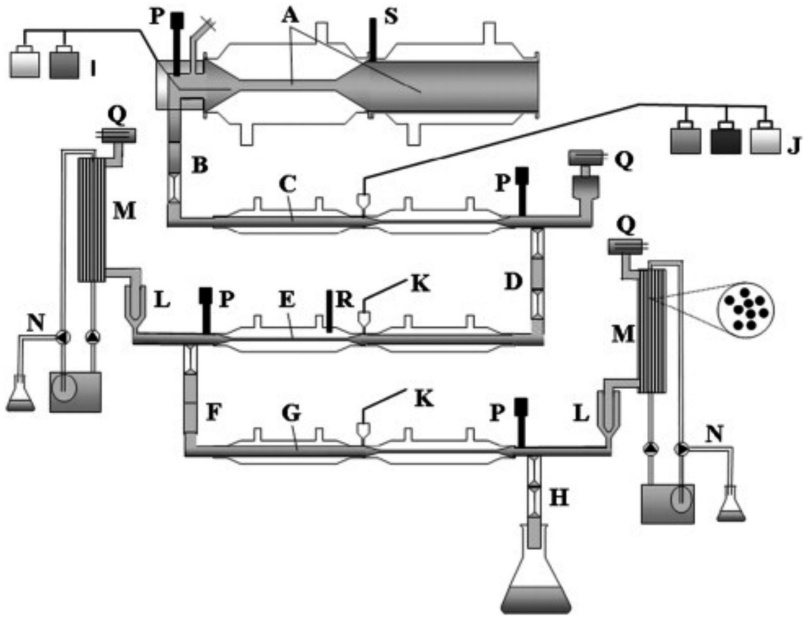

Figure 1. Schematic view of the TIM-1 system. A: gastric compartment; B: pyloric sphincter; C: duodenum compartment; D: peristaltic valve; E: jejunum compartment; F: peristaltic valve; G: ileum compartment; H: ileo-cecal valve; I: stomach secretion of water, enzymes and acid; J: duodenum secretion bottles with bile, pancreatin and bicarbonate; K: secretion of bicarbonate to control the intestinal pH; L: pre-filter system; M: semi-permeable membrane system (hollow fibers); N: closed dialyzing and water absorption system; O: dialysate; P: pH electrodes; Q: level sensor; R: temperature sensor; S: pressure sensor.[11,13]

Experiment	Condition
1	PP + placebo
2	PP + placebo + BS
3	PP + placebo + PE + BS
4	PP + probiotics
5	PP + probiotics + BS
6	PP + probiotics + PE
7	PP + probiotics + PE + BS
8	MF + probiotics
9	MF + probiotics + PE + BS

Table 1. Experimental conditions used. PP: Nutrison Protein Plus, MF: Nutrison Multi Fibre, BS: bile salts, PE: pancreatic enzymes

Enteral nutrition

To resemble the clinical situation of patients with acute pancreatitis, enteral nutrition (Nutrison Multi Fibre or Nutrison Protein Plus, Nutricia, Zoetermeer, the Netherlands) was administered directly into the duodenal compartment, bypassing the gastric compartment. By using a syringe pump, enteral nutrition was continuously administered to the duodenal compartment with an infusion rate of 33 ml per hour (as in proportion to the *in vivo* situation in patients). Preliminary batch experiments (not shown) did not reveal a difference in lactic acid production by the probiotic mixture between both types of enteral nutrition. Although Nutrison Multi Fibre was used in the PROPATRIA study, nowadays in the clinical setting protein enriched-enteral nutrition is preferred above fibre-enriched enteral nutrition. Therefore, and since there was no difference between the two nutrition types in batch experiments, most of the experiments were performed with Nutrison Protein Plus.

Experimental design

Each experiment with TIM-1 lasted six hours, in an attempt to copy the slower transit time through the small intestinal tract of a pancreatitis patient. Table 1 shows the different conditions for each experiment. During the TIM-1 experiments, the pH in each small intestinal compartment was continuously monitored and kept at constant values (duodenum 6.3, jejunum 6.8, and ileum 7.2) by addition of bicarbonate, an integral part of exocrine pancreatic secretion. The amount of bicarbonate administered during an experiment consists of the amount required for maintaining the pH in the compartments plus the amount for neutralizing the extra pH decrease, mainly caused by the acid produced by probiotic fermentation. Each experiment was performed in duplicate. Lactic acid and SCFA production were measured to monitor metabolic activity of the probiotics. To that end, every hour, the dialysate (of both the jejunum and ileum compartment) and the ileal efflux were collected. Samples were taken and stored at -80°C until analysis.

Measurement of (L- and D-) lactic acid and short-chain fatty acids

Dialysis and effluent samples were centrifuged (12000 rpm, 5 min) and both L- and D-lactic acid were determined enzymatically in the supernatant (based on Boehringer, UV-method, Cat. No. 1112821) by a Cobas Mira plus autoanalyzer (Roche, Almere, The Netherlands). The dialysis samples were analyzed by gaschromatography to determine the SCFA concentrations in a Chrompack CP9001 gas chromatograph using an automatic sampler (Chrompack liquid sampler CP9050; Varian Chrompack). This methodology allows detection of acetic acid, propionic acid, butyric acid and valeric acid.[14]

Statistical analysis

Per experiment, the total amount of bicarbonate added to the TIM-1 system during the experiment was measured and the total amount of lactic acid and SCFA in each compartment was calculated. The Mann-Whitney Test was used to test statistical differences between groups. All statistics were performed with IBM® SPSS Statistics version 20.0.0 (IBM, Chicago, Illinois, USA).

RESULTS

Bicarbonate consumption

In experiments in which pancreatic enzymes were added, significantly more bicarbonate was needed to maintain the target pHs in each compartment compared to experiments in which pancreatic enzymes were absent (Figure 2) ($P<0.001$). Type of enteral nutrition and the presence or absence of probiotics and bile salts did not influence the total amount of bicarbonate needed.

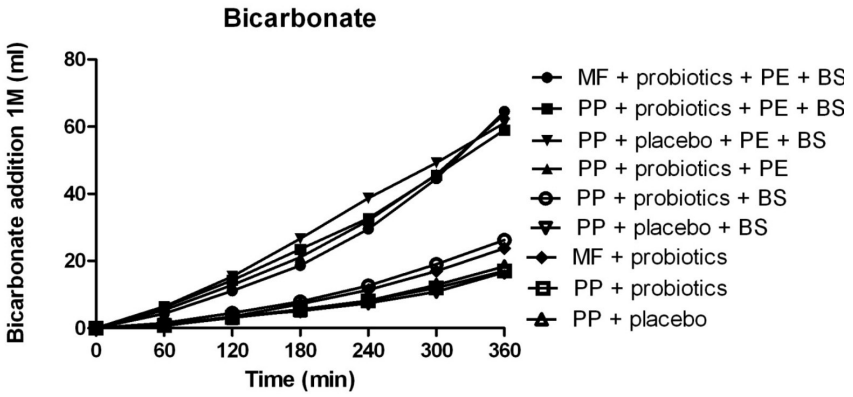

Figure 2. Addition of bicarbonate to the small intestinal compartments of the TIM-1 system, used to maintain the pH in these compartments (n=2). MF: Nutrison Multi Fibre, PP: Nutrison Protein Plus, PE: pancreatic enzymes, BS: bile salts

Total lactic acid production

Figure 3A shows the cumulative lactic acid production under different conditions with probiotics and Nutrison Protein Plus. Lactic acid production was detected from 60 minutes after start of the experiments onwards, which is directly after addition of the probiotics into the duodenal lumen. In the presence of pancreatic enzymes, lactic acid production was further increased, indicative for enhanced metabolic activity of the probiotics. Bile salts, however, decreased total lactic acid production. This inhibitory effect was partially compensated by the presence of pancreatic enzymes. The two types of enteral nutrition were compared under two different conditions (i.e., in the presence of bile salts and pancreatic enzymes, and in the absence of both), as shown in Figure 3B. Lactic acid production was increased when Nutrison Multi Fibre was used compared to Nutrison Protein Plus. This increased production in favour of Nutrison Multi Fibre was also observed when pancreatic enzymes and bile salts were added to the system. In none of the experiments with placebo (i.e. without the addition of live bacteria), lactic acid production was detected (data not shown).

It is important to note that the differences in lactic acid production between the different conditions became apparent after a test period of four hours or more. In the first four hours after start of the experiment, no clear differences between the various experimental conditions were detectable. In the last two hours of an experiment, under conditions with pancreatic enzymes (PE), metabolic activity of the probiotics increased (Fig. 3A and 3B).

SCFA production

Production of SCFA, mainly acetic acid (data on proportions of SCFA not shown), was measured in four selected experiments in which both types of enteral nutrition were compared under two different conditions. The cumulative amount of SCFA is shown in Figure 4. In general, the cumulative production of SCFA (Fig. 4) was higher than that of lactate (Fig. 3B). As for lactic acid, production of SCFA started almost directly after introduction of the probiotic mixture in TIM-1. However, again, clear differences between the experiments were generally seen four hours after start of the experiment (i.e., three hours after administration of the probiotic mixture into the duodenum). In the presence of Nutrison Multi Fibre, SCFA production was increased compared to Nutrison Protein Plus, as was observed for lactate.

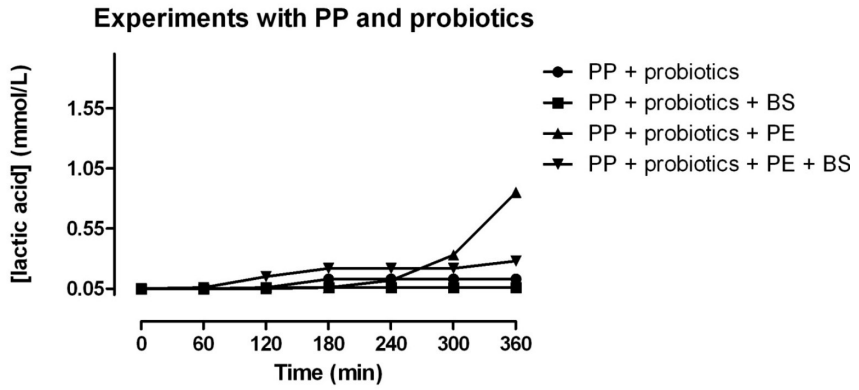

Figure 3A. Effect of pancreatic enzymes and bile salts on production of lactic acid by probiotics in protein-enriched enteral nutrition. The lowest detection level for lactic acid was 0.05 mmol/L. Pancreatic enzymes enhanced lactic acid production by the probiotic bacteria. Once combined with bile salts, the effect of pancreatic enzymes was neutralized. PP: Nutrison Protein Plus, PE: pancreatic enzymes, BS: bile salts

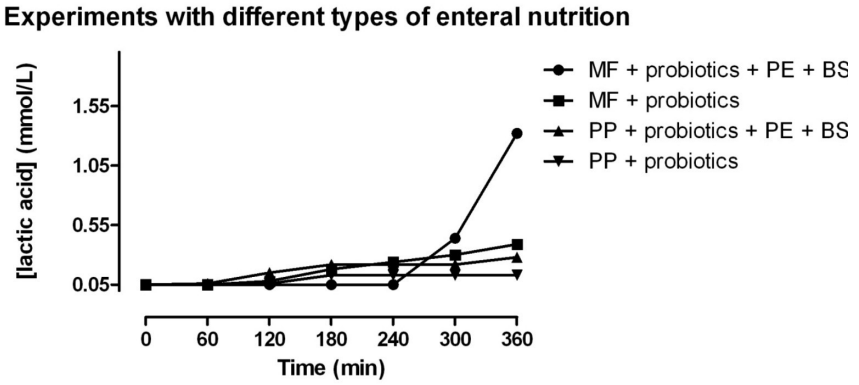

Figure 3B. Effect of pancreatic enzymes and bile salts on production of lactic acid by probiotics in different types of enteral nutrition. There was no statistical significant difference in metabolic activity of the probiotic bacteria in the presence of a fibre-rich as compared to protein-rich type of enteral nutrition of enteral feeding. Once bile salts and pancreatic enzymes were added to the substrate, more lactic acid was produced in the presence of fibre-enriched enteral nutrition. MF: Nutrison Multi Fibre, PP: Nutrison Protein Plus, PE: pancreatic enzymes, BS: bile salts

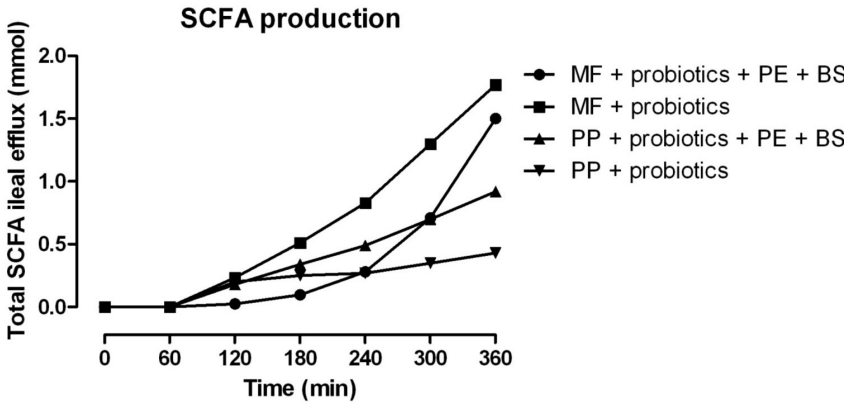

Figure 4. Production of SCFA with different types of enteral nutrition. The presence of a fibre-rich type of enteral nutrition resulted in a higher higher production of SCFA by the probiotics as compared to a protein-rich type of enteral nutrition. SCFA: Short-chain fatty acids, PP: Nutrison Protein Plus, PE: pancreatic enzymes, BS: bile salts

DISCUSSION

This study was designed to study the metabolic activity of probiotic strains in a controlled environment to investigate whether the combination of acute pancreatitis and administration of probiotics with enteral feeding would enhance lactic acid and SCFA production. To this end, several circumstances such as absence of pancreatic enzymes, and changes in bile salt excretion were mimicked. The probiotic mixture was identical to that used in the PROPATRIA study (Ecologic® 641).[10]

The findings of this study can be summarized as follows: 1) in the presence of pancreatic enzymes the metabolic activity of probiotics is higher. Once combined with bile salts, the effect of pancreatic enzymes was abolished. So based on these two findings, it seems that pancreatic enzymes play a dominant role in the probiotic-lowering of the pH in this model; 2) in the presence of Nutrition Protein Plus and probiotics, again the addition of pancreatic enzymes has the dominant effect on the pH, demonstrated by the highest production of lactic acid under these circumstances. Addition of bile salts seems to mitigate this lactic acid production; 3) when Nutrison Protein Plus is replaced by Nutrison Multifibre, pancreatic enzyme addition, again, leads to increase of lactic acid production; 4) SCFA production was highest after administration of pancreatic enzymes, irrespective of the type of enteral feeding added to the TIM-1 system. So, on balance it seems that, in a study to compare the *in vitro* effect of composition of enteral feeding, bile salts, pancreatic enzymes and probiotics, only the addition of pancreatic enzymes has an overriding effect on the lowering of the pH in the lumen of the duodenum and jejunum by

the probiotics. Apparently, the pancreatic enzymes digest components of the enteral nutrition such that the probiotics can metabolize them into lactic acid and SCFA.

In the current study, a validated *in vitro* model for gastrointestinal passage was used. The advantage of the TIM-1 system is the ability to investigate the metabolic activity of the probiotics under specific conditions, such as presence or absence of bile and pancreatic juice. Samples from different compartments could be taken any time during the experiment and transit times of the system could be adapted, allowing mimicking patients with acute pancreatitis. Since the system does not contain host cells, the metabolites produced by the bacteria are not used by the host cells and therefore the production of metabolites can be monitored. Consequently, the results of this study are very useful for further *in vitro* and *in vivo* studies. Extrapolation of the results to the clinical situation, however, is difficult because of the absence of bowel mucosa and resident intestinal microbiota. Furthermore, the temporal changes in bile salt secretion and/or pancreatic enzyme production during acute pancreatitis have not been studied in detail and published data are controversial.

How can our findings – dominant effect of pancreatic enzymes leading to lowering of intraluminal pH by the probiotics – be put into the clinical perspective of a higher death rate and a possible induction of small and large bowel ischemia in patients with severe pancreatitis receiving the combination of enteral feeding and enteral probiotics? Or can our findings be balanced with our hypothesis that lactic acid and SCFA may have a toxic effect on bowel mucosa leading to mucosal necrosis and ensuing complications like bacteriemia with infection of pancreatic necrosis, bowel perforation and increase of mortality as a result of these complications? Data in the literature on enzyme secretion in acute pancreatitis are scarce. In 2005, O'Keefe *et al* showed that patients with acute pancreatitis have lower rates of secretion of digestive enzymes in the duodenum compared to healthy volunteers.[15] However, it was also shown that patients with severe necrotizing pancreatitis synthesized newly pancreatic enzymes more rapidly than patients with mild pancreatitis or even healthy volunteers.[15] Unfortunately, only 12 patients were included in this study and at least three patients suffered from recurrent attacks of acute pancreatitis. One may question whether these patients with recurrent attacks did not already suffer from exocrine dysfunction due to a subclinical chronic pancreatitis. So, no clue is given on the exact changes in secretion during acute pancreatitis and its potential effect on complications. In contrast to these clinical data, Czako *et al* found that the basal pancreatic fluid secretion in the duodenum was greatly increased in a mouse model of experimental pancreatitis. However, the concentration of pancreatic enzymes in the pancreatic fluid was dependent of the severity of the pancreatitis, with a lower enzyme concentration in rodents that show a more severe pancreatitis.[16] In our *in vitro* study, two 'extreme' conditions regarding pancreatic enzyme

secretion were tested, i.e. with and without pancreatic enzymes in the duodenum. Although in the literature no exact data are available on the exact level of increase or decrease in duodenal concentrations of pancreatic enzymes, it is generally accepted that there still is some residual pancreatic enzyme secretion in the duodenum during an attack of acute pancreatitis.[15,16] Therefore, we think that in our study the conditions with the presence of pancreatic enzymes reflect the pathophysiological situation, like in acute pancreatitis, best.

There is also limited information on the effect of lactic acid production in case of severe pancreatitis. In the last two decades, the heterofermentative capacity of lactic acid producing bacteria has extensively been investigated in the food industry.[17-19] In addition to lactic acid, lactobacilli produce acetate. In the current study, acetic acid was the most predominant SCFA present in the ileal efflux of the TIM-1system (60-80%). The probiotic strain which is thought to be mainly responsible for production of the acetate in our experiments is *L. casei*, although bifidobacteria also produce acetate. All other *Lactobacillus* and *Lactococcus* species in Ecologic 641 are homofermentative. It is to be expected that the SCFA production by bifidobacteria in Ecologic 641, would mainly take place in the colon and not in the small bowel.[20,21]

Up to now, it is completely unknown what underlying mechanism can possibly be held responsible for a probiotic-induced bowel ischemia in the patients with pancreatitis. The current study shows that a considerable amount of lactic acid and SCFA are produced when enteral feeding and a probiotic mixture are administered in the artificial bowel lumen. Addition of pancreatic enzymes seems to further accelerate the metabolic activity of the probiotics, reflected in a further increase of lactic acid and SCFA production. However, whether these production levels of lactic acid and SCFA are comparable with the clinical situation is not known, since concentrations *in vivo* cannot be measured. Moreover, whether or not these lactic acid and SCFA concentrations can be harmful in man in case of pancreatitis cannot be tested in the type of experiment that we have conducted.

In the TIM-1 experimental set-up, it is not possible to test the cytotoxic effect of lactic acid and SCFA on enterocytes, because of the absence of gut epithelial cells in the TIM-1 system. Although it was assumed that the probiotic strains in Ecologic® 641 would produce lactic acid and SCFA, it is unknown at what concentration these become toxic to the gut mucosa of AP patients.

This *in vitro* study has obvious limitations. Only one single dose of probiotics was administered, whereas in the PROPATRIA study probiotics were administered for 28 consecutive days.[10] Whether such prolonged administration of probiotics and thus prolonged exposure of enterocytes to lactic acid or other microbial metabolites have led to the dramatic results of the PROPATRIA study remains unclear. The facts that TIM-1 does not carry endogenous intestinal microbiota, has no intestinal mucosa and only allows

for acute experiments, are distinct drawbacks and limitations. However, this model was chosen, since TIM-1 has been shown to be very useful for metabolic studies in general and, as a consequence, to study the effect of probiotics under several experimental conditions, impossible to be conducted *in vivo*. Furthermore, since bowel mucosa is absent, a full mass balance of the produced microbial metabolites could be made without interference of other local metabolic processes induced or facilitated by resident microbiota in the ileum of patients with acute pancreatitis. The main strength of the current study, is that in the TIM-1 model probiotic activity can directly be tested under several preset conditions in each compartment of the digestive tract, thus allowing mechanistic studies on pathophysiological phenomena in the gut. To answer the question on the relation between concentration of lactic acid/SCFA and cytotoxicity and whether this could contribute to non-occlusive mucosal ischemia, *in vitro* experiments with cell cultures (e.g., Caco-2 cells) would have to be performed.

In summary, the current study shows that pancreatic enzymes increase the probiotic activity. The administration of fibre-rich enteral nutrition resulted in an increase of lactate and SCFA production more than protein-rich enteral nutrition. Future experiments are directed towards effects of microbial metabolites on *in vitro* cell cultures to unravel the underlying mechanism of probiotic-induced bowel ischemia.

Acknowledgements

We thank Winclove Probiotics B.V. for providing the placebo and probiotic mixture (Ecologic® 641) and Mark Jelier (TNO) for his skillful assistance in the experimental design and the experimental implementation.

References

1. Ammori BJ, Leeder PC, King RF, et al. Early increase in intestinal permeability in patients with severe acute pancreatitis: correlation with endotoxemia, organ failure, and mortality. J Gastrointest Surg 1999; 3(3)252-262.
2. Deitch EA. The role of intestinal barrier failure and bacterial translocation in the development of systemic infection and multiple organ failure. Arch Surg 1990; 125(3)403-404.
3. Van Leeuwen PA, Boermeester MA, Houdijk AP, et al. Clinical significance of translocation. Gut 1994; 35(1 Suppl) S28-S34.
4. Petrov MS, van Santvoort HC, Besselink MG, et al. Enteral nutrition and the risk of mortality and infectious complications in patients with severe acute pancreatitis: a meta-analysis of randomized trials. Arch Surg 2008 Nov;143(11): 1111-1117.
5. Al-Omran M, Albalawi ZH, Tashkandi MF, et al. Enteral versus parenteral nutrition for acute pancreatitis. Cochrane Database Syst Rev 2010 Jan 20;(1).
6. De Vries AC, Besselink MG, Buskens E, et al. Randomized controlled trials of antibiotic prophylaxis in severe acute pancreatitis: relationship between methodological quality and outcome. Pancreatology 2007;7(5-6)531-538.
7. Wittau M, Mayer B, Scheele J, et al. Systematic review and meta-analysis of antibiotic prophylaxis in severe acute pancreatitis. Scand J Gastroenterol 2011;46(3)261-270.
8. Olah A, Belagyi T, Issekutz A, et al. Randomized clinical trial of specific lactobacillus and fibre supplement to early enteral nutrition in patients with acute pancreatitis. Br J Surg 2002;89(9) 1103-1107.
9. Oláh A, Belágyi T, Pótó L, et al. Synbiotic control of inflammation and infection in severe acute pancreatitis: a prospective, randomized, double blind study. Hepatogastroenterology. 2007 Mar;54(74):590-594.
10. Besselink MG, van Santvoort HC, Buskens E, et al. Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. Lancet 2008;371(9613)651-659.
11. Minekus M, Marteau P, Havenaar P, et al. A multi compartmental dynamic computer-controlled model simulating the stomach and small intestine. Alternatives to Laboratory Animals (ATLA) 1995; 23: 197-209.
12. Marteau P, Minekus M, Havenaar S, et al. Survival of lactic acid bacteria in a dynamic model of the stomach and small intestine: Validation and the effects of bile. J Dairy Sci 1997; 80(6): 1031-1037
13. Martinez RC, Aynaou AE, Albrecht S, et al. In vitro evaluation of gastrointestinal survival of Lactobacillus amylovorus DSM 16698 alone and combined with galactooligosaccharides, milk and/or Bifidobacterium animalis subsp. lactis Bb-12. Int J Food Microbiol 2011 Sep 15;149(2):152-8.
14. Jouany JP. Volatile fatty acids and alcohols determination in digestive contents, silage juice, bacterial culture and anaerobic fermenter contents. Scientific Aliments 1982(2): 31-44.
15. O'Keefe SJ, Lee RB, Li J et al. Trypsin secretion and turnover in patients with acute pancreatitis. Am J Physiol Gastrointest Liver Physiol 2005 Aug;289(2):G181-187

16. Czakó L, Yamamoto M, Otsuki M. Exocrine pancreatic function in rats after acute pancreatitis. Pancreas 1997 Jul;15(1): 83-90.
17. Watanabe K, Fujimoto J, Tomii Y, et al. Lactobacillus kisonensis sp. nov., Lactobacillus otakiensis sp. nov., Lactobacillus rapi sp. nov. and Lactobacillus sunkii sp. nov., heterofermentative species isolated from sunki, a traditional Japanese pickle. Int J Syst Evol Microbiol 2009 Apr;59(Pt 4):754-760
18. Schwab C1, Gänzle M. Lactic acid bacteria fermentation of human milk oligosaccharide components, human milk oligosaccharides and galactooligosaccharides. FEMS Microbiol Lett 2011 Feb;315 (2):141-148.
19. Sohier D, Jamet E, Le Dizes AS, et al. Polyphasic approach for quantitative analysis of obligately heterofermentative Lactobacillus species in cheese. Food Microbiol 2012 Sep;31(2):271-277
20. Rossi M, Corradini C, Amaretti A, et al. Fermentation of fructooligosaccharides and inulin by bifidobacteria: a comparative study of pure and fecal cultures. Appl Environ Microbiol 2005 Oct;71(10): 6150-6158.
21. Falony G, Verschaeren A, De Bruycker F, et al. In vitro kinetics of prebiotic inulin-type fructan fermentation by butyrate-producing colon bacteria: implementation of online gas chromatography for quantitative analysis of carbon dioxide and hydrogen gas production. Appl Environ Microbiol 2009 Sep;75(18):5884-5892.

Chapter 3

Association between probiotics and enteral nutrition in an experimental acute pancreatitis model in rats

Accepted for publication Pancreatology

Mark C van Baal[1]
Michiel J van Rens[1]
Christopher B Geven[1]
Francien M van de Pol[2]
Ilona W van den Brink[2]
Gerjon Hannink[1]
Iris D Nagtegaal[3]
Wilbert H Peters[4]
Ger T Rijkers[1]
Hein G Gooszen[1]

[1] Dept. Operating Rooms/Evidence Based Surgery, Radboudumc, Nijmegen
[2] Dept. of Anesthesiology, Radboudumc, Nijmegen
[3] Dept. of Pathology, Radboudumc, Nijmegen
[4] Dept. of Gastroenterology, Radboudumc, Nijmegen
All: the Netherlands

ABSTRACT

Background / Objectives: Recently, a randomized controlled trial showed that probiotic prophylaxis was associated with an increased mortality in enterally fed patients with predicted severe pancreatitis. In a rat model for acute pancreatitis, we investigated whether an association between probiotic prophylaxis and enteral nutrition contributed to the higher mortality rate.

Methods: Male Sprague-Dawley rats were allocated to four groups: 1) acute pancreatitis (n=9), 2) acute pancreatitis and probiotic prophylaxis (n=10), 3) acute pancreatitis and enteral nutrition (n=10), and 4) acute pancreatitis, probiotic prophylaxis and enteral nutrition (n=11). Acute pancreatitis was induced by intraductal glycodeoxycholate and intravenous cerulein infusion. Enteral nutrition, saline, probiotics and placebo were administered through a permanent jejunal feeding. Probiotics or placebo were administered starting 4 days before induction of pancreatitis and enteral nutrition 1 day before start until the end of the experiment, 6 days after induction of pancreatitis. Tissue samples and body fluids were collected for microbiological and histological examination.

Results: In all animals, serum amylase was increased six hours after induction of pancreatitis. After fulfilling the experiment, no differences between groups were found in histological severity of pancreatitis, degree of discomfort, weight loss, histological examination of small bowel and bacterial translocation (all $p>0.05$). Overall mortality was 10% without differences between groups ($p=0.54$).

Conclusion: No negative association was found between prophylactic probiotics and enteral nutrition in acute pancreatitis. No new clues for a potential mechanism responsible for the higher mortality and bowel ischemia in the PROPATRIA study were found.

INTRODUCTION

Acute pancreatitis runs a mild course in the majority of patients. However, 20% of patients develop a severe pancreatitis with the presence of peripancreatic or pancreatic necrosis and (multiple) organ failure.[1] If the necrosis becomes infected, this is associated with a mortality of 15-25% and a morbidity rate of 50-100%.[2-5] Infection of necrotic pancreatic tissue is caused by bacterial translocation from the intestines and is thought to be preceded by three pathophysiological processes: 1) bacterial overgrowth of the small bowel due to decreased bowel motility, 2) dysfunction of the local mucosal and systemic immune system, and 3) increased intestinal permeability, resulting in bacterial translocation to other sites, such as the pancreas.[6-8] Reduction of bacterial translocation may reduce the rate of secondary infection of the pancreatic necrosis and decrease mortality and morbidity.

In 2006, our study group started a multicenter placebo-controlled randomized trial (PROPATRIA) on probiotic prophylaxis in patients with predicted severe pancreatitis.[9] Based on the results of a smaller trial, the aim of the study was to reduce the number of infectious complications. However, no difference in infection rate between the two groups was observed.[10] Strikingly, probiotic prophylaxis turned out to be associated with an unexpected high mortality rate, possibly related to the presence of bowel ischemia (4% vs. 0% in the placebo arm, p=0.004).[10] These unexpected and unexplained findings prompted others to stop planned and ongoing trials on probiotic prophylaxis in severely ill patients.[11,12]

In previous experiments we observed that prophylactic use of probiotics improved survival in rats.[13,14] The underlying mechanism for the negative effect of the combination of enteral probiotics with enteral nutrition in patients with predicted severe pancreatitis is unknown and stands in strong contrast to the previous findings of the protective effect of prophylactic probiotics in rats with acute pancreatitis. In order to address this mechanism, we investigated the relation between probiotic administration and enteral nutrition in a rat model of acute pancreatitis.

MATERIALS AND METHODS

Animals

Male specific pathogen-free Sprague-Dawley rats (Harlan, Horst, the Netherlands) with a mean bodyweight of 328 grams (range 91 grams) and age between 10-12 weeks were kept under constant housing conditions (temperature 22°C), relative humidity (60%) and a 12-hour light-dark cycle). Prior to the first surgical procedure, rats were allowed to adjust to these conditions for at least one week. During this week, all animals had unlimited access to water and food. Rats were randomly divided into four groups: 1) acute pancreatitis (jejunal cannula, 0.9% sodium chloride and placebo, n=9), 2) acute pancreatitis and probiotics (jejunal cannula and 0.9% sodium chloride, n=10),

3) acute pancreatitis and enteral nutrition (jejunal cannula and placebo, n=11) and 4) acute pancreatitis, probiotics and enteral nutrition (jejunal cannula, n=11). Animals were terminated at the end of the experiment, 6 days after induction of acute pancreatitis. The experimental design, as shown in Figure 1, was approved by the Regional Animal Ethics Committee of the Radboud UMC and was conducted under the guidelines of the Dutch Council for Animal Care and the National Institutes of Health.

Enteral nutrition

The animals allocated to group 3 and 4 received sterile enteral nutrition (Nutrison Multi Fibre, Nutricia, Zoetermeer, the Netherlands). Animals in groups one and two received (sterile) saline as substitution to the enteral nutrition. The saline and enteral nutrition were infused through the permanent jejunal cannula from day -1 to the end of the experiment on day 6 (Figure 1). On day -12, a swivel jacket was fitted and the rat was able to adjust to the jacket for one week. The swivel jackets were checked daily and, if necessary, adapted to the body size of the rat. From day -1 to the end of the experiment, each animal was connected to a swivel system for nine hours per day (usually from 9.00 a.m. to 6.00 p.m.). As shown in Figure 2, the complete swivel system consisted of a syringe pump, a swivel device and a swivel mount, all interconnected by tubing. The system was used to allow free movement of the animal through the cage during connection (all parts of the swivel system and swivel jacket: Instech Laboratries Inc, Plymouth, PA, USA). When connected, the syringe pump continuously administered the enteral nutrition or the saline with an infusion rate of 1.5 ml/hour. During the nine hours of connection to the swivel system rats were withheld from other food. However, when disconnected, animals had unlimited access to food (RMH 11110, Hope Farms, Woerden, The Netherlands). Throughout the whole experiment, whether connected or disconnected to the swivel system, all animals had free access to water.

Probiotics and placebo

The probiotics (*Ecologic® 641*, Winclove Probiotics, Amsterdam, the Netherlands) consisted of six viable and freeze-dried probiotic strains; four lactobacilli (*Lactobacillus acidophilus* (W70), *Lactobacillus casei* (W56), *Lactobacillus salivarus* (W24), *Lactobacillus lactis* (W58)), and two bifidobacteria strains (*Bifidobacterium bifidum* (W23) and *Bifidobacterium infantis* (W52)). The placebo consisted of carrier substance only (corn-starch). Directly before administration of the probiotics and the placebo, both products were reconstituted in sterile water for 15 minutes at 37°C. A single probiotics dose in a volume of 1.0 ml contained a total of 5 x 10^9 Colonic Forming Unit (CFU) bacteria. According to van Minnen *et al*, both probiotics and placebo were administered once daily through the permanent jejunal cannula, starting five days prior to induction of the pancreatitis until the end of the experiment.[13]

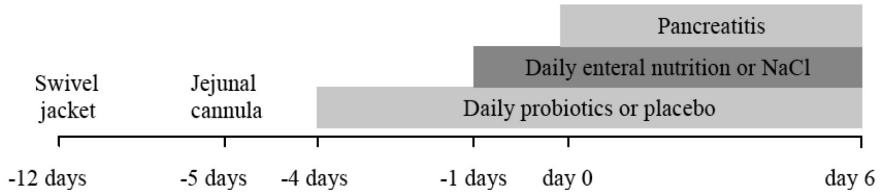

Figure 1. Experimental design. At the start of the experiment, 12 days prior to induction of acute pancreatitis, a swivel jacket was fitted to all animals, which were subsequently allowed to adjust to this jacket for seven days. On day -5, a jejunal cannula was fitted. From day -4 onwards, daily probiotics or placebo were administered through the permanent cannula. One day before induction of the pancreatitis, animals received daily enteral nutrition or saline which continued until the end of the experiment. On day 0, acute pancreatitis was induced and seven days later, on day 6, all surviving animals were anesthetized to allow sterile removal of organs and blood samples, followed by termination.

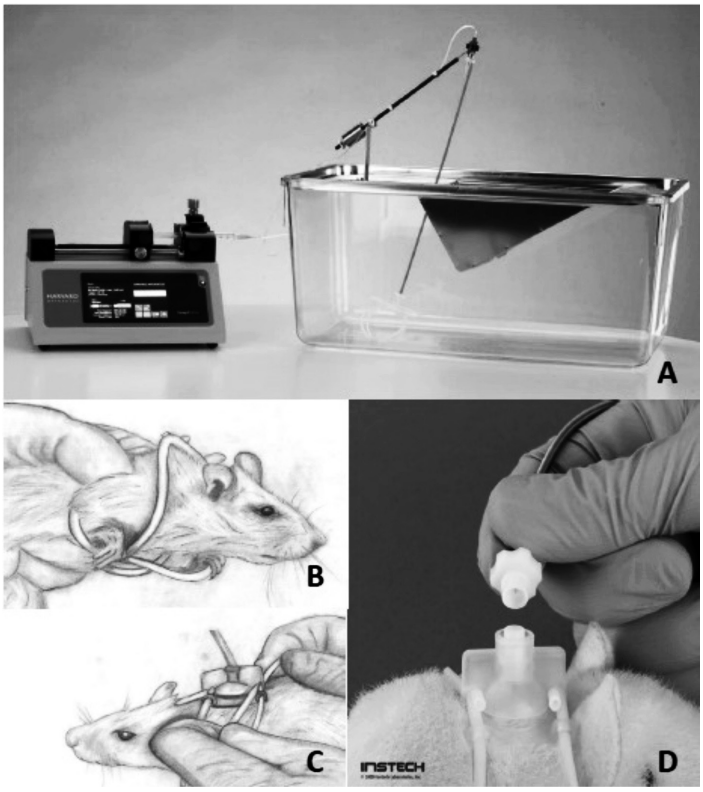

Figure 2. Swivel system. A: The complete swivel system consisted of a syringe pump, a swivel mount with a swivel device, all interconnected with tubing. B and C: fitting the swivel jacket to the proportion of the rat. D: Connection of the swivel arm to the swivel jacket. Illustrations provided by Instech Laboratories Inc, Plymouth, PA, USA.

Surgical procedures

All surgical procedures were performed by the responsible researcher (MCvB), usually with assistance of members of the technical staff (MvR, FvdP or IvdB). Every procedure was conducted under general anaesthesia, using continuous intravenous administration of 30 mg/kg/h Alfaxan® (alfaxalone 10 mg/ml, Vetoquinol UK limited, Buckinham, United Kingdom) via a tail vein. However, rarely, in case of very poor vascular access, gas anaesthesia through a snout mask was used as an alternative (2% isoflurane gas, flow: 0.5 l/min O2, 1.5 l/min air). At least at 30 minutes prior to every surgical procedure, as well as up to three days post-operatively, once daily intra-muscular injections of 5 mg/kg Rimadyl (Carprofen 50 mg/ml, Pfizer Animal Health, West Ryde, New South Wales, Australia), a long-acting non-steroidal anti-inflammatory drug (NSAID), was administered for pain relief. All surgical procedures were performed on a heated operating table with sterile instruments under aseptic conditions. Body temperature of all animals was kept between 37 and 38.5 degrees Celcius.

Jejunal cannulation

Seven days after fitting the swivel jacket, rats were anaesthetized to fit the permanent silicone jejunal cannula (outer diameter 1.4 mm, inner diameter 0.6 mm, Instech Laboratories Inc, Plymouth, PA, USA). Under general anaesthesia, a 1.5 cm midline laparotomy was made to insert the end of the jejuna cannula into the stomach through a puncture in the greater curvature. Subsequently, the cannula was tunneled through the stomach and the sphincter and moved up manually through the duodenum into the jejunum. The cannula was securely fixed with a purse-string suture in the stomach wall. A puncture hole was made in the muscular layer, through which the other end of the cannula entered the subcutaneous space. Subsequently, the cannula was tunneled subcutaneously from the abdominal wall to the back, penetrating the skin between the scapulae, just underneath the connection part of the swivel jacket. The cannula was connected to the swivel jacket and the abdomen was closed in two layers. All animals were allowed to recover for one day before administration of the probiotics or placebo and for five days before induction of the pancreatitis.

Induction of acute pancreatitis

On day 0, five days after fitting the permanent jejunal cannula, acute pancreatitis was induced by the internationally well accepted model for acute pancreatitis, described by Schmidt *et al*.[18] Under general anaesthesia, with a midline relaparotomy, the papilla of Vater was cannulated transduodenally (24G Abbocath®-T i.v. infusion cannula, Abbott, Sligo, Republic of Ireland). Subsequently, the common bile duct was clamped and 0.5 ml (infusion rate: 3 ml/hour) sterilized glycodeoxycholic acid in glycylglycine-NaOH-buffered solution (10 mmol/l, pH 8.0, 37°C, chemicals obtained from Sigma-Aldrich

Chemie BV, Zwijndrecht, the Netherlands) was infused into the common bile duct and pancreas. Directly after infusion, the clamp was removed and the puncture hole in the duodenum was sutured. After closure the abdomen in two layers, rats were kept under general anesthesia to allow intravenous cerulein infusion with 5 µg/kg/hr for six hours (Cerulein, Sigma-Aldrich Chemie BV, Zwijndrecht, the Netherlands). During these six hours, body temperature was maintained with a heating pad and rats were connected to a pulse-oxy device to check vital parameters every 15 minutes. Due to ventilation problems which were encountered in earlier experiments (not published), all animals were intubated to maintain a free airway and glycopyrrolate (0.04mg/kg body weight every 2 hours during general anesthesia) (Sigma-Aldrich Chemie BV, Zwijndrecht, the Netherlands) was administered to reduce salivary, pharyngeal and tracheobroncheal secretions. Fluid substitution was provided by hourly subcutaneously injections of 2 ml 0.9% sodium chloride. After six hours of cerulein infusion, all animals were disconnected and were allowed to recover in their home cage.

Pain relief

As mentioned before, prior to every surgical procedure and up to three days postoperatively, a long-acting NSAID was administered (Rimadyl). Since in humans acute pancreatitis is a very painful disease, all animals received daily subcutaneous injections of this long-acting NSAID, starting at induction of the pancreatitis to the end of the experiment.

Animal welfare

Animal welfare was assessed by daily repeated observations and bodyweight measurements. For scoring the clinical response a multiple aspect, 0-2 points scoring system was used: Grooming behaviour: normal = 0 points, moderate decrease = 1 point, severe decrease = 2 points. Mobility: normal = 0 points, small-moderate decrease = 1 point, severe decrease = 2 points. Response to external stimuli: normal = 0 points, small-moderate hypo/hyperresponsive = 1 point, severe hypo/hyperresponsive = 2 points. Pain posture: none = 0 points, moderate pain posture = 1 points, severe pain posture = 2 points. A low cumulative score reflects mild discomfort, whereas a high cumulative score reflects severe discomfort. Aspects of this scoring system are well recognized behavioural parameters expressing health or morbidity, including abdominal and visceral pain.[15] A cumulative score of 7 or more was defined as extreme suffering (and considered to be the humane endpoint). In case of extreme suffering, rats were euthanized without delay by suffocation with CO_2, followed by cervical dislocation. Obduction was performed in all euthanized animals.

Collection of tissue and fluid samples

Blood samples (0.2 ml) were taken from the tail vein immediately before (t

= 0 hrs), and after 6 hours (t = 6 hrs), 24 hours (t = 24 hrs) and at the end of the experiment, 6 days after induction of acute pancreatitis. In case of survival without extreme suffering, animals were terminated at the end of the experiment, six days after induction of the acute pancreatitis. These animals were anesthetized to allow sterile removal of the pancreas. In order to control for potential organ damage, all organs were subjected to macroscopic inspection. After tissue collection, rats were euthanized by terminal blood loss, followed by cervical dislocation.

Histological examination

Samples from the jejunum, ileum and pancreas were collected and stored in formalin and subsequently embedded in paraffin for histopathological analysis. A standard haematoxylin and eosin (H&E) staining was performed. Histopathological severity of acute pancreatitis was assessed based on the acute pancreatitis scoring system as previously described (Table 1).[16] Intestinal histology was assessed by the following parameters: crypt-villus proportion, ischemia throughout the different layers of the bowel wall, presence of inflammatory cells, localisation of the inflammatory cells, hemorrhagic changes, epithelial destruction, oedema, arteriitis, and the presence of peritonitis (Table 2). Scores of the jejunum and ileum were added to obtain a final score. Severe tissue damage is reflected by a high cumulative score, whereas minor damage is reflected by a low cumulative score.

DNA isolation and real-time PCR assay for total bacterial quantification

Pancreas biopsies were stored at -80°C prior to DNA extraction. Total DNA was extracted from pancreas biopsies using the repeated bead beating method in combination with the QiaAmp DNA Mini Stool Kit (Qiagen, Hilden, Germany). DNA was quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop® Technologies, Wilmington, DE) and adjusted to 10-20 ng/µl as template for subsequent 16S rRNA gene PCR analysis. Quantitative PCR (qPCR) was performed in 384-well PCR plates (Bio-Rad Laboratories, Hercules, CA) sealed with Microseal B film (Bio-Rad) using a CFX384 Touch™ Real-Time PCR Detection System with CFX manager™ software version 2.0 (Bio-Rad). Each reaction was carried out in a total volume of 25 µl using iQ™ SYBR Green Supermix (Bio-Rad) according to the manufacturer's instructions with 200 nM of each primer and 5 µl template DNA. The universal primer set Bact1369 (5'-CGG TGA ATA CGT TCY CGG-3') and Prok1492 (5'-GGW TAC CTT GTT ACG ACT T-3') was used for quantification of total bacterial 16S rRNA gene copies. The amplification program consisted of an initial denaturation step at 95°C for 10 min, followed by 40 cycles of 15 s at 95°C, 30 s at 56°C, and 30 s at 72°C, followed by a melt-curve analysis. All reactions were performed in triplicate. Standard curves were generated from a dilution series of 16S rRNA gene

Item description	Score
Peripancreatic	
No pathology	0
Fat inflammation / mild peritonitis	1
Fat necrosis / peritonitis	2
Edema	
None	0
Interlobular expansion	1
Interacinar expansion	2
Ducti / Ductuli	
No pathology	0
Inflammation	1
Inflammatory infiltrate	
None	0
1 - 10 intralobular or perivascular leukocytes / HPF	1
11 - 20 intralobular or perivascular leukocytes / HPF	2
21 - 30 intralobular or perivascular leukocytes / HPF	3
> 30 intralobular or perivascular leukocytes / HPF	4
Acinar cell pathology	
None	0
Focal cytoplasmatic changes	1
Extensive cytoplasmatic changes	2
Degeneration with nuclear changes	3
Focal necrosis	4
Extensive necrosis	5
Acinar dilatation	
None	0
Focal	1
Extensive	2
Hemorrhagic changes	
None	0
Focal interlobular erythrocytes	1
Extensive interlobular erythrocytes	2
Focal parenchymal erythrocytes	3
Extensive parenchymal erythrocytes	4
TOTAL SCORE	**Max 20**

Table 1. Histopathologic scoring criteria pancreas. HPF: High power field (400x). Modified from Schmidt *et al*.[18]

fragments amplified from the target sequence (108 to 100 copies/µl). Number of rRNA gene copies per gram pancreatic tissue were calculated and compared between groups.

Serum amylase assay

Serum amylase concentrations were determined in blood samples collected on t = 0 hrs, t = 6 hrs, t = 24 hrs and at the end of the experiment, 6 days after induction of acute pancreatitis. After sample collection, blood was centrifuged on 13.000 rpm for 10 minutes and the serum was stored at -80°C until analysis. For amylase quantification an amylase kit (DAMY-100 QuantiChromTM α-Amylase Assay Kit, Gentaur, Kampenhout, Belgium) was used according to the manufacture's guidelines. All samples were tested at 1:2, 1:4, 1:15 and 1:40 dilutions and the amylase concentration was calculated from the dilution(s) with a reading in the linear part of the calibration curve.

Statistical analysis

Mortality was analyzed with the Chi-square test. Health scores and body weights were analyzed with repeated measurement ANOVA, followed by Bonferroni posthoc analyses. Histological scores and bacterial translocation were compared between groups using ANOVA. Only animals which completed the experiment or animals euthanized because of reaching the humane endpoint were used to calculate the histological scores. All other scores were calculated with animals which completed the experiment. P-values <0.05 were considered statistically significant. All analyses were performed using SPSS® for Windows® version 20 (IBM, Chicago, Illinois, USA).

RESULTS

Morbidity and mortality

Placement of the jejunal canula on day -5 resulted in mild post-operative discomfort, as indicated in Figure 3. On day 0, the time of induction of acute pancreatitis, mean average degree of discomfort was 0.68 (range 0-1), indicating that all animals recovered well from surgery. After induction of pancreatitis, all animals showed an increase in discomfort which lasted until the end of the experiment. Although the degree of discomfort fluctuated, no clear-cut pattern was observed. No difference in degree of discomfort was found between groups throughout the experiment, suggesting that probiotics did not reduce clinical severity of acute pancreatitis and that enteral feeding has no additional effect.

Next to the behaviour scoring system as parameter for clinical severity of experimental pancreatitis, overall change in bodyweight also was taken as marker for severity of acute pancreatitis. Figure 4 shows the changes in mean bodyweight per group throughout the experiment. Prior to induction of acute pancreatitis, all animals regained their initial bodyweight after insertion of the

Item description	Score
Crypt-villi proportion	
Normal	0
Crypt hyperplasia	1
Partial villous atrophy	2
Total villous atrophy	3
Ischemia	
None	0
Thrombi	1
Necrosis	2
Inflammation	
None	0
Focal neutrophylic infiltration	1
Extensive neutrophylic infiltration	2
Localisation of inflammation	
None	0
Mucosal	1
Submucosal	2
Muscular	3
Hemorrhagic changes	
None	0
Mild	1
Severe	2
Epithelial destruction	
None	0
Focal	1
Extensive	2
Edema submucosa	
None	0
Focal	1
Extensive	2
Edema lamina propria	
None	0
Focal	1
Extensive	2
Arteriitis	
None	0
Mild	1
Severe	2
TOTAL SCORE	**Max 20**

Table 2. Histopathologic scoring criteria of the small bowel.

jejunal canula. From induction of pancreatitis until the end of the experiment, all animals lost 9% body weight, on average (mean loss of body weight of 29 grams). Animals treated with probiotics and enteral feeding started to gain weight from day 4 onwards, however, the differences in total bodyweight at the end of the experiment were not significant.

Overall mortality during the experiment (within the 6 days after induction of acute pancreatitis) was 4/41 animals (10%); in group 1, one animal died; in group 3, two animals died and in group 4, one animal died. All animals in group 2 (administration of probiotics) survived. Mortality did not differ between the groups (p=0.57).

Induction and severity of acute pancreatitis

All animals showed a significant (on average >10-fold) increase in serum amylase concentrations 6 hours after induction of pancreatitis, indicating a successful induction of acute pancreatitis (mean concentration serum amylase 483 IU/L at t=0 vs. 5014 IU/L at t=6). Serum amylase concentrations decreased during the next 18 hours after induction and returned to almost normal levels 6 days after induction (Figure 5). No difference in serum amylase levels was found between the four experimental groups.

Histological analysis (H-E staining) was performed on pancreatic tissue at the end of the experiment. As shown in Figure 6, histological scores were comparable between groups, suggesting that all animals developed acute pancreatitis of equal severity (p=0.55).

Intestinal inflammation and ischemia

Histopathological analysis of the jejunum and ileum at 6 days after induction of acute pancreatitis showed signs of mild to moderate serositis in nearly all animals. All rats developed serositis of different extent and with wide inter-individual variation; in some animals inflammatory changes included the entire bowel wall, including the mucosa. Histopathological scores are shown in Figure 7, without differences between groups (p=0.52). In none of the surviving animals signs of bowel ischemia were observed. Post-mortem analysis of the animals that died during the experiment or preliminary terminated the experiment, showed large bowel obstruction in combination with severe peritonitis and necrotic changes and perforation of the terminal ileum in one animal (group 3). In all other diseased animals no signs of (non-occlusive) bowel ischemia were found.

Bacterial translocation

One of the hallmarks of the pathophysiology of acute pancreatitis is bacterial translocation from the intestine to necrotic pancreas tissue. Determination of the bacterial load in the pancreas was performed by bacterial counts in the pancreas by qPCR for 16S rRNA. Although the process of bacterial

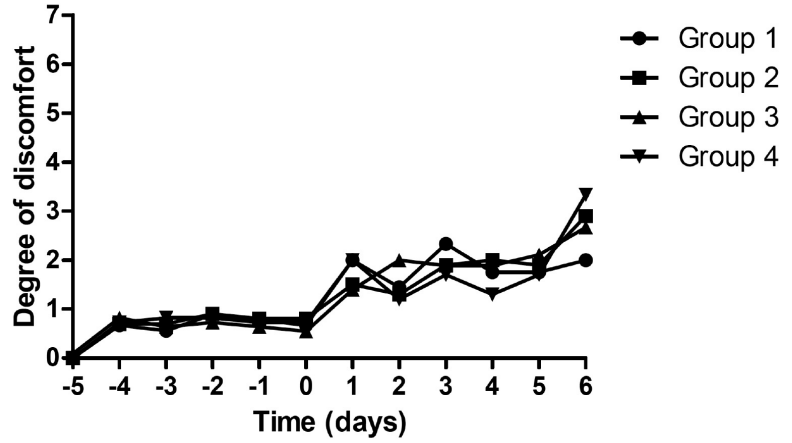

Figure 3. Morbidity induced by the acute pancreatitis was evaluated by daily measurement of the degree of discomfort. In all 4 experimental groups (Group 1, acute pancreatitis, saline and placebo (n=9); group 2, acute pancreatitis, saline and probiotics (n=10); group 3, acute pancreatitis, enteral nutrition and placebo (n=10); group 4, acute pancreatitis, probiotics and enteral nutrition (n=11)) induction of pancreatitis caused discomfort during the 6 days afterwards. This discomfort was present from day 1 onwards and persisted during the whole observation period. No difference between the 4 experimental groups was seen in degree of discomfort ($p>0.05$).

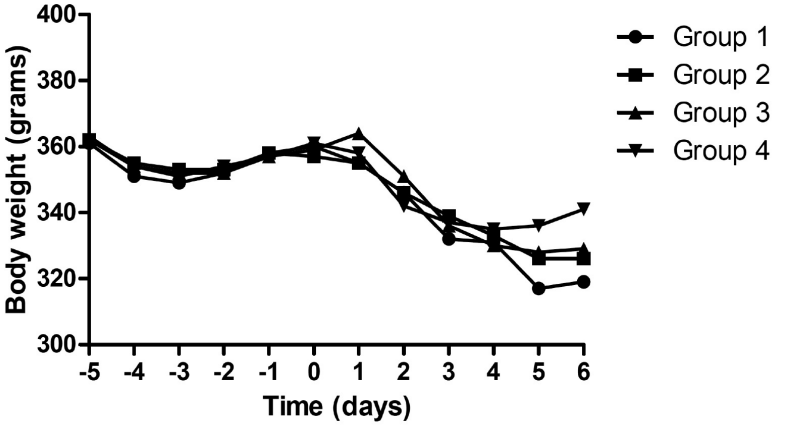

Figure 4. Change in bodyweight during acute pancreatitis. From day -5 until the end of the experiment, all animals were weighed daily. All rats initially lost, but subsequently regained bodyweight in the period between placement of the jejunal cannula (t = -5 days) and induction of acute pancreatitis (t = 0 days). Bodyweight decreased after induction of acute pancreatitis (Group 1, acute pancreatitis, saline and placebo (n=9); group 2, acute pancreatitis, saline and probiotics (n=10); group 3, acute pancreatitis, enteral nutrition and placebo (n=10); group 4, acute pancreatitis, probiotics and enteral nutrition (n=11)). At the end of the experiment, mean body weight in group 4 was 20 grams higher than in the other 3 experimental groups, however these differences were not significant ($p>0.05$).

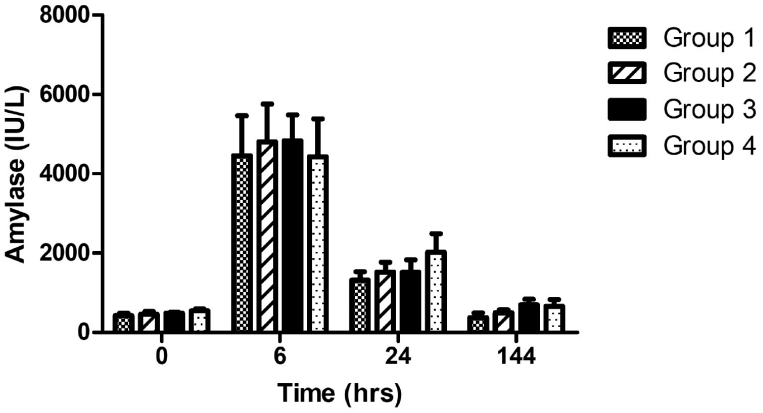

Figure 5. Serum amylase concentrations during acute pancreatitis. A peak in serum amylase activity is seen 6 hours after induction of acute pancreatitis in all 4 experimental groups (Group 1, acute pancreatitis, saline and placebo (n=9); group 2, acute pancreatitis, saline and probiotics (n=10); group 3, acute pancreatitis, enteral nutrition and placebo (n=10); group 4, acute pancreatitis, probiotics and enteral nutrition (n=11)). After 24 hrs, amylase activity has decreased significantly but is still elevated as compared to t = 0. Six days after induction of acute pancreatitis, amylase levels normalized. No difference between experimental groups was observed on any of the time points. Data are presented as mean +/- SEM.

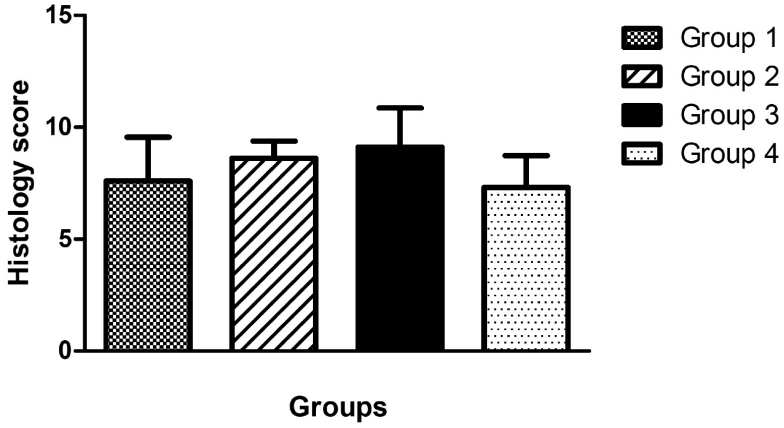

Figure 6. Histology scores of the pancreas. Pancreas tissue was obtained during autopsy at day 6 after induction of pancreatitis. Tissue sections were scored for histopathological signs of pancreatitis according to the scheme in Table 1 (maximal score is 20). All animals showed a moderate to severe pancreatitis score. No differences in histology scores were found between the groups, indicating that the severity of the pancreatitis was similar in all animals (p=0.55). Data are presented as mean +/- SEM.

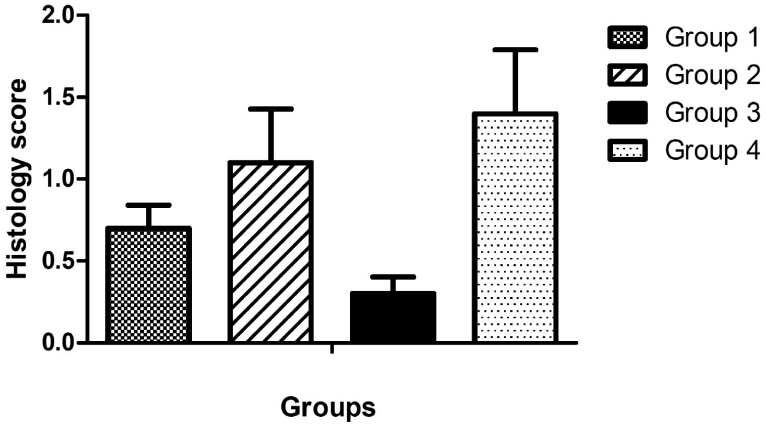

Figure 7. Intestinal histopathology. Ileum and jejunum tissue samples were obtained during autopsy at day 6 after induction of pancreatitis. Tissue sections were scored for inflammatory and ischemic lesions according to the scheme in Table 2 (range 0-20). The scores of ileum and jejunum were added. Animals showed minimal intestinal histopathological changes and no differences in histology scores were found between the groups (p=0.52). Data are presented as mean +/- SEM.

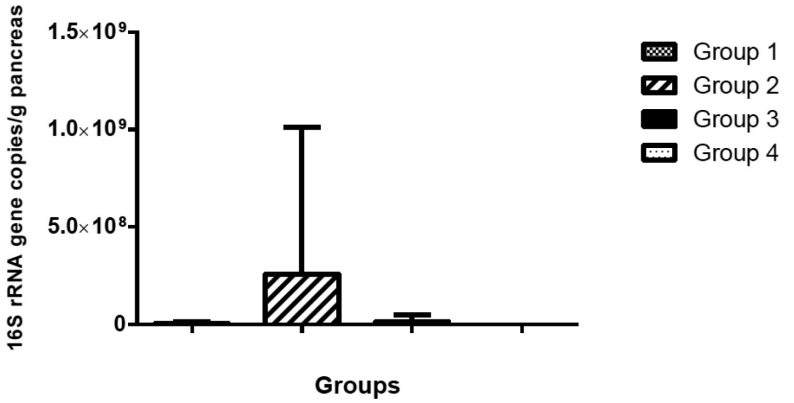

Figure 8. Total bacterial count of the pancreas. Pancreatic tissue samples were obtained during autopsy at day 6 after induction of pancreatitis. Total 16S rRNA gene copies per gram pancreatic tissue are shown. No differences in bacterial counts were found between the groups (p=0.13). Data are presented as mean +/- SD.

translocation was not measured directly in this study, we have analyzed a major consequence of this process, i.e. bacterial contamination of pancreatic tissue. No difference between the four groups in terms of rRNA gene copies per gram pancreatic tissue (p=0.13) was observed, as shown in Figure 8. So, if this form and extent of pancreatic contamination is the result of bacterial translocation, no difference between the four groups was found.

DISCUSSION

The current study was performed to address the underlying mechanisms responsible for the high mortality rate and high incidence of non-occlusive bowel ischemia in patients who received the combination of probiotic prophylaxis and enteral nutrition in the PROPATRIA study.[10] All animals developed a moderate to severe acute pancreatitis with a significant increase in serum amylase levels in the first 24 hours after induction of pancreatitis. No differences were found in terms of animal discomfort, bacterial translocation, bowel ischemia and mortality between the four experimental groups, whether or not probiotics and enteral nutrition were administered. With the results of the current study, with mortality rates in the same range as seen in previous experiments and probiotics having no adverse effect on morbidity and mortality and no interaction between probiotics and enteral feeding, no new insights were obtained in search for the underlying mechanisms responsible for the increased incidence of non-occlusive bowel ischemia and mortality in patients treated with probiotics and enteral nutrition.

Of all experimental models for acute pancreatitis, the current model is most robust and has the highest percentage of pancreatic necrosis with short- and long-term survival rates to make observation/intervention studies feasible. This model, in our hands, initially was associated with a mortality rate of approximately 30%.[13] This was deemed unacceptably high and has led to some minor, but essential modifications in the model, leading to an improved survival rate of around 10% for all four groups. This finding may indicate that now too few animals developed a really severe pancreatitis with not enough mucosal damage and bacterial translocation to be detected in the different groups, as a consequence.

Now that we have found no differences in the animal model, the question arises whether this experimental design can be extrapolated to a clinical study in human with four groups of 10 patients with predicted severe acute pancreatitis as a set up to investigate the possible rerlation between probiotics and enteral feeding. It is likely that such a clinical study would probably need much more patients to be randomized, otherwise no meaningful difference between groups can be shown and no clinically relevant conclusion can be drawn. So, is this experimental study seriously underpowered as a reason for not having shown any meaningful effect of intervention? In sharp contrast to the clinical reality, all animals were genetically nearly identical, were born,

fed and kept under identical conditions and received the same standardized experimental induction of pancreatitis. Probably, this has an impact on the number of rats needed per treatment arm (i.e., one could imagine that less animals are needed to observe differences between groups), although we have no clue about the influence of these factors on the number of rats needed to be randomized per arm.[17]

In human, prophylaxis – antibiotics, enteral feeding or probiotics – is expected to minimize bacterial translocation and to reduce late infection of pancreatic necrosis. This renders extrapolation of our findings in this rat model to clinical human pancreatitis to be seriously flawed. No biphasic pattern with the sequence of SIRS, recovery, 'second hit' with necrosis getting infected, leading to late mortality after having survived the first phase of SIRS is shown in rats and there is no pattern of intra- and juxtapancreatic necrosis. However, despite the abovementioned shortcommings, up to now, the current model is the best available model studying severe acute pancreatitis in rats.

In order to resemble the situation during the PROPATRIA study in our model, we have used the identical mixture of probiotics (but from a newly produced batch) and identical enteral nutrition, Nutrison Multi Fibre.[9] Although technically it would have been possible to administer the enteral nutrition continuously during seven days, we used a maximum period of 9 hrs enteral feeding per day. Consequently, animals were given access to conventional food during the remainder of the day. It is unlikely that this may have affected the results.

Another major difference with the clinical situation is the timing of administration of probiotics. As in the study of van Minnen *et al*, in the current study animals were pretreated with probiotics, while in PROPATRIA all patients received the first dose of probiotics when the pancreatitis was already ongoing for 24 to 72 hours.[13] In a mouse model for acute pancreatitis, it has been shown that pretreatment with probiotics abolishes the intestinal barrier dysfunction, while treatment with probiotics does not.[18] So the timing of administration of probiotics is essential for their effects. Although this relationship between timing of administration of probiotics and start of the pancreatitis has only been shown in experimental models, it is perceivable that the same holds for the human situation.

One of the most unexpected findings in the PROPATRIA trial which needs to be resolved, was the occurrence of non-occlusive mesenteric ischemia.[10] It has been speculated that the combination of enteral nutrition and probiotics may have contributed to the occurrence of this otherwise rare complication of acute pancreatitis. In our rat model, we therefore paid particular attention to any potential ischemic lesions occurring along the complete length of the intestinal tract. Although small numbers per group, in both the surviving and the deceased animals no signs of transmural bowel ischemia was observed neither macroscopically nor histopathologically.

None of the previously published studies about probiotic prophylaxis and experimental pancreatitis have shown negative effects of probiotics, including the current study.[13,14,19] Therefore, it is unlikely that future experimental animal studies will provide us with knowledge to understand any of the possibly negative effects of probiotics observed in the PROPATRIA study.

In summary, this experimental study in rats has not shown any effect of probiotics with or without enteral feeding on morbidity and mortality in rats subjected to acute pancreatitis. In accordance with previous experimental studies, no negative effects of probiotics in acute pancreatitis were shown. So, in conclusion, it is not likely that new *in-vivo* experimental studies will bring us closer to an explanation of the still unexplained findings in the PROPATRIA trial. With the current knowledge and techniques no explanation could be found for the increased mortality and incidence of bowel ischemia as found in enterally fed patients with predicted severe pancreatitis and prophylactically treated with probiotics in the PROPATRIA trial.

Acknowledgements

We thank Daphne Reijnen and Debby Smits for assisting with the animal experiments, Coline Gerritsen for performing the assays for bacterial translocation and Hennie Roelofs for assisting with the amylase assays. In addition, we thank Nutricia (Zoetermeer, the Netherlands) for providing the enteral nutrition and Winclove Probiotics (Amsterdam, the Netherlands) for providing the probiotics and placebo.

References

1. Banks PA, Freeman ML; Practice Parameters Committee of the American College of Gastroenterology. Practice guidelines in acute pancreatitis. Am J Gastroenterol 2006; 101: 2379-2400.
2. Rau B, Bothe A, Beger HG. Surgical treatment of necrotizing pancreatitis by necrosectomy and closed lavage: changing patient characteristics and outcome in a 19-year, single-center series. Surgery 2005; 138:28-39.
3. Büchler MW, Gloor B, Müller CA, et al. Acute necrotizing pancreatitis: treatment strategy according to the status of infection. Ann Surg 2000; 232:619-626.
4. Rodriguez JR, Razo AO, Targarona J, et al. Debridement and closed packing for sterile or infected necrotizing pancreatitis: insights into indications and outcomes in 167 patients. Ann Surg 2008; 247:294-299.
5. van Santvoort HC, Besselink MG, Bakker OJ, et al. A step-up approach or open necrosectomy for necrotizing pancreatitis. N Engl J Med 2010; 362:1491-1502
6. Ammori BJ, Leeder PC, King RF, et al. Early increase in intestinal permeability in patients with severe acute pancreatitis: correlation with endotoxemia, organ failure, and mortality. J Gastrointest Surg 1999; 3(3)252-262.
7. Deitch EA. The role of intestinal barrier failure and bacterial translocation in the development of systemic infection and multiple organ failure. Arch Surg 1990; 125(3)403-404.
8. Van Leeuwen PA, Boermeester MA, Houdijk AP, et al. Clinical significance of translocation. Gut 1994; 35(1 Suppl) S28-S34.
9. Besselink MG, Timmerman HM, Buskens E, et al. Probiotic prophylaxis in patients with predicted severe acute pancreatitis (PROPATRIA): design and rationale of a double-blind, placebo-controlled randomised multicenter trial [ISRCTN38327949]. BMC Surg 2004; Sep 29;4:12.
10. Besselink MG, van Santvoort HC, Buskens E, et al. Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. Lancet 2008; 371:651-659.
11. Sharma B, Srivastava S, Singh N, et al. Role of probiotics on gut permeability and endotoxemia in patients with acute pancreatitis: a double-blind randomized controlled trial. J Clin Gastroenterol 2011; 45(5)442-448.
12. Lata J, Stiburek O. [Prophylactic antibiotics and probiotics in acute pancreatitis]. Vnitr Lek 2010; 56(6)582-584.
13. van Minnen LP, Timmerman HM, Lutgendorff F, et al. Modification of intestinal flora with multispecies probiotics reduces bacterial translocation and improves clinical course in a rat model of acute pancreatitis. Surgery 2007; 141:470-80.
14. Lutgendorff F, Trulsson LM, van Minnen LP, et al. Probiotics enhance pancreatic glutathione biosynthesis and reduce oxidative stress in experimental acute pancreatitis. Am J Physiol Gastrointest Liver Physiol 2008; 295:G1111-1121.
15. Stam R, van Laar TJ, Wiegant VM. Physiological and behavioural responses to duodenal pain in freely moving rats. Physiol Behav 2004; 81(1):163-9.

16. Schmidt J, Rattner DW, Lewandrowski K, et al. A better model of acute pancreatitis for evaluating therapy. Ann Surg 1992; 215(1):44-56.
17. Hooijmans CR, de Vries RB, Rovers MM, et al. The effects of probiotic supplementation on experimental acute pancreatitis: a systematic review and meta-analysis. PloS One 2012; 7(11):e48811
18. Rychter JW, van Minnen LP, Verheem A, et al. Pretreatment but not treatment with probiotics abolishes mouse intestinal barrier dysfunction in acute pancreatitis. Surgery 2009; 145(2):157-167.
19. Lutgendorff F, Nijmeijer RM, Sandström PA, et al. Probiotics prevent intestinal barrier dysfunction in acute pancreatitis in rats via induction of ileal mucosal glutathione biosynthesis. PLoS One 2009; 4:e4512.

Chapter 4

Probiotic treatment with Probioflora in patients with predicted severe acute pancreatitis without organ failure

Pancreatology 2012; 12(5):458-462

Mark C van Baal[1]
Pavel Kohout[2]
Marc G Besselink[3,4]
Hjalmar C van Santvoort[3]
Zdnek Benes[2]
Roman Zazula[5]
Ger T Rijkers[1]
Hein G Gooszen[1]

[1] Dept. Operating Room/Evidence Based Surgery, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands
[2] Dept. of Internal Medicine, Faculty Thomayer´s Hospital, Prague, Czech Republic
[3] Dept. of Surgery, University Medical Center Utrecht, Utrecht, the Netherlands
[4] Dept. of Surgery, Academic Medical Center Amsterdam, Amsterdam, the Netherlands
[5] Dept. of Anesthesiology and Intensive Care, Faculty Thomayer´s Hospital, Prague, Czech Republic

ABSTRACT

Background: We previously demonstrated that probiotic prophylaxis, in patients with predicted severe pancreatitis, did not prevent infectious complications but unexpectedly increased the risk of bowel ischemia and mortality. The suggestion that these negative findings are only observed in the presence of organ failure at the start of probiotic treatment has not been confirmed.

Methods: In a retrospective analysis, all patients with predicted severe acute pancreatitis without initial organ failure admitted to a medium care facility of a teaching hospital in Prague from January 2003 to December 2010 were included. All patients routinely received probiotic treatment with Probioflora. Total parenteral nutrition (TPN) was routinely started and shifted towards total enteral nutrition. Infectious complications, mortality and the incidence of bowel ischemia were recorded.

Results: 99 consecutive patients, mean age 56 years, were included. Infectious complications occurred in 42 patients (42%), consisting of bacteraemia (n=40), pneumonia (n=11) and infected necrosis (n=11). Bowel ischemia was detected in two patients (2%). Overall mortality was 8%.

Conclusion: In this retrospective study no apparent positive or negative impact of probiotic treatment with Probioflora was demonstrated when administered to patients with predicted severe acute pancreatitis without initial organ failure.

INTRODUCTION

In 80% of patients, acute pancreatitis runs a mild course, but 20% of patients develop necrotizing pancreatitis.[1] If pancreatic or peripancreatic necrosis becomes infected, intervention is generally required. This is associated with high morbidity and mortality.[1] Next to infected necrotizing pancreatitis, other infectious complications, such as bacteraemia and pneumonia, are risk factors for mortality in acute pancreatitis.[2] Therefore, any treatment regimen capable of lowering the infection rate in acute pancreatitis could potentially reduce both morbidity and mortality.

Some authors propose that bacterial translocation from the small bowel is the main route of infection but others do not agree.[3-6] Prophylactic measures using antibiotics or probiotics and early start of enteral feeding have been hypothesized to reduce the infection rate, each by different mechanisms. Several recent meta-analyses have found no beneficial effect of systemic antibiotic prophylaxis in terms of reducing infectious complications and mortality.[7,8] In patients with acute pancreatitis, routine enteral nutrition, when compared to routine parenteral nutrition, is associated with a lower mortality.[9,10] However, it is suggested that this difference is a reflection of the fact that patients with parenteral nutrition may suffer from gut failure.[11]

Several clinical studies have assessed the effect of probiotics prophylaxis with contradictory results. Some suggested that probiotics prophylaxis reduces the infection rate and the need for surgical intervention in patients with necrotizing pancreatitis.[12] The unexpected outcome of the Dutch PROPATRIA study[13] resulted in a premature termination of several randomized trials in which probiotics were administrated to patients with acute pancreatitis[14,15] including a termination of a prospective study with the same probiotics used as in the PROPATRIA study in the Faculty Thomayer´s Hospital, Prague, Czech Republic.

Although the use of probiotics in acute pancreatitis is still a delicate issue, the negative outcome of the PROPATRIA study requires elucidation of the mechanism responsible for the negative outcomes observed.[16] Since the PROPATRIA publication, no new studies have been performed on probiotics in patients with severe pancreatitis. In 2010, we became aware of the fact that in the Faculty Thomayer´s Hospital, the use of probiotic treatment in patients with predicted severe pancreatitis had continued, but only in patients without initial organ failure. As a part of our mutual interest in the impact of probiotics on these patients we retrospectively analyzed these patient data with the aim to unravel the mechanism of probiotic-induced bowel ischemia.

MATERIALS AND METHODS

Patient selection

A retrospective analysis was performed including all consecutive patients with acute pancreatitis who were admitted to the Faculty Thomayer´s Hospital, between January 1st, 2003 and December 31st, 2010. All patients with predicted severe pancreatitis without initial organ failure were admitted to the medium care unit where they routinely received probiotic treatment. Patients with organ failure on hospital admission were admitted to the intensive care unit (ICU) where they did not receive probiotic treatment. These patients were excluded from analysis. The database of the medium care was searched for patients with the ICD-10 code for acute pancreatitis (K85,0).

Definitions

Acute pancreatitis was defined as acute upper abdominal pain with a serum amylase level at least three times higher than the upper limit of normal. Predicted severity of the pancreatitis was assessed by the Imrie score[17] and the serum C-reactive protein level[18]. Patients with an Imrie score of 3 or more, or with C-reactive protein over 150 mg/L, both within 48 hours after hospital admission, were classified as 'predicted severe acute pancreatitis'. In case of clinical deterioration a contrast-enhanced CT scan (CECT) was performed to detect pancreatic or peripancreatic necrosis.

Nutrition

Immediately after hospital admission, all patients (regardless of organ failure) received total parenteral nutrition (TPN). Within the first days after admission, a nasojejunal tube was inserted to administer probiotics and eventually enteral feeding as well. Total parenteral nutrition was provided for at least ten days, with, if tolerated, a gradual shift towards total enteral nutrition. Depending on the calculated dietary needs of the patient, enteral nutrition consisted of Nutrison standard, Nutrison Multi Fibre or Nutrison Protein Plus (all Nutricia, Prague, Czech Republic). Patients received enteral nutrition until all signs of pancreatitis (e.g., fluid collections) were resolved on CECT. Consequently, patients could be discharged from hospital while on total enteral nutrition. These patients were followed clinically and radiologically in the outpatient department for weeks to months.

Probiotics

Probiotics were administered upon admission to the medium care through a nasojejunal tube and continued until the last day of hospital admission. The probiotic mixture, containing $3.0*10^9$ bacteria per gift, was administered twice daily and consisted of seven different probiotic strains (i.e., *Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus casei, Bifidobacterium bifidum, Bifidobacterium longum, Bifidobacterium lactis,* and *Streptococcus*

termophilus; Probioflora, Goldim, Prague, Czech Republic). When patients developed organ failure and needed ICU treatment, they were transferred to the ICU and treatment with Probioflora was continued. As these patients did not have organ failure at the time the probiotics were started, they were included in the analysis, according to the intention-to-treat principle. After hospital discharge, patients were advised to continue the use of these probiotics, which in the Czech Republic are available over the counter. Since Probioflora was administered as part of a routine daily clinical practice in patients with abdominal diseases, including acute pancreatitis, no ethical approval for this retrospective study was required.

Procedures and definitions

On admission, standard laboratory tests and abdominal ultrasound were performed to establish the cause of the pancreatitis. In case of cholangitis and/or choledocholithiasis, endoscopic retrograde cholangiography (ERC) with endoscopic sphincterotomy (ES) was performed.

Infection of (peri-)pancreatic necrosis was defined as a positive bacterial culture of necrotic tissue obtained during intervention (i.e., percutaneous catheter drainage (PCD) or open necrosectomy), gas in the fluid collection on CECT, or a positive bacterial culture obtained with fine-needle aspiration (FNA). In case of (suspected) infection, patients were treated with intravenous antibiotics. Intervention was performed if patients deteriorated in spite of maximum conservative treatment.

Single organ failure was defined as pulmonary failure (PaO2<60mm Hg, despite FiO2 of 0.30, or need for mechanical ventilation), circulatory failure (circulatory systolic blood pressure <90mm Hg, despite adequate fluid resuscitation, or need for inotropic support) or renal failure (creatinine level >177µmol/L after rehydration or need for hemofiltration or hemodialysis). Multiple organ failure was defined as failure of two or three organ systems at the same time. Disseminated intravascular coagulation (platelet count $<100x10^9$/L), severe metabolic disturbance (calcium level <1.87 mmol/L) and gastrointestinal bleeding (>500ml of blood/24 hr) were reported as systemic complications.[19] For reasons of comparability of the data from this study with those of the PROPATRIA study[13], infectious complications were defined as infected (peri-)pancreatic necrosis, bacteraemia, pneumonia, urosepsis and infected ascites.

Data collection

The following data were extracted from the patient records: co-morbidity, length and body weight, American Society of Anesthesiologists classification (ASA classification), etiology, day of onset of symptoms, day of hospital admission, length of hospital stay, need for ICU admission, relevant laboratory findings (i.e., amylase, lipase, C-reactive protein, white blood cell count,

blood glucose, liver enzymes, calcium, hematocrit, arterial oxygen pressure, base deficit, urea, serum albumin), bacterial cultures, outcome of fine-needle aspiration (FNA), findings on CECT scan, presence of cholangitis or cholecystitis, presence of (multiple) organ failure and systemic complications, performance of ERCP and ES, length of administration of enteral nutrition and probiotics, type of intervention, presence of infectious complications, presence of bowel ischemia and mortality.

Statistical analysis

Absolute frequency and percentages were calculated for infectious complications, mortality and bowel ischemia. Normally distributed data are presented as means (±SD). Non-normally distributed data are presented as median with interquartile range (IQR: P25–P75). Statistical analyses were performed using SPSS® for Windows® version 16.0.2 (SPSS, Chicago, Illinois, USA).

RESULTS

Baseline characteristics

From January 1st, 2003 to December 31st 2010, 99 consecutive patients with predicted severe pancreatitis without organ failure were admitted to the medium care unit. All these patients received enteral nutrition and Probioflora as described. Baseline characteristics are shown in Table 1. Male:female ratio was 1:1, with a median age of 56 years (IQR 44-68 years). Median time between onset of symptoms and hospital admission was 0 days (IQR 0-0 days), and since patients were immediately admitted to the medium care unit, the time between hospital admission and admission to the medium care was also 0 days (IQR 0-2 days). The twice daily, single dose of Probioflora and enteral nutrition was initiated after a median of 4 days (IQR 2-8 days). CECT was performed after a median of 6 days (IQR 4-7 days) in hospital.

Outcomes

Table 2 shows the patient outcomes. Sixty patients (60%) developed necrotizing pancreatitis, with a median CT severity index of 5 (IQR 3-6). Infectious complications were diagnosed in 42 patients (42%): bacteraemia in 40 patients (40%), pneumonia in 11 patients (11%) and infected necrosis in 11 patients (11%). No patients developed urosepsis or infected ascites. Severe systemic complications occurred in 48 patients (48%): severe metabolic disturbance in 47 patients (47%), disseminated intravascular coagulation in 11 patients (11%) and severe gastrointestinal bleeding in 2 patients (2%). New onset organ failure during hospital admission, but *after* administration of Probioflora, was reported in 27 patients: seven patients (7%) developed single organ failure and 20 patients (20%) developed multiple organ failure.

	N=99
Age (years)	56 (44-68)
Sex (male)	58 (59%)
BMI (kg/m2)*	30.7 (5.9)
Cause of pancreatitis	
Biliary	51 (52%)
Alcohol	25 (25%)
Other	23 (23%)
American Society of Anaesthesiologists classification	
I (healthy status)	25 (25%)
II (mild systemic disease)	43 (43%)
III (severe systemic disease)	31 (31%)
Severity of pancreatitis	
Imrie score (first 48h)	3.1 (1.4)
Ranson score (first 48h)**	3.8 (1.9)
C-reactive protein (mg/L) (highest first 48h)**	221 (81)
Time from first symptoms to admission (days)	0 (0-0)
Time from admission to first dose (days)	4 (2-8)
Time from admission to enteral nutrition (days)	4 (2-8)

Table 1. Baseline characteristics of 99 patients with predicted severe pancreatitis receiving probiotic treatment with Probioflora. Data are n (%), mean (SD), or median (IQR). Unless noted, data were available in all 99 patients. *Data available in 35/99 patients. **Data available in 98/99 patients.

	N=99
Contrast-enhanced CT	
Necrotizing pancreatitis	60 (60%)
<30% necrosis	36 (36%)
>30% necrosis	24 (24%)
No contrast-enhanced CT performed	3 (3%)
CT severity index	5 (0-10)
Any infectious complication	42 (42%)
Infected necrosis	11 (11%)
Bacteraemia	40 (40%)
Pneumonia	11 (11%)
Percutaneous drainage	8 (8%)
Surgical intervention, any indication	9 (9%)
Necrosectomy	7 (7%)
Intensive care admission	16 (16%)
Intensive care stay (days)	25 (12-36)
Hospital stay (days)	19 (13-30)
OF during admission, any onset*	27 (27%)
MOF during admission, any onset*	20 (20%)
Bowel ischemia	2 (2%)
Mortality	8 (8%)

Table 2. Clinical outcome. Data are n (%), mean (SD), or median (IQR). OF: Organ failure. MOF: Multiple organ failure. *New onset (multiple) organ failure during hospital admission, but after administration of probiotics was started.

16/99 patients (16%) who were admitted to the medium care unit needed transfer to the ICU because of development of organ failure. Overall median length of hospital admission was 19 days (IQR 13-30 days). Eight patients (8%) died. Except for one patient, all eight patients had new onset multiple organ failure during hospitalization and in 3/8 patients (38%) infected necrosis was diagnosed. In 6/8 patients (75%) autopsy was performed and no signs of bowel ischemia were detected.

Infectious complications

The pathogens isolated from the patients with infectious complications were mainly Staphylococcus spp. and Enterococcus spp. (Table 3). The overall median time between hospital admission and diagnosis of any infectious complication was 11 days (IQR 8-19 days). Median time between admission and diagnosis of bacteraemia was 9 days (IQR 6-15 days), pneumonia 7 days (IQR 3-13 days) and infected necrosis 19 days (IQR 13-31). In patients with infected necrosis, infection was diagnosed in 1/11 patients (9%) in the first week after admission, in 2/11 patients (18%) in the second week after admission, in 4/11 patients (36%) in the third week after admission and in 4/11 patients (36%) after three weeks of admission.

Bowel ischemia

Bowel ischemia was diagnosed in two patients who received Probioflora and enteral nutrition. As no data were available on histopathological examination, no specific cause for the bowel ischemia could be reported.

The first patient was a 51-year old male with an alcoholic pancreatitis without comorbidity. Open necrosectomy due to infected necrosis was performed in six separate procedures on day 34, 44, 52, 62, 90 and 109. During necrosectomy on day 62, multiple necrotic perforations of the descending colon were found. Ultimately, this patient recovered.

The second patient who developed bowel ischemia was a 62-year old male with a history of gastro-esophageal reflux disease and axonal neuropathy, who was admitted with biliary pancreatitis. On day 38, a laparotomy was performed and a small part of the duodenum and the proximal jejunum were resected because of ischemic alterations at the site of the tip of the nasojejunal feeding tube. On day 42, an ischemic colitis was found during the second open procedure. The patient died on day 62 and no autopsy was performed.

Gram-positive bacteria	
Staphylococcus *spp.*	26
Staphylococcus epidermidis	9
Staphylococcus hominis	6
Staphylococcus aureus	6
Enterococcus *spp.*	5
Corinebacterium *spp.*	4
Streptococcus *spp.*	3
Gram-negative bacteria	
Enterobacter *spp.*	17
Klebsiella *spp.*	10
Escherichia coli	5
Pseudomonas *spp.*	4
Other gram-negative microorganisms*	6
Fungi	0

Table 3. Pathogens isolated from 42/99 patients with an infectious complication. When different organisms were cultured from different sites of a single patient, all microorganisms are listed. If a single organism was cultured from different sites of a single patient, this organism was listed only once. *Acinobacter spp. (1), and Sphingobacter spp. (1).

DISCUSSION

This cohort of patients with predicted severe pancreatitis without initial organ failure was analyzed retrospectively in order to obtain clues for the yet unknown mechanism behind probiotic-induced bowel ischemia, as shown in the PROPATRIA study. Although in the current study treatment of the patients was not state-of-the-art (e.g. initial total parenteral nutrition in every patient), we focused on potential negative effects of probiotic treatment with Probioflora and did not find an increased incidence of bowel ischemia and mortality.

This conclusion is based on comparison with the literature.[13,20] Unfortunately, a direct comparison with the patients included in the PROPATRIA trial[13] is not realistic given the major differences between both cohorts: 1) PROPATRIA included patients with initial organ failure whereas this study did not, 2) the mixture of probiotics used was different and 3) parenteral nutrition was administered routinely in the current study, whereas in PROPATRIA enteral nutrition was used.

In the current study, probiotics were administered after a median of four days of hospital admission. Administration of probiotics starting four days after admission is outside the time interval considered “the window for prophylaxis”, suggested to be as small as 48 hours. In a mouse model for acute pancreatitis, Rychter *et al*. showed that the effectiveness of probiotics strongly depends on the timing of administration; administration of probiotics before induction of pancreatitis prevents intestinal barrier dysfunction to occur, while administration started after induction of disease was ineffective in this respect.[21]

The percentage of infectious complications reported in this study (42%) is higher than reported in two large randomized controlled trials (30% and 31%).[13,22] The high rate of infections may be related to the prolonged use of TPN as this has been documented to increase the incidence of positive blood cultures.[9]

In the current study, ultimately 27 patients developed (late) organ failure, while receiving Probioflora and ultimately 16 of these patients were admitted to the ICU. In none of these patients with organ failure was bowel ischemia diagnosed. However, one has to bear in mind that there was no formal strategy to detect bowel ischemia in these patients. The overall incidence of bowel ischemia was 2% and mortality 8%. Both this frequencies are within the expected range as described in the literature in patients with predicted severe acute pancreatitis.[20] The bowel ischemia, however, was diagnosed after several weeks after hospital admission (i.e. day 38 and day 62), whereas in the PROPATRIA study bowel ischemia was diagnosed within the first 2 weeks of admission. Furthermore, in PROPATRIA in all but one of the 9 patients who developed bowel ischemia already suffered from organ failure on the first or second day of admission. These findings might suggest that the patients in the current study may have suffered from another type of bowel ischemia and not specifically probiotic-related.

In 2009, Besselink *et al*. discussed whether the negative outcome of the PROPATRIA study may be related to the presence of organ failure at the time the probiotics were administered.[6] The post-hoc sub-group analysis of the PROPATRIA cohort showed that a higher degree of enterocyte damage occurred in patients who received probiotics when they already had organ failure. These findings may suggest that probiotics do not increase the incidence of bowel ischemia when administered to patients without early organ failure. Although the current study supports this hypothesis there is no clear mechanism available to explain these findings.

In conclusion, this study describes the first analysis of a cohort of patients with predicted severe pancreatitis without initial organ failure treated with probiotic treatment with Probioflora after the unexpected outcome of the PROPATRIA study in 2008. Although we acknowledge that patient care was not conform current guidelines and comparison with the PROPATRIA study is not realistic, we could not find a clear positive or negative impact of probiotic treatment with Probioflora on the incidence of bowel ischemia and mortality in patients with predicted severe pancreatitis without organ failure.

Acknowledgements

We thank the nursing staff of the department of Internal Medicine, Faculty Thomayer´s Hospital, Prague, for their hospitality and assistance.

References

1. UK guidelines for the management of acute pancreatitis. Gut 2005;54 Suppl 3iii1-iii9.
2. Besselink MG, van Santvoort HC, Boermeester MA, et al. Timing and impact of infections in acute pancreatitis. Br.J.Surg. 2009; 96(3)267-273.
3. Ammori BJ, Leeder PC, King RF, et al. Early increase in intestinal permeability in patients with severe acute pancreatitis: correlation with endotoxemia, organ failure, and mortality. J.Gastrointest.Surg. 1999;3(3)252-262.
4. Deitch EA. The role of intestinal barrier failure and bacterial translocation in the development of systemic infection and multiple organ failure. Arch.Surg. 1990;125(3)403-404.
5. Van Leeuwen PA, Boermeester MA, Houdijk AP, et al. Clinical significance of translocation. Gut 1994;35(1 Suppl) S28-S34.
6. Besselink MG, van Santvoort HC, Renooij W, et al. Intestinal barrier dysfunction in a randomized trial of a specific probiotic composition in acute pancreatitis. Ann Surg. 2009 Nov;250(5):712-9.
7. de Vries AC, Besselink MG, Buskens E, et al. Randomized controlled trials of antibiotic prophylaxis in severe acute pancreatitis: relationship between methodological quality and outcome. Pancreatology. 2007;7(5-6)531-538.
8. Wittau M, Mayer B, Scheele J, et al. Systematic review and meta-analysis of antibiotic prophylaxis in severe acute pancreatitis. Scand.J.Gastroenterol. 2011;46(3)261-270.
9. Petrov MS, van Santvoort HC, Besselink MG, et al. Enteral nutrition and the risk of mortality and infectious complications in patients with severe acute pancreatitis: a meta-analysis of randomized trials. Arch Surg. 2008 Nov;143(11):1111-7.
10. Al-Omran M, Albalawi ZH, Tashkandi MF, et al. Enteral versus parenteral nutrition for acute pancreatitis. Cochrane Database Syst Rev. 2010 Jan 20;(1).
11. Gatt M, MacFie J. Randomized clinical trial of gut-specific nutrients in critically ill surgical patients. Br J Surg. 2010 Nov;97(11):1629-36.
12. Olah A, Belagyi T, Issekutz A, et al. Randomized clinical trial of specific lactobacillus and fibre supplement to early enteral nutrition in patients with acute pancreatitis. Br.J.Surg. 2002;89(9)1103-1107.
13. Besselink MG, van Santvoort HC, Buskens E, et al. Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. Lancet 2008;371(9613)651-659.
14. Sharma B, Srivastava S, Singh N, et al. Role of probiotics on gut permeability and endotoxemia in patients with acute pancreatitis: a double-blind randomized controlled trial. J.Clin.Gastroenterol. 2011;45(5)442-448.
15. Lata J, Stiburek O. [Prophylactic antibiotics and probiotics in acute pancreatitis]. Vnitr.Lek. 2010;56(6)582-584.
16. Sand J, Nordback I. Probiotics in severe acute pancreatitis. Lancet 2008;371(9613)634-635.
17. Blamey SL, Imrie CW, O'Neill J, et al. Prognostic factors in acute pancreatitis. Gut 1984;25(12)1340-1346.

18. Werner J, Hartwig W, Uhl W, et al. Useful markers for predicting severity and monitoring progression of acute pancreatitis. Pancreatology. 2003;3(2)115-127.
19. Bradley EL, III. A clinically based classification system for acute pancreatitis. Summary of the International Symposium on Acute Pancreatitis, Atlanta, Ga, September 11 through 13, 1992. Arch. Surg. 1993;128(5)586-590.
20. Hirota M, Inoue K, Kimura Y, et al. Non-occlusive mesenteric ischemia and its associated intestinal gangrene in acute pancreatitis. Pancreatology 2003; 3: 316–22.
21. Rychter JW, van Minnen LP, Verheem A, et al. Pretreatment but not treatment with probiotics abolishes mouse intestinal barrier dysfunction in acute pancreatitis. Surgery 2009;145(2)157-167.
22. Isenmann R, Runzi M, Kron M, et al. Prophylactic antibiotic treatment in patients woth predicted severe acute pancreatitis; a placebo-controlled, double-blind trial. Gastroenterology. 2004;126:997-1004.

Part II

Diagnosis of infection and interventional strategies in acute pancreatitis

Chapter 5

The role of routine fine-needle aspiration in the diagnosis of infected necrotizing pancreatitis

Surgery 2014; 155(3):442-448

Mark C van Baal[1]
Thomas L Bollen[2]
Olaf J Bakker[3]
Harry van Goor[4]
Marja A Boermeester[5]
Cees H Dejong[6]
Hein G Gooszen[1]
Erwin van der Harst[7]
Casper H. van Eijck[8]
Hjalmar C van Santvoort[3]
Marc G Besselink[5]
for the Dutch Pancreatitis Study Group

[1] Dept. Operating Room/Evidence Based Surgery, Radboud University Nijmegen Medical Centre, Nijmegen
[2] Dept. of Radiology St. Antonius Hospital, Nieuwegein
[3] Dept. of Surgery, University Medical Center Utrecht, Utrecht
[4] Dept. of Surgery, Radboud University Nijmegen Medical Centre, Nijmegen
[5] Dept. of Surgery, Academic Medical Center, Amsterdam
[6] Dept. of Surgery, Maastricht University Medical Center, Maastricht
[7] Dept. of Surgery, Maasstad Hospital, Rotterdam
[8] Dept of Surgery, Erasmus Medical Center, Rotterdam
All: the Netherlands

ABSTRACT

Background: Diagnosing infected necrotizing pancreatitis (INP) may be challenging. The aim of this study was to determine the added value of routine fine needle aspiration (FNA) next to clinical and imaging signs of infection in patients who underwent intervention for suspected INP.

Methods: Post-hoc analysis of 208 consecutive patients from a prospective multicenter database who underwent intervention because of suspected INP. In retrospect, three groups were constructed based on the patients preoperative characteristics: clinical, imaging and FNA. Patients in the clinical group had clinical signs of infection but no gas on preoperative CT and no FNA performed prior to intervention. Patients in the imaging group had gas bubbles on the preoperative CT scan but no FNA performed and patients in the FNA group had a positive FNA prior to intervention. The reference standard for infection was the culture taken during the first intervention (either catheter drainage or necrosectomy).

Results: The initial intervention for INP was performed a median of 27 days (IQR 20-39 days) after admission, without difference between the three groups (P=0.15). Infection was confirmed in 80% of 92 patients of the clinical group, in 94% of 88 patients of the imaging group, and in 86% of 28 patients of the FNA group (P=0.07). Mortality was 19% without differences between groups (P=0.39).

Conclusion: INP can generally be diagnosed based on clinical or imaging signs of infection. FNA can be performed on indication, in patients with unclear clinical signs and no imaging signs of INP.

INTRODUCTION

Acute pancreatitis is the most common gastrointestinal condition requiring acute hospitalization in the United States.[1] Twenty percent of patients develop necrotizing pancreatitis.[2] The 2012 revised Atlanta classification defines necrotizing pancreatitis by the presence of either pancreatic parenchymal or only peripancreatic necrosis.[3] In approximately 30% of these patients infection of the necrosis occurs (infected necrotizing pancreatitis, INP) which requires radiological or surgical intervention in the vast majority of patients.[2,4] Interventions in these often critically ill patients carry a morbidity of 50-100% and a mortality of 15-25%.[4-9] Therefore, many studies have focused on prevention of INP. Surprisingly, only few studies have addressed the topic of diagnosing INP.

Suspicion of infected necrosis can be based on clinical signs only (e.g., fever, organ failure), on imaging signs namely gas bubbles in peripancreatic collections on computed tomography (CT), on positive microbiological culture obtained by fine-needle aspiration (FNA), or on a combinations of these factors.[10,11] Since the initial Atlanta classification[12] in 1993, only one retrospective study reported on the incidence of gas in peripancreatic collections (24% of 42 patients) in relation to patient outcome in patients with necrotizing pancreatitis.10 On the other hand, several studies reported on the use of FNA in diagnosing infected necrosis.[6,11,13-17] As a result of these studies, some authors propose routine FNA in patients with necrotizing pancreatitis, as reflected by the high use of FNA in the literature (40-100%).[5,17-19] The accuracy of FNA to diagnose infected necrosis may be high (ranging from 67 to 98%)[19-21], but, for several reasons, the added value of routine FNA may be limited[7]. First, with the current preferred approach of delayed intervention, even in case of infected necrosis, FNA has limited therapeutic implications.[4,22] Second, false-negative and false-positive (contamination) rates have been reported up to 25% and 15%, respectively.[7,18,23] Finally, although FNA is a considered to be a safe and minimally invasive procedure, it does carry a small risk of procedure-related complications (e.g., bleeding, perforation, iatrogenic infection).[24]

The aim of this study was to determine the individual role and (added) value of clinical and imaging signs and, especially, FNA in diagnosing INP.

PATIENTS AND METHODS

Patients

We performed a post-hoc analysis in a prospective database of 639 patients with necrotizing pancreatitis, included between March 2004 and November 2008 in all eight Dutch university medical centres and 13 large teaching hospitals of the Dutch Pancreatitis Study Group. This cohort has been described previously.[4] During the study period, all patients admitted with acute pancreatitis were registered in a prospective database.[8,25] Patients were selected for the current study if they underwent intervention for suspected infection of peripancreatic

or pancreatic necrosis. Patients were excluded if intervention was performed for other indications than (suspected) infection (e.g., abdominal compartment syndrome, bleeding, bowel ischaemia or perforation).

Definitions and groups

A definitive diagnosis of INP was established by a positive microbiological culture obtained at the first intervention (either via percutaneous drainage or surgical necrosectomy). For this post-hoc analysis, the intervention culture was considered to be the reference standard for infection, regardless of other subsequent cultures obtained by drainage or re-interventions. A positive FNA culture prior to intervention or the presence of gas in peripancreatic collections on CT was not considered definite proof for infection, being the diagnostic variables under study. However, in clinical practice, a positive FNA culture was indicative for infected necrosis. Patients without intervention for suspected infected necrosis were excluded from further analysis, since the reference standard was lacking in these patients. All included patients were divided in three groups: 1) clinical signs of infection (clinical group); 2) gas in peripancreatic collections on CT and clinical signs (imaging group); and 3) FNA and clinical signs (FNA group).

To facilitate the analysis between the study groups, patients who had both gas in peripancreatic collections on CT and in whom FNA cultures were performed (*n*=16) were included in the imaging group, because FNA requires an additional intervention whereas information on the presence of gas in peripancreatic collections can be derived from the CT that is already performed routinely. However, in the clinical situation, this positive FNA was not ignored and used in the diagnostic work-up to establish the diagnosis of infected necrosis, therefore an additional sensitivity analysis was performed to determine the impact of including these 16 patients in the FNA group.

Treatment protocol

The treatment protocol has been described in detail previously.[4] In short, patients received broad-spectrum antibiotics in case of (suspected) INP initially. In case of clinical improvement, the antibiotic treatment regimen was narrowed based on culture results (if available). The majority of patients received broad-spectrum antibiotics for several weeks. Due to the multicenter character of this study it was not possible to record the exact use of antibiotics in all patients. Intervention was postponed, if possible for at least four weeks after onset of symptoms, allowing for demarcation and encapsulation of the infected collection, so called walled-off necrosis. The minority of the present cohort (*n*=88) was included in the PANTER trial and was assigned to open necrosectomy (*n*=45) or to the step-up approach (*n*=43).[8] Since 2006, a multidisciplinary expert panel, consisting of eight gastrointestinal surgeons, one gastroenterologist and three radiologists guided decisions on intervention.

Patients with (suspected) INP were evaluated by the expert panel and the treating physician was informed about the individual recommendations of the members of the panel. Notably, in every case the ultimate decision for treatment and intervention was made by the treating physician.

Clinical group

Patients who had no gas in peripancreatic collections on CT and in whom no FNA was performed, were classified as patients in whom the suspicion of INP was based on clinical signs. Unfortunately, no algorithm exists for establishing the diagnosis of infected necrosis only based on clinical signs and therefore it is not possible to provide clear cut-off points of vital, biochemical and mechanical outcome parameters to define infection. Usually, clinical deterioration was an important observation in patients with (suspected) infected necrosis. Examples of clinical signs are persisting sepsis, (new or prolonged) organ failure, increased need for cardiovascular and/or respiratory and/or renal support, leucocytosis, increased C-reactive protein levels and fever. Moreover, no other infectious focus must be found or held responsible for the clinical deterioration. However, since an experienced clinical judgment is needed in these complex and usually critically ill patients, in the majority of patients the decision to intervene was advised by the expert panel.

Imaging group

Patients with gas bubbles on CT were included in this group. CTs were performed at the discretion of the treating physician. One dedicated abdominal radiologist (TLB) reviewed all CTs, blinded for the clinical background and treatment.

Fine-needle aspiration group

With the policy of postponing intervention regardless the presence of (suspected) infection, routine FNA was not used routinely. The indication for performing FNA was left to the treating physician and therefore FNA was only performed in case of unclear clinical and radiological signs of infection. FNA was performed with ultrasound-guidance or CT-guidance.

Data collection

The following data were extracted from the prospective database: patient demographics, patient history, American Society of Anesthesiologists (ASA) class, etiology, day of hospital admission, length of hospital stay, laboratory findings, CT findings from the initial hospital and second review by an experienced abdominal radiologist, presence of infectious complications, presence of (multiple) organ failure, clinical course, type of intervention(s), cultures from FNA and first intervention, and mortality.

Statistical analysis

All patients were analyzed in the three predefined groups. Per group, all data were pooled and baseline characteristics were listed. Percentages were calculated for baseline characteristics and outcomes and all intervention cultures were compared. Continuous data were presented as mean ± standard deviation (SD) and non-normally distributed data were presented as median (interquartile range, (IQR)). Differences were compared with the Chi-square or Mann-Whitney U tests, as appropriate. A p-value of 0.05 or less was considered as statistically significant. All statistical analyses were performed using SPSS® for Windows® version 16.0.2 (SPSS, Chicago, Illinois, USA).

RESULTS

Baseline characteristics

Of 639 consecutive patients with necrotizing pancreatitis, 208 patients (32%) underwent either percutaneous drainage or surgical necrosectomy for suspected INP, and could therefore be evaluated for the reference standard of infected necrosis. Median age was 60 years (IQR 48-69) with male:female ratio 2:1. The clinical group consisted of 92 (44%) of 208 patients, these patients had neither gas bubbles on CT nor was FNA performed. Gas in peripancreatic collections on CT was seen in 88 (42%) of 208 patients (imaging group) and FNA was performed in 28 (13%) of 208 patients (FNA group). Baseline characteristics are shown in Table 1.

Timing of intervention

There was no difference in timing of the first intervention between the three groups. In the clinical group (*n*=92), intervention was performed at a median of 27 days (IQR 21-38 days) after hospital admission versus 31 days (IQR 22-46 days) in the imaging group and 31 days (IQR 18-38 days) in the FNA group (P=0.15).

Gas in collections with necrosis (*n*=88) was seen after a median of 22 days (IQR 13-37 days) after hospital admission and the first intervention was performed a median of 10 days later. The first FNA (*n*=28) was performed a median of 17 days (IQR 10-28 days) after hospital admission and the first intervention was performed a median of 14 days later.

Diagnostic accuracy

Infected necrosis was documented by a positive culture of material obtained during the first intervention (i.e., the reference standard) in 74 (80%) of 92 patients of the clinical group, in 83 (94%) of 88 patients of the imaging group, and in 24 (86%) of 28 patients of the FNA group (P=0.07).

In 19 of the 28 patients (68%) in the FNA group the FNA-cultures matched with the intervention cultures. In 8 (29%) of 28 patients, other (new) micro-organisms were found during intervention culture, in these patients the FNA

Characteristic	All patients (*n*=208)	Clinical group (*n*=92)	Imaging group (*n*=88)	FNA group (*n*=28)
Age – yr	60 (48-69)	58 (45-69)	61(51-72)	57 (43-64)
Male sex	142 (68)	60 (64)	60 (68)	22 (79)
Etiology				
Biliary	101 (49)	36 (39)	49 (56)	16 (57)
Alcohol abuse	44 (21)	30 (33)	10 (11)	4 (14)
Other	18 (9)	6 (8)	7 (8)	5 (18)
Unknown	45 (21)	20 (21)	22 (25)	3 (11)
ASA-class on admission				
I (healthy status)	57 (27)	21 (23)	22 (25)	14 (50)
II (mild systemic disease)	113 (54)	53 (59)	50 (57)	10 (36)
III (severe systemic disease)	38 (18)	18 (18)	16 (18)	4 (14)
Predicted severity of pancreatitis				
APACHE-II score on admission	8 (5-11)	9 (5-11)	8 (5-11)	6 (4-10)
APACHE-II score >8 on admission	95 (46)	47 (50)	42 (48)	6 (21)
Imrie-score on admission				
Imrie-score ≥3 on admission	4 (3-5)	4 (2-5)	3 (3-5)	4 (2-5)
Highest CRP level in first 48h of admission (mg/L)	158 (76) 295 (212-380)	69 (75) 289 (210-372)	67 (76) 289 (205-381)	22 (79) 335 (245-438)
CRP >150 (mg/L)	179 (86)	77 (78)	77 (88)	25 (89)
CT-severity index	8 (6-10)	8 (4-10)	6 (6-10)	7 (6-8)
Pancreatic necrosis	156 (75)	66 (72)	70 (80)	20 (71)
Peripancreatic necrosis alone	52 (25)	26 (28)	18 (20)	8 (29)
Extent of pancreatic necrosis				
<30%	102 (49)	40 (45)	46 (52)	16 (57)
30-50%	53 (25)	28 (30)	17 (19)	8 (29)
>50%	53 (25)	24 (25)	25 (28)	4 (14)

Table 1. Baseline characteristics. Continuous variables are in median (interquartile range), percentages are in parenthesis. CRP: C-reactive protein, APACHE: Acute Physiology And Chronic Health Evaluation, ASA: American Society of Anaesthesiologists

culture was considered to be false-negative. In one patient (4%) a false-positive culture was found. When all 44 patients who underwent FNA before intervention were analyzed (including all 16 patients with both gas bubbles and FNA), 27% of FNA cultures (12 out of 44 patients) did not match with cultures taken from the intervention, regardless of the presence of gas in peripancreatic collections on CT. In 11 (25%) of 44 patients other (new) microorganisms were found during intervention culture compared to the FNA culture was (median time between FNA and intervention 9 days, IQR 5-20 days) and in 1 (2%) of 44 patients a positive FNA culture was found with subsequently a negative intervention culture. In this patient, the time interval

between FNA and intervention was five days. These data are shown in detail in Table 2.

Mortality

Overall mortality was 19% (40 out of 208 patients) without differences between the groups: 18% (17 out of 92 patients) in the clinical group, 17% (15 out of 88 patients) in the imaging group, and 28% (8 out of 28 patients) in the FNA group (P=0.39). Mortality in all 44 patients who underwent FNA prior to intervention was 27% (12 out of 44 patients).

Microbiology

In 184 (88%) of 208 patients infected necrosis was confirmed by culture taken at the first intervention. In 114 of these 184 patients (62%) the infection was monomicrobial whereas in 70 patients (38%) two or more bacteria/fungi were cultured. The mortality between these groups did not differ (18%, (21 out of 114 patients) with monomicrobial culture *vs.* 21% (15 out of 70 patients) with polymicrobial culture, P=0.62). *Escherichia coli* was most frequently cultured (40%), followed by *Staphylococcus spp.* (28%) and *Enterococcus spp.* (25%). Yeasts were cultured in 9% of patients, predominantly *Candida spp.* No data were provided about the resistance pattern of micro-organisms cultured from the necrosis.

DISCUSSION

This study suggests that the diagnosis of INP can be based on clinical and imaging signs in the majority of patients. FNA can be used selectively in patients in whom the clinical signs are unclear and have no imaging signs of infection.

Routine use of FNA has previously been advocated in patients suspected of having INP.[26,27] This recommendation dates from a time period where the diagnosis of infected necrosis demanded immediate surgical treatment or interventional drainage. In current series, however, intervention is usually postponed, if clinically possible, until the necrosis has become walled-off.[4,6,22,28] Thus, even after confirmation of the diagnosis of infected necrosis, intervention is postponed. This is reflected by our data showing that the median timing of intervention was 29 days (IQR 22-41 days), without difference between the groups. Apparently, FNA did not lead to earlier intervention, whereas mortality was comparable between groups. Notably, no mortality was observed in the 11 patients with gas bubbles and/or positive FNA in whom intervention was postponed and ultimately waived because of successful conservative treatment.[4] These findings support the philosophy that the diagnosis of INP does not mandate an emergency intervention and are in line with previous studies.[4,7,29] Future studies should determine whether earlier intervention after positive FNA, without the current 10-14 days delay, can reduce morbidity or mortality.

Patient	FNA culture	Sensitivity profile	Antibiotics started after culture	Intervention culture	Sensitivity profile	Antibiotics started after culture	Time between FNA and intervention (days)
1	Moraxella spp.	PENI	IMIP, FLUCO	No growth	*n.a.*	IMIP, FLUCO	5
2	C. albicans E. faecium	VANCO, FLUCO	VANCO, FLUCO	C. albicans, E. faecium, P. aeruginosa	MERO, FLUCO	VANCO MERO, FLUCO	1
3	H. influenza	*missing*	*missing*	S. salivarius, Prefotella spp.	AUGM, PENI, COTRIM	*missing*	1
4	No growth	*n.a.*	*missing*	Klebsiëlla spp.	AUGM, CIPRO	MERO, CIPRO	5
5	P. mirabilis	PENI, AUGM	IMIP	C. albicans E. faecalis	AUGM, VANCO	IMIP	7
6	No growth	*n.a.*	none	Enterococcus spp.	VANCO	VACO	8
7	E. cloacae	*missing*	MERO	E. cloacae E. faecium	*missing*	VANCO, COTRIM	9
8	Streptococcus spp. Enterococcus spp.	*missing*	VANCO, FLUCO, CEFTA	B. fragilis, E. faecium	*missing*	VANCO	11
9	No growth	*n.a.*	none	Citrobacter spp. E. faecalis	AUGM, IMIP	IMIP, TEICO, FLUCO	15
10	No growth	*n.a.*	TAZO	Stenotrophomonas spp.	TAZO, COTRIM	CLINDA, COTRIM	20
11	E. coli	*missing*	*missing*	E. coli, E. cloacae Streptococcus spp.	*missing*	*missing*	36
12	No growth	*n.a.*	none	S. aureus	FLUCLOX	FLUCLOX	44

Table 2. Data of 12 patients with discrepancy between FNA culture and intervention culture. AUGM: Augmentin, CEFTA: Ceftazidim, CIPRO: Ciproxin, CLINDA: Clindamycine, COTRIM: Cotrimoxazol, FLUCO: Fluconazol, FLUCLOX: Flucloxacilline, IMIP: Imipenem, MERO: Meroponem, PENI: Penicilline, TAZO: Tazocin, TEICO: Teicoplanine, VANCO: Vancomycine, n.a.: Not applicable

The presence of gas in peripancreatic collections is considered by many as pathognomonic for INP.[11] Only three studies reported on the incidence of gas in peripancreatic collections.[10,11,20] Two studies published prior to 1993 included only a small number of patients (less than 30).[11,20] The third study, describing 42 patients with pancreatic necrosis on CT, found gas bubbles in 20 patients (48%).[10] However, because no consecutive series was described, the actual incidence of gas bubbles in patients with necrotizing pancreatitis

remained unclear. In the current study, only patients in whom an intervention was performed for suspected INP were included (208 out of 639 patients). Even though this was a selected subgroup of patients, it enabled us to compare the CT (and FNA) findings with the reference standard.

Infection of necrosis can occur at any given time after onset of symptoms but has a peak incidence between the third and fourth week.[30] Therefore, FNA performed early in the disease course often renders negative results. Moreover, negative FNA cultures are obviously only reliable for a short period of time. Cut-off points varying from 1-27 days have been reported[19,21,31], but most of the studies do not actually report on the time between FNA culture and intervention.[6,11,13-17]

The role of antibiotics in patients with suspected INP is still a topic of debate. In the current study, almost all patients with suspicion of infected necrosis received broad-spectrum antibiotics as part of the conservative treatment strategy. Consequently, outcome of FNA cultures may be influenced and false-negative FNA cultures could occur. This may be partly the reason for the high false-negative rate of 29%. Conversely, prolonged antibiotic treatment before intervention could result in a negative intervention culture and thus false-positive FNA cultures and false-positive gas bubbles in peripancreatic collections. Whether antibiotics substantially influenced the intervention cultures remains unclear, although it may partly explain the false-positive outcomes of both FNA and CT findings.

Our results show that in almost 40 per cent of patients with INP multiple micro-organisms were found at cultures taken from the first intervention and that in 27% of patients these findings did not (fully) correspond with the micro-organisms found with FNA culture. This may indicate that translocation of other intestinal micro-organisms occurred in the time period between FNA and intervention. These findings do not support the routine narrowing the antibiotic treatment based on FNA cultures.

This study has some limitations. First, since not all patients with necrotizing pancreatitis underwent a routine FNA and a subsequent intervention, this study cannot be seen as a purely diagnostic study. However, it seems rather unlikely that such a study will be ever performed given the clear ethical problems with such an approach. Second, both FNA and CTs were performed on the discretion of the treating physician. We cannot exclude that only patients without obvious clinical signs and no gas bubbles on CT scan underwent a FNA. This could lead to selection bias but has not further implications to the management of the individual patient. The main strength of this paper, however, lies in the use of a multicentre, prospective database specifically focussed on intervention in necrotizing pancreatitis in a consecutive series of patients.

In conclusion, this study showed that in the majority of patients INP can be diagnosed based on clinical and imaging signs and that FNA may be reserved for patients with unclear clinical signs without imaging signs of infection.

Although FNA may lead to an earlier diagnosis of INP, it is unclear whether this is of additional value. This could be addressed in future studies.

Collaborators

Members of the expert panel include: M. A. Boermeester, T. L. Bollen, C. H. Dejong, C. H. van Eijck, H. van Goor, H. G. Gooszen, H. S. Hofker, J. S. Laméris, M. S. van Leeuwen, A. F. Schaapherder, R. Timmer, and V. B. Nieuwenhuijs.

In addition to the authors, the following clinicians participated in this study: Maastricht University Medical Center, Maastricht: R. M. van Dam, J. P. Rutten, J. H. Stoot, Y. Keulemans, R. Vliegen; University Medical Center Utrecht, Utrecht: A. Roeterdink, U. Ahmed Ali, A. M. Schrijver, H. G. Rijnhart, G. A. Cirkel, K. J. van Erpecum, F. P. Vleggaar, L. M. Akkermans, M. S. van Leeuwen, G. T. Rijkers; St Antonius Hospital, Nieuwegein: M. J. Wiezer, B. L. Weusten, H. D. Biemond, B. van Ramshorst; University Medical Center Groningen, Groningen: R. J. Ploeg, H. T. Buitenhuis, S. U. van Vliet, S. Ramcharan, H. M. van Dullemen, H. S. Hofker, V. B. Nieuwenhuijs; Academic Medical Center, Amsterdam: O. van Ruler, W. Laméris, J. S. Laméris, D. J. Gouma, O. R. Busch, P. Fockens; Leiden University Medical Center, Leiden: A. Haasnoot, R. Veenendaal, A. F. Schaapherder; Gelderse Vallei Hospital, Ede: B. J. Witteman, P. M. Kruyt; Medical Center Leeuwarden, Leeuwarden: J. P. Pierie, P. Spoelstra, J. A. Dol, R. T. Gerritsen, E. R. Manusama; Maasstad Hospital, Rotterdam: J. F. Lange, N. A. Wijffels, L. A. van Walraven, P. P. Coene, F. J. Kubben; Amphia Hospital, Breda: J. H. Wijsman, R. M. Crolla, A. W. van Milligen de Wit, M. C. Rijk, G. P. van der Schelling; Reinier de Graaf Hospital, Delft: L. P. Stassen, T. Karsten; Gelre Hospital, Apeldoorn: H. Buscher, E. J. Hesselink; St. Elisabeth Hospital, Tilburg: J. Heisterkamp, H. van Oostvogel, M. J. Grubben; Canisius Wilhelmina Hospital, Nijmegen: A. C. Tan, C. Rosman; Erasmus Medical Center, Rotterdam: J. B. van der Wal, M. J. Morak, E. J. Kuipers, J. W. Poley, M. Bruno; Radboud University Nijmegen Medical Centre, Nijmegen: J. B. Jansen, J. J. Hermans, S. P. Strijk, C. J. van Laarhoven, V. Zeguers; Rijstate Hospital, Arnhem: P. J. Wahab; Jeroen Bosch Hospital, Den Bosch: D. Lips, J. G. Olsman, I. P. van Munster, K. Bosscha; Medisch Spectrum Twente, Enschede: J. J. Kolkman, A. B. Huisman, R. J. de Wit; Medical Center Alkmaar, Alkmaar: H. A. Tuynman, B. M. Wiarda, A. P. Houdijk; Meander Medical Center, Amersfoort: E. J. Consten, M. P. Schwartz, M. A. Brink; Vrije Universteit Medical Center, Amsterdam: D. L. van der Peet, C. J. Mulder, M. A. Questa.

References

1. Peery AF, Dellon ES, Lund J, et al. Burden of gastrointestinal disease in the United States: 2012 update. Gastroenterology 2012; 143: 1179-1187.
2. Banks PA, Freeman ML; Practice Parameters Committee of the American College of Gastroenterology. Practice guidelines in acute pancreatitis. Am J Gastroenterol 2006; 101: 2379-2400.
3. Banks PA, Bollen TL, Dervenis C, et al. Classification of acute pancreatitis--2012: revision of the Atlanta classification and definitions by international consensus. Gut 2012; Oct 25
4. van Santvoort HC, Bakker OJ, Bollen TL, et al. A conservative and minimally invasive approach to necrotizing pancreatitis improves outcome. Gastroenterology 2011; 141: 1254-1263.
5. Rau B, Bothe A, Beger HG. Surgical treatment of necrotizing pancreatitis by necrosectomy and closed lavage: changing patient characteristics and outcome in a 19-year, single-center series. Surgery 2005; 138: 28-39.
6. Büchler MW, Gloor B, Müller CA, et al. Acute necrotizing pancreatitis: treatment strategy according to the status of infection. Ann Surg 2000; 232: 619–626.
7. Rodriguez JR, Razo AO, Targarona J, et al. Debridement and closed packing for sterile or infected necrotizing pancreatitis: insights into indications and outcomes in 167 patients. Ann Surg 2008; 247: 294-299.
8. van Santvoort HC, Besselink MG, Bakker OJ, et al. A step-up approach or open necrosectomy for necrotizing pancreatitis. N Engl J Med 2010; 362: 1491–1502.
9. Bakker OJ, van Santvoort HC, van Brunschot S, et al. Endoscopic transgastric vs surgical necrosectomy for infected necrotizing pancreatitis: a randomized trial. JAMA 2012; 307: 1053-1061.
10. Baril NB, Ralls PW, Wren SM, et al. Does an infected peripancreatic fluid collection or abscess mandate operation? Ann Surg 2000; 231: 361–367.
11. Sarr MG, Nagorney DM, Mucha P Jr, et al. Acute necrotizing pancreatitis: management by planned, staged pancreatic necrosectomy/debridement and delayed primary wound closure over drains. Br J Surg 1991; 78: 576-581.
12. Bradley EL 3rd. A clinically based classification system for acute pancreatitis. Summary of the International Symposium on Acute Pancreatitis, Atlanta, Ga, September 11 through 13, 1992. Arch Surg 1993; 128: 586-590.
13. Bradley EL, Allen K. A prospective longitudinal study of observation versus surgical intervention in the management of necrotizing pancreatitis. Am J Surg 1991;161: 19-25.
14. Pederzoli P, Bassi C, Vesentini S, et al. A randomized multicenter clinical trial of antibiotic prophylaxis of septic complications in acute necrotizing pancreatitis with imipenem. Surg Gyn Obst 1993; 176: 480-483.
15. Banks PA, Gerzof SG, Langevin RE, et al. CT-guided aspiration of suspected pancreatic infection: bacteriology and clinical outcome. Int J Pancreatol 1995; 18: 265-270.
16. Kalfarentzos FE, Kehagias J, Kakkos SK, et al. Treatment of patients with severe acute necrotizing pancreatitis based on prospective evaluation. Hepatogastroenterol 1999; 46: 3249-3256.

17. Olah A, Belagyi T, Issekutz A, et al. Value of procalcitonin quick test in the differentiation between sterile and infected forms of acute pancreatitis. Hepatogastroenterol 2005; 52: 243-245.
18. Nordback I, Paajanen H, Sand J. Prospective evaluation of a treatment protocol in patients with severe acute necrotising pancreatitis. Eur J Surg 1997; 163: 357-364.
19. Ashley SW, Perez A, Pierce EA, et al. Necrotizing pancreatitis: contemporary analysis of 99 consecutive cases. Ann Surg 2001; 234: 572–579.
20. Sostre CF, Flournoy JG, Bova JG, et al. Pancreatic phlegmon – clinical features and course. Dig Dis Sci 1985; 30: 918-927.
21. Delattre JF, Chazal NL, Lubrano D, et al. Percutaneous ultrasound-guide drainage in complications of acute pancreatitis. Ann de Chir 2004; 129: 497-502.
22. Besselink MG, Verwer TJ, Schoenmaeckers EJ, et al. Timing of surgical intervention in necrotizing pancreatitis. Arch Surg 2007; 142: 1194-1201.
23. Paye F, Rotman N, Radier C, et al. Percutaneous aspiration for bacteriological studies in patients with necrotizing pancreatitis. Br J Surg 1998; 85: 755-759.
24. Banks PA, Pappas TN. Is computerized tomographic fine needle aspiration helpful in the management of infected pancreatic necrosis? Am J Gastroenterol 2005; 100:2371–2374.
25. Besselink MG, van Santvoort HC, Buskens E, et al. Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. Lancet 2008; 371: 651-659.
26. Uhl W, Warshaw A, Imrie C, et al. IAP Guidelines for the Surgical Management of Acute Pancreatitis. Pancreatology 2002; 2: 565-573.
27. Forsmark CE, Baillie J. AGA Institute technical review on acute pancreatitis. Gastroenterol 2007;132: 2022-2044.
28. Horvath K, Freeny P, Escallon J, et al. Safety and efficacy of video-assisted retroperitoneal debridement for infected pancreatic collections: a multicenter, prospective, single-arm phase 2 study. Arch Surg 2010;145: 817-825.
29. Mouli VP, Sreenivas V, Grag PK. Efficacy of concervative treatment, without necrosectomy, for infected pancreatic necrosis: a systematuc review and meta-analysis. Gastroenterol 2012, Oct 12 epub ahead of print.
30. Besselink MG, van Santvoort HC, Boermeester MA, et al. Timing and impact of infections in acute pancreatitis. Br J Surg 2009; 96:267-273.
31. Rau B, Pralle U, Mayer JM, et al. Role of ultrasonographically guided fine-needle aspiration cytology in the diagnosis of infected pancreatic necrosis. Br J Surg 1998; 85: 179-184.

Chapter 6

Systematic review of percutaneous catheter drainage as primary treatment for necrotizing pancreatitis

British Journal of Surgery 2011; 98(1):18-27

Mark C van Baal[1]
Hjalmar C van Santvoort[1]
Thomas L Bollen[2]
Olaf J Bakker[1]
Marc G Besselink[1]
Hein G Gooszen[3]
for the Dutch Pancreatitis Study Group

[1] Dept of Surgery, University Medical Center Utrecht
[2] Dept of Radiology, St Antonius Hospital Nieuwegein
[3] Dept of OR/Evidence Based Surgery, Radboud University Nijmegen Medical Centre, Nijmegen
All: the Netherlands

ABSTRACT

Objective: To evaluate the role of percutaneous catheter drainage (PCD) in patients with (infected) necrotizing pancreatitis.

Methods: A systematic literature search was performed. Inclusion criteria: 1) consecutive cohort of patients with necrotizing pancreatitis undergoing PCD as primary treatment of peripancreatic collections; 2) indication for PCD: (suspected) infected necrosis or symptomatic sterile pancreatic necrosis; 3) outcomes reported: percentage of infected peripancreatic collections, need for additional surgical necrosectomy, complications and mortality. Exclusion criteria: 1) cohorts of <5 patients; 2) cohorts including chronic pancreatitis; 3) studies on a selected subgroup of patients with acute pancreatitis: 'pseudocysts' or 'pancreatic abscesses' and/or exclusively sterile pancreatic necrosis; 4) cohort of patients in whom PCD was always combined with another minimally invasive strategy.

Results: Eleven studies, including 384 patients, fulfilled the inclusion criteria. Only one study was a randomized controlled trial (RCT). Four studies reported on the presence of organ failure prior to PCD; in 67% of 116 patients. Infected necrosis was proven in 271/384 patients (71%). In 214/384 patients (56%) no additional surgical necrosectomy was required after PCD. Complications consisted mostly of internal and external pancreatic fistulas. Overall mortality was 17% (67/384 patients). Nine of eleven studies separately reported mortality in patients with infected necrosis undergoing PCD: 15% (27/105 patients).

Conclusion: This study reviewed the outcome of PCD in a mixed group of patients with both sterile and infected pancreatic necrosis, largely in retrospective studies. A considerable number of patients can be treated with PCD without the need for surgical necrosectomy.

INTRODUCTION

Around 20% of patients with acute pancreatitis develop necrosis of the pancreas or peripancreatic fat tissue with associated peripancreatic collections.[1,2] Sterile necrosis can generally be managed conservatively and mortality is relatively low (12%).[1,3] Approximately 30% (range 14-62%) of patients with necrotizing pancreatitis, however, develop secondary infection of peripancreatic collections which is associated with sepsis and organ failure and is indication for intervention.[1]

For long, the first choice intervention in infected necrotizing pancreatitis has been surgical necrosectomy by laparotomy with the aim to remove all infected necrosis.[4-6] This approach is associated with considerable morbidity (34-95%) and mortality (11-39%).[1,7-9] Some patients with sterile necrosis will ultimately also undergo surgical necrosectomy in case of clinical deterioration (i.e. multiple organ failure) despite maximal supportive therapy on the basis of suspected infection. Others undergo necrosectomy because of persistent symptomatic external hepatobiliary or duodenal compression by peripancreatic collections.[5]

In 1998, Freeny et al. first described a consecutive series of patients exclusively with infected pancreatic necrosis who were primarily treated with imaging-guided percutaneous catheter drainage (PCD), as an alternative to primary surgical necrosectomy.[10] The rationale for PCD was to temporize sepsis and thereby postpone the need for surgical necrosectomy. In their retrospective cohort study, PCD was successful in postponing surgical intervention for a median of 4 weeks and even in obviating the need for surgical necrosectomy in almost half of the patients. In addition, PCD seems technically feasible in the vast majority of patients with necrotizing pancreatitis.[11] In the last decade, several cohort studies on PCD and minimally invasive necrosectomy (e.g. percutaneous necrosectomy[12], video-assisted retroperitoneal debridement (VARD)[13,14], transluminal endoscopic necrosectomy[15]) have been published. Recently, a minimally invasive 'step-up approach' consisting of PCD as first step and, if necessary, followed by VARD has proven to be more effective than primary open necrosectomy in a randomised trial (PANTER trial).[16] More than a third of patients with infected necrotizing pancreatitis had been successfully treated with PCD only.

This systematic review focuses on PCD as primary treatment for (infected) necrotizing pancreatitis. Primary aim was to determine the proportion of patients that can be treated with PCD without the need for additional necrosectomy from the published literature.

METHODS

Study selection

A systematic literature search was performed in EMBASE, MEDLINE and the Cochrane libraries from January 1st, 1992 to May 31st, 2010. The search was

limited to this episode, because before 1992, no universally accepted definitions were available for acute pancreatitis and pancreatic collections, frustrating the comparison of studies on PCD. In 1992, the Atlanta symposium provided definitions for acute pancreatitis and the different local complications such as pancreatic necrosis, pseudocysts and pancreatic abscesses.[17] Although it is recognized today that the Atlanta classification has considerable shortcomings and is currently under revision[18,19], the definitions have been widely used in the literature since 1992.

The MEDLINE and EMBASE search-terms were "(radiologic OR percutaneous OR drainage) AND pancreatitis". Search-terms for the Cochrane library were: "pancreatitis AND (radiologic OR percutaneous OR drainage)", restricted to title, abstract, keywords and English language. From the studies identified by the initial literature search, all titles and abstracts were screened to select studies reporting on patients undergoing PCD of peripancreatic collections associated with pancreatitis. Subsequently, full-text papers of the selected studies were independently screened by three authors (MvB, HvS and TB) to assess eligibility.

Inclusion criteria were: 1) a consecutive cohort of patients with acute necrotizing pancreatitis (ANP) undergoing PCD as a primary treatment of peripancreatic collections; 2) indication for PCD: (suspected) infected necrosis or symptomatic sterile pancreatic necrosis (e.g. clinical deterioration or significant mechanical obstruction); 3) essential outcomes reported: percentage of infected peripancreatic collections, need for additional surgical necrosectomy, complications and mortality.

Exclusion criteria were: 1) very small cohorts (< 5 patients); 2) cohorts including chronic pancreatitis (and results for acute pancreatitis not reported separately); 3) studies on a selected subgroup of patients with acute pancreatitis, classified as 'pseudocysts' or 'pancreatic abscesses' (as defined by the Atlanta classification) or sterile pancreatic necrosis exclusively; 4) cohorts of patients undergoing minimally invasive surgical necrosectomy which included previous PCD and cases treated by PCD only were not separately reported.

All cross-references were screened for potential relevant studies not identified by the initial literature search. The final decision on eligibility was reached by consensus.

Data extraction

From the included articles the following variables were extracted (if available): number of patients with ANP undergoing PCD, etiology, predictive severity scores (e.g. Ranson score[20], Acute Physiology And Chronic Health Evaluation [APACHE]-II score[21]) prior to PCD, percentage of patients with organ failure, CT severity scores (i.e. CT severity index [CTSI][22], modified CTSI[23], and Balthazar grade[24]), percentage of patients with infected necrosis, indication for PCD, time between hospital admission and PCD, drain size, total number of

drains placed, time to removal of drains, success of PCD (defined as survival without the need for additional surgical necrosectomy), total hospital stay, complications and mortality.

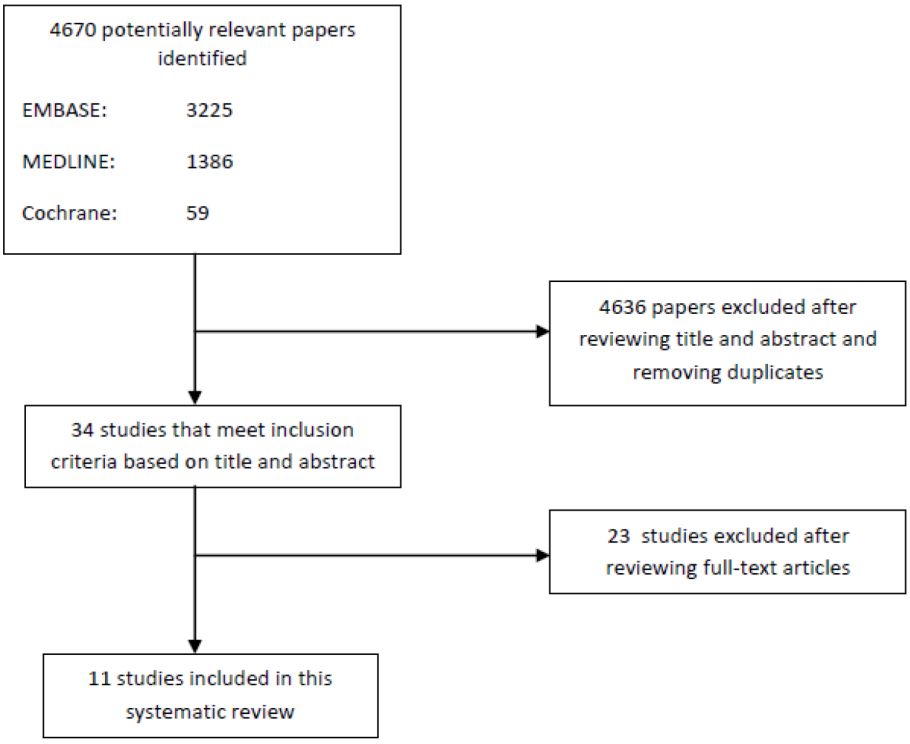

Figure 1. Study selection

RESULTS

Included studies

The results of the literature search are depicted in Figure 1. Of these 34 papers reporting on PCD of peripancreatic collections associated with pancreatitis, 23 were excluded for the following reasons: cohort of less than 5 patients (n= 1)[25], cohorts including patients with 'pseudocysts' (n= 1)[26], 'pancreatic abscesses' (n= 3)[27-29] or both (n= 1)[30], cohorts including patients with sterile pancreatic necrosis only (n= 2)[31,32], cohorts of mixed chronic and acute pancreatitis and outcomes not reported separately (n= 1)[33], one or more essential outcome not reported (n= 6)[34-39], PCD in combination with transgastric drainage (n= 2)[40,41] and cohorts where primary PCD was part of a 'step-up' approach in which PCD was always followed by minimally invasive surgical necrosectomy (n= 6)[42-47]. Finally, eleven studies were included in the current systematic review.[10,16,48-56] Nine studies were retrospective, non-controlled case-series[10,48,50-56], one study was

a prospective, non-controlled case-series[49] and one study was a multicentre RCT16 (Oxford, level 4 evidence and level 1b evidence, respectively).[57] Study characteristics of the eleven included studies are summarized in Table 1.

Patient characteristics

The pooled data from the 11 studies included in this study, comprised a total of 384 patients undergoing PCD as primary treatment for (suspected) infected necrosis or symptomatic sterile pancreatic necrosis (range of number of patients per study 8-80). Patient characteristics are summarized in Table 2.

Etiology of ANP was reported in seven studies and five other studies reported data on clinical details and CT severity (Table 2). In these 11 studies, various predictive severity scores and CT severity scores were used. Four studies (116 patients) reported on percentage of patients suffering from organ failure prior to PCD. Seventy-eight of 116 patients (67%) ,had organ failure (34 patients with single and 44 with multiple organ failure) prior to PCD. Out of the total of 384 patients, 271 patients (71%) had infected peripancreatic necrosis, defined as the presence of gas in the peripancreatic collection on CT or as a positive culture at fine needle aspiration in all studies. For PCD both the Seldinger and the Tandem trocar techniques were used and the majority of the radiological interventions were CT-guided. The size of the drains used, however, ranged from 8 to 28 French (2.7 to 9.3 mm).

Outcome

Table 3 shows outcomes as reported in the studies. Whenever possible, outcomes for infected and sterile necrosis are presented separately. The success rate of PCD, defined as the percentage of patients surviving without additional surgical necrosectomy, was 214/384 patients (56%). Eight studies reported specific data on patients with infected necrosis (n= 166): 87/166 patients (52%) recovered after PCD only. Five series reported on the time between insertion and removal of drains, varying from 16 to 98 days. Additional surgical necrosectomy was needed in 133/384 patients (35%). The remaining 9% (37 patients) were considered unfit for surgery or died before necrosectomy could be performed. The time interval between first PCD and surgery was reported in six series and ranged from 18 to 109 days. In those studies reporting on the number of catheters placed (7 studies), two or more catheters were usually placed per patient, with a maximum of 14 catheters.[54] No accurate mean number of procedures can be presented, since most series do not provide these data. Generally, drains were flushed with saline every eight hours and were replaced when occluded. In the one RCT on this subject a median of 1 catheter was placed with a median size of 14 French. Ultimately, drains were upgraded or replaced in 11 patients (26%).[16]

The complication rate was described in all but one series. One-hunderd

Author	Year published	Country	Study design	Study period	Inclusion criteria	Technique used
Freeny[10]	1998	United States	Retrospective, non-controlled case-series	1991 - 1995	- medically uncontrolled sepsis	CT-guided drainage, 10-28 Fr drains
Gambiez[48]	1998	France	Retrospective, non-controlled case-series	1990 - 1995	- secondary outbreak or persistence (>7 days) of signs of sepsis unexplained by a source of infection other than abdominal abscess - organ failure despite medical treatment - bacterial proof of infection	CT-guided drainage
Fotoohi[49]	1999	United States	Retrospective, non-controlled case-series	1988 - 1997	- sepsis - pain - biliary obstruction	CT-guided drainage, Seldinger / Trocar technique used, 8-24 Fr drains
Baril[50]	2000	United States	Retrospective, non-controlled case-series	1993 - 1997	- (suspected) pancreatic or peri-pancreatic sepsis	CT-guided drainage, Seldinger technique used, 10-12 Fr drains
Cheung[51]	2005	Hong Kong	Retrospective, non-controlled case-series	2001 - 2002	- deteriorated clinical condition - symptomatic pancreatic collections	CT-guided drainage, 20 Fr drains
Navalho[52]	2006	Portugal	Retrospective, non-controlled case-series	1993 - 2003	- infected pancreatic necrosis	US / CT-guided drainage, Trocar technique used, 12-14 Fr drains
Lee[53]	2008	Korea	Prospective, non-controlled case-series	2000 - 2004	- infected pancreatic necrosis	CT-guided drainage, 14 Fr drains stepwise dilated to 20 Fr drains
Bruennler[54]	2008	Germany	Retrospective, non-controlled case-series	1992 - 2004	- infected pancreatic necrosis	CT-guided drainage, Seldinger
Mortele[55]	2009	United States	Retrospective, non-controlled case-series	n.r	- infected pancreatic necrosis - suspected pancreatic necrosis, raised by fever, elevated WBC, or general clinical deterioration refractory to standard medical care	CT-guided drainage, Seldinger / Trocar technique used, up to 14 Fr drains
Rocha[56]	2009	United States	Retrospective, non-controlled case-series	2001 - 2005	*n.r.*	*n.r.*
Van Santvoort[16]	2010	The Netherlands	RCT	2005 - 2008	- infected pancreatic necrosis - persistent sepsis or progressive clinical deterioration despite maximal support on ICU, without documentation of infected necrosis	US / CT-guided, Seldinger technique used, minimal drain size 12 Fr

Table 1. Study characteristics of the included studies on percutaneous catheter drainage as primary treatment for necrotizing pancreatitis. n.r.: Not reported, WBC: White Blood cell Count, ICU: Intensive Care Unit, RCT: Randomized Controlled Trial, CT: Computed Tomography, US: Ultrasound, Fr: French.

Study	No. of patients	Etiology	Predictive severity scores	Organ failure	CT severity scores	Infected (peri)-pancreatic collections
Freeny[10] 1998	34	7/34 alcoholic 12/34 biliary 15/34 other	*n.r.*	*n.r.*	CTSI: mean 8.2	34/34 (100%)
Gambiez[48] 1998	10	*n.r.*	Ranson: mean 3.4	5/10 (50%)	Balthazar: Gr. D: 16 Gr. E: 37	3/10 (33%)
Fotoohi[49] 1999	60	20/60 alcoholic 6/60 biliary 34/60 other	*n.r.*	*n.r.*	*n.r.*	44/60 (73%)
Baril[50] 2000	38	*n.r.*	*n.r.*	*n.r.*	*n.r.*	25/38 (66%)
Cheung[51] 2005	8	1/8 alcoholic 5/8 biliary 2/8 other	Ranson: mean 5.9 (range 3-9)	*n.r.*	*n.r.*	4/8 (50%)
Navalho[52] 2006	30	9/30 alcoholic 17/30 biliary 4/30 other	*n.r.*	*n.r.*	*n.r.*	30/30 (100%)
Lee[53] 2008	18	*n.r.*	*n.r.*	*n.r.*	*n.r.*	18/18 (100%)
Bruennler[54] 2008	80	32/80 alcoholic 26/80 biliary 22/80 other	Ranson: median 2 (range 0-4) APACHE: median 18 (range 1-38)	65/80 on ICU	CTSI: median 6 (range 4-10)	52/80 (65%)
Mortele[55] 2009	35	8/35 alcoholic 12/35 biliary 15/35 other	Atlanta: mean 0.92 (range 0-3)	16/35 (46%)	Modified CTSI: mean 9.4 (range 8-10)	13/35 (37%)
Rocha[56] 2009	28	*n.r.*	*n.r.*	21/28 (75%)	*n.r.*	*9/28 (32%)*
van Santvoort[16] 2010	43	3/43 alcoholic 26/43 biliary 14/43 other	APACHE: mean 15 MODS: median 2 (range 0-9) SOFA: median 3 (range 0-11)	36/43 (84%)	CTSI: median 8 (range 4-10)	39/43 (91%)

Table 2. Patient characteristics of the included studies on percutaneous catheter drainage as primary treatment for necrotizing pancreatitis. n.r.: Not reported, Ranson: Severity score for acute pancreatitis[20], Atlanta: Severity score for acute pancreatitis[17], APACHE: Acute Physiology and Chronic Health Evaluation[21], CTSI: Computed Tomography Severity Index[22], Modified CTSI: Modified Computed Tomography Severity Index[23], Balthazar: CT severity score for acute pancreatitis[24], ICU: Intensive Care Unit

Study	No. of patients	Time admission until PCD (days)	Successful PCD	Need for additional surgery	Time between PCD and necrosectomy (days)	Patients with one or more complications	Mortality
Freeny[10] 1998	34	Mean 9 (1-48)	16 (47%) All IPN	18 (53%) All IPN	Mean 32 (6-78)	9 (26%)	4 (12%) All IPN
Gambiez[48] 1998	10	Mean 17 (10-25)	3 (30%) IPN: 0/3 SPN: 3/7	7 (70%) IPN: 3/3 SPN: 4/7	*n.r.*	6 (60%)	2 (20%) IPN: 2/3 SPN: 0/7
Fotoohi[49] 1999	60	*n.r.*	54 (90%)	3 (5%)	*n.r.*	6 (10%)	3 (5%)
Baril[50] 2000	38	*n.r.*	30 (79%) IPN: 18/25 SPN: 12/13	7 (18%) IPN: 6/25 SPN: 1/13	*n.r.*	1 (3%)	2 (5%) IPN: 2/25 SPN: 0/13
Cheung[51] 2005	8	Mean 55 (21-154) IPN: 30 SPN: 81	3 (38%) IPN: 1/4 SPN: 2/4	5 (63%) IPN: 3/4 SPN: 2/4	Mean 70 (1-161) IPN: 59 SPN: 88	4 (50%)	1 (13%) IPN: 1/4 SPN: 0/4
Navalho[52] 2006	30	Mean 18	19 (63%) All IPN	10 (33%) All IPN	Mean 18	*n.r.*	5 (17%) All IPN
Lee[53] 2008	18	Median 10 (1-58)	14 (78%) All IPN	3 (17%) All IPN	*n.r.*	2 (11%)	1 (6%) All IPN
Bruennler[54] 2008	80	Median 3.5 (1-40)	38 (48%)	24 (30%)	*n.r.*	23 (29%)	27 (34%)
Mortele[55] 2009	35	Mean 11 (2-33) IPN: 12 SPN:10	17 (49%) IPN: 6/13 SPN: 11/22	13 (37%) IPN: 7/13 SPN: 6/22	Mean 69 (3-445) IPN: 42 SPN: 101	4 (11%)	6 (17%) IPN: 1/13 SPN: 5/22
Rocha[56] 2009	28	*n.r.*	5 (18%)	17 (61%)	Median 109 (1-600)	3 (11%)	8 (29%) IPN: 4/9 SPN 4/19
van Santvoort[16] 2010	43	Median 30 (11-71)	15 (35%) IPN:13/39 SPN: 2/4	26 (60%) IPN: 25/39 SPN: 1/4	Median 10 (1-52)	17 (40%)	8 (19%) IPN: 7/8 SPN: 1/8

Table 3. Outcome of the included studies on percutaneous catheter drainage as primary treatment for necrotizing pancreatitis. n.r.: Not reported, PCD: Percutaneous catheter drainage, IPN: Infected pancreatic necrosis, SPN: Sterile pancreatic necrosis, MOF:Multiple organ failure

and three complications occurred in 75/354 patients (21%). The majority of complications were pancreatico-cutaneous and pancreatico-enteric fistulas (n= 53, 51% of all 103 complications). In total, nine other procedure-related complications were described. This included four bleeding complications of which two were self-limiting, and two consisted of massive bleeding due to puncture of the splenic artery. These two patients suffered from subsequent hemorrhagic shock and died. One colonic perforation, which required surgical intervention, was reported. The remaining four complications were considered minor and consisted of transient abdominal pain (n= 1), self-limiting pneumothorax (n= 1) and catheter dislodgement (n= 2).

The mortality rate was 17% (67 of 384) across studies. Nine of eleven studies reported the mortality for PCD in patients with infected necrosis: 15% (27 of 175 patients). In these studies, mortality for PCD in patients with sterile necrosis was 15% as well (10 of 69 patients).

DISCUSSION

This study reviewed the outcome of PCD in a mixed group of patients with both sterile and infected pancreatic necrosis, by enlarge in retrospective studies. The bundle of data, including the PCD step-up arm of the only RCT on the subject, supports the conclusion that a considerable number of patients can be treated with PCD without the need for surgical necrosectomy. Approximately half of the patients were treated with PCD, without the need for further surgical necrosectomy. In patients who did require surgical intervention, PCD allowed for postponing additional intervention for several weeks.

The across study mortality was 17% (reported in eleven studies), and 15% in patients with infected necrosis (reported in nine studies). Although not all studies provided data on mortality of patients with infected necrosis, mortality of 15% is similar to numbers reported for open “conventional” and minimally invasive necrosectomy. A recent review (1994-2008) of 11 series with more than 100 patients undergoing open necrosectomy demonstrated a mean mortality of 19%.[58] The same study reported 19% mortality in their series of 137 patients undergoing minimally invasive necrosectomy. Furthermore, in the one RCT on this subject mortality in the ‘step-up’ arm was 19% and multi organ failure was present in 35% of patients prior to intervention.[16]

Over the last two decades, PCD is increasingly used as primary minimally invasive treatment for necrotizing pancreatitis with proven or suspected infection. The rationale of PCD treatment is to improve the clinical condition of these usually seriously ill patients by drainage of “infected fluid (pus) under pressure” to either postpone surgical intervention or to even obviate the need for surgical necrosectomy. Postponing surgical intervention has been shown to improve outcome in patients needing necrosectomy for infected necrosis.[59] In 2000, Büchler et al. proposed that (surgical) intervention should be restricted to patients with infected peripancreatic or pancreatic necrosis.[4] This has

resulted in a much more critically ill group of patients undergoing intervention. The indication for PCD, however, differed between the 11 included series. Although all 384 patients suffered from necrotizing pancreatitis, only 71% indeed had infected peripancreatic collections proven by bacterial culture. Other indications for intervention were symptomatic 'organized necrosis' and 'severe clinical deterioration despite maximum conservative treatment'. These last two indications are not very well-defined and one may question whether these patients could not have been successfully treated conservatively.

In the pooled data of this systematic review, the percentage of patients with one or more complications was 21%, with only nine reported procedure-related complications. Series on surgical necrosectomy report a considerable higher complication rate, ranging from 34% to 68%.[3,60,61] Furthermore, in the current study, only 15% of patients developed a pancreatic fistula, compared to 22-47% in the studies on surgical necrosectomy.[62-64] However, mostly only early complications were described in the included studies. Except for the only RCT[16], no follow-up was reported in other studies and as a result, late complications (e.g. pseudocysts, pancreatic duct amputations, pancreatic insufficiency and chronic pancreatitis) were likely missed. In the PANTER trial, a 6-months complication rate of 30% was reported in the step-up arm, consisting of incisional hernias (7%), endocrine insufficiency (16%) and need for pancreatic enzyme suppletion (7%).[16] These results indicate that late complications do occur, while only short-term complications were reported in ten of the eleven included studies.

It is conceivable, that drain placement into a sterile peripancreatic collection can introduce bacteria resulting in secondary infection. Walser et al. showed that initially culture negative collections more frequently become infected after percutaneous drainage (13/22, 59%), than after simple fine needle aspiration (3/15, 20%, $p < 0.03$).[31] Not all such infections can be classified as iatrogenic, as sterile necrosis could become 'spontaneously' infected due to bacterial translocation through the bowel wall or by systemic infection.[65] A recent randomized study found an increased risk of developing infected necrosis by routine prolonged percutaneous catheter drainage of sterile peripancreatic collections (11/20, 55%) as compared to conservative treatment (4/20, 20%, $p = 0.048$).[32,66] None of the studies included reported on the rate of iatrogenic infection, but underreporting is likely to have occurred.

In the current study, almost a half of the patients still needed surgical necrosectomy after PCD. Although PCD may have ameliorated the patient's condition and may have improved outcome, it may also have caused unnecessary delay in a subgroup that is better off with primary surgical intervention. This review, however, does not allow for detecting and classifying this specific subgroup.

The size of the drains used varied from 8 to 28 French and in only one study the drainage tract was stepwise dilated.[53] Occluded or dislocated drains were

replaced by the interventional radiologist in all series. There is a tendency over time to use large bore catheters with a lower rate of catheter occlusion and need for replacement. In the future, it would be interesting to evaluate whether the use of large-size catheters will reduce the need for catheter replacement.

In most series, catheters were flushed daily with saline, in general every eight hours. The daily flushing in combination with the frequent need for catheter replacement makes PCD a relatively intensive and time-consuming therapy for the patient, surgeon and interventional radiologist.[67] However, in the RCT, 56% of patients in the step-up arm only needed one catheter placement with a median size of 14 French.[16]

In this review, studies reporting on PCD as part of a step-up approach for minimally invasive necrosectomy were not included in order not to mix the effects and complications of drainage only with those of minimally invasive necrosectomy (with or without videoscopic assistance) only.[42-46] With minimally invasive necrosectomy, not only the fluid compartment will be drained by the catheter, but also the remaining necrotic debris will be removed. This technique is becoming increasingly popular over the last decade.

A limitation of the current systematic review is that many of the included studies were small and retrospective. Moreover, in some series essential data were not presented (e.g. total number of interventions, outcome related to infectious status of collections, percentage of patients with organ failure at time of PCD). A formal assessment of methodological quality could not be performed because the papers did not provide enough detailed information for such an assessment.[68] This makes comparison with studies reporting on open necrosectomy even more difficult. It is likely that the success of PCD with the need for further surgery is overestimated due to selection bias in the overall results of this review, as success rate was 56% compared with 35% in the one RCT. Nevertheless, many patients with (suspected) infected necrotizing pancreatitis can recover with PCD as first and only intervention.

References

1. Banks PA, Freeman ML. Practice Parameters Committee of the American College of Gastroenterology. Practice guidelines in acute pancreatitis. Am J Gastroenterol 2006; 101(10): 2379-2400
2. British Society of Gastroenterology. United Kingdom guidelines for the management of acute pancreatitis. Gut 1998; 42 Supplement 2: S1-13
3. Nieuwenhuijs VB, Besselink MG, van Minnen LP et al. Surgical management of acute necrotizing pancreatitis: a 13-year experience and a systematic review. Scand J Gastroenterol Suppl 2003; 239: 111-116
4. Büchler MW, Gloor B, Müller CA et al. Acute necrotizing pancreatitis: treatment strategy according to the status of infection. Ann Surg 2000; 232(5): 619-626
5. Uhl W. et al, International Association of Pancreatology. IAP Guidelines for the surgical management of acute pancreatitis. Pancreatology 2002; 2(6): 565-573
6. Werner J, Feuerbach S, Uhl W et al. Management of acute pancreatitis: from surgery to interventional intensive care. Gut 2005; 54(3):426-436
7. Rau B, Bothe A, Beger HG. Surgical treatment of necrotizing pancreatitis by necrosectomy and closed lavage: changing patient characteristics and outcome in a 19-year, single-center series. Surgery 2005; 138:28-39
8. Rodriguez JR, Razo AO, Targarona J et al. Debridement and closed packing for sterile or infected necrotizing pancreatitis: insights into indications and outcomes in 167 patients. Ann Surg 2008; 247:294-299
9. Tsiotos GG, Luque-de León E, Sarr MG. Lon-term outcome of necrotizing pancreatitis treated by necrosectomy. Br J Surg 1998; 85:1650-1653
10. Freeny PC, Hauptmann E, Althaus SJ et al. Percutaneous CT-guided catheter drainage of infected acute necrotizing pancreatitis: techniques and results. AJR Am J Roentgenol 1998; 170(4): 969-975
11. Besselink MG, van Santvoort HC, Schaapherder AF et al, Dutch Acute Pancreatitis Study Group. Feasibility of minimally invasive approaches in patients with infected necrotizing pancreatitis. Br J Surg 2007; May;94(5):604-608
12. Carter CR, McKay CJ, Imrie CW. Percutaneous necrosectomy and sinus tract endoscopy in the management of infected pancreatic necrosis: an initial experience. Ann Surg 2000 Aug;232(2):175-180
13. Horvath KD, Kao LS, Ali A et al. Laparoscopic assisted percutaneous drainage of infected pancreatic necorsis. Surg Endosc 2001 Jul;15(7):677-682
14. van Santvoort HC, Besselink MG, Horvath KD et al, Dutch Acute Pancreatitis Study Group. Videoscopic assisted retroperitoneal debridement in infected necrotizing pancreatitis. HPB (Oxford) 2007;9(2):156-159
15. Seifert H, Biermer M, Schmitt W et al. Transluminal endoscopic necrosectomy after acute pancreatitis: a multicentre study with long-term follow-up (the GEPARD Study). Gut 2009 Sep;58(9): 1260-1266
16. van Santvoort HC, Besselink MG, Bakker OJ et al, Dutch Pancreatitis Study Group. A step-up approach or open necrosectomy for necrotizing pancreatitis. NEJM 2010;362:1491-1502

17. Bradley EL IIIrd. A clinically based classification system for acute pancreatitis. Summary of the International Symposium on Acute Pancreatitis, Atlanta, Ga, September 11 through 13, 1992. Arch Surg 1993; 128(5): 586-590
18. Bollen TL, van Santvoort HC, Besselink MG et al. Update on acute pancreatitis: ultrasound, computed tomography, and magnetic resonance imaging features. Semin Ultrasound CT MR 2007; 28(5): 371-383
19. Bollen TL, van Santvoort HC, Besselink MG et al, Dutch Acute Pancreatitis Study Group. The Atlanta Classification of acute pancreatitis revisited. Br J Surg 2008; 95(1): 6-21
20. Ranson JH, Rifkind KM, Roses DF et al. Prognostic signs and the role of operative management in acute pancreatitis. Surg Gynecol Obstet 1974; 139(1):69-81
21. Knaus WA, Draper EA, Wagner DP et al. APACHE II: a severity of disease classification system. Crit Care Med 1985;13(10):818-829
22. Balthazar EJ, Robinson DL, Megibow AJ et al. Acute pancreatitis: value of CT in establishing prognosis. Radiology 1990; 174(2):331-336
23. Mortele KJ, Wiesner W, Intriere L et al. A modified CT severity index for evaluating acute pancreatitis: improved correlation with patient outcome. AJR Am J Roentgenol 2004; 183(5):1261-1265
24. Balthazar EJ, Ranson JH, Naidich DP et al. Acute pancreatitis: prognostic value of CT. Radiology 1985; 156(3):767-772
25. Ashley SW, Perez A, Pierce EA et al. Necrotizing pancreatitis: contemporary analysis of 99 consecutive cases. Ann Surg 2001; 234(4):572-579; discussion 579-580
26. Cantasdemir M, Kara B, Kantarci F et al. Percutaneous drainage for treatment of infected pancreatic pseudocysts. South Med J 2003; 96(2):136-140
27. Mithöfer K, Mueller PR, Warshaw AL. Interventional and surgical treatment of pancreatic abscess. World J Surg 1997; 21(2):162-168
28. Srikanth G, Sikora SS, Baijal SS et al. Pancreatic abscess: 10 years experience. ANZ J Surg 2002; 72(12):881-886
29. vanSonnenberg E, Wittich GR, Chon KS et al. Percutaneous radiologic drainage of pancreatic abscesses. AJR Am J Roentgenol 1997; 168(4): 979-984.
30. Malecka-Panas E, Juszynski A, Chrzastek J et al. Pancreatic fluid collections: diagnostic and therapeutic implications of percutaneous drainage guided by ultrasound. Hepatogastroenterology 1998; 45(21):873-878
31. Walser EM, Nealon WH, Marroquin S et al. Sterile fluid collections in acute pancreatitis: catheter drainage versus simple aspiration. Cardiovasc Intervent Radiol 2006; 29(1):102-107
32. Zerem E, Imamovic G, Omerovic S et al. Randomized controlled trial on sterile fluid collections management in acute pancreatitis: should they be removed? Surg Endosc 2009; 23(12):2770-2777
33. Heider R, Meyer AA, Galanko JA et al. Percutaneous drainage of pancreatic pseudocysts is associated with a higher failure rate than surgical treatment in unselected patients. Ann Surg 1999; 229(6):781-787; discussion 787-789

34. Berzin TM, Banks PA, Maurer R et al. CT-guided percutaneous catheter drainage in necrotizing pancreatitis: outcomes among patients discharged with drains in place. J Vasc Interv Radiol 2008; 19(7):1002-1006
35. Kumar P, Mukhopadhyay S, Sandhu M et al. Ultrasonography, computed tomography and percutaneous intervention in acute pancreatitis: a serial study. Australas Radiol 1995; 39(2):145-152
36. Lee MJ, Rattner DW, Legemate DA et al. Acute complicated pancreatitis: redefining the role of interventional radiology. Radiology 1992; 183(1):171-174
37. Szentkereszty Z, Kerekes L, Hallay J et al. CT-guided percutaneous peripancreatic drainage: a possible therapy in acute necrotizing pancreatitis. Hepatogastroenterology 2002; 49(48):1696-1698
38. Szentkereszty Z, Kotán R, Pósán J et al. Therapeutic tactics in the treatment of acute necrotizing pancreatitis. Hepatogastroenterology 2008; 55(81): 266-269
39. Ai X, Qian X, Pan W et al. Ultrasound-guided percutaneous drainage may decrease the mortality of severe acute pancreatitis. J Gastroenterol 2010;45:77-85
40. Becker V, Huber W, Meining A et al. Infected necrosis in severe pancreatitis - combined non-surgical multi-drainage with directed transabdominal high-volume lavage in critically ill patients. Pancreatology 2009;9:280-286
41. Ross A, Gluck M, Irani S et al. Combined endoscopic and percutaneous drainage of organized pancreatic necrosis. Gastrointest Endosc 2010;71:79-84
42. Bruennler T, Langgartner J, Lang S et al. Percutaneous necrosectomy in patients with acute, necrotizing pancreatitis. Eur Radiol 2008; 18(8):1604-1610
43. Bucher P, Pugin F, Morel P. Minimally invasive necrosectomy for infected necrotizing pancreatitis. Pancreas 2008; 36(2):113-119
44. Chang YC, Tsai HM, Lin XZ et al. No debridement is necessary for symptomatic or infected acute necrotizing pancreatitis: delayed, mini-retroperitoneal drainage for acute necrotizing pancreatitis without debridement and irrigation. Dig Dis Sci 2006; 51(8):1388-1395
45. Echenique AM, Sleeman D, Yrizarry J et al. Percutaneous catheter-directed debridement of infected pancreatic necrosis: results in 20 patients. J Vasc Interv Radiol 1998; 9(4):565-571
46. Endlicher E, Völk M, Feuerbach S et al. Long-term follow-up of patients with necrotizing pancreatitis treated by percutaneous necrosectomy. Hepatogastroenterology 2003; 50(54):2225-2228
47. Bala M, Almogy G, Klimov A et al. Percutaneous “stepped” drainage technique for infected pancreatic necrosis. Surg Laparosc Endosc Percutan Tech 2009; 19(4):113-118
48. Gambiez LP, Denimal FA, Porte HL et al. Retroperitoneal approach and endoscopic management of peripancreatic necrosis collections. Arch Surg 1998; 133(1):66-72
49. Fotoohi M, D’Agostino HB, Wollman B et al. Persistent pancreatocutaneous fistula after percutaneous drainage of pancreatic fluid collections: role of cause and severity of pancreatitis. Radiology 1999; 213(2):573-578

50. Baril NB, Ralls PW, Wren SM et al. Does an infected peripancreatic fluid collection or abscess mandate operation? Ann Surg 2000; 231(3):361-367
51. Cheung MT, Ho CN, Siu KW et al. Percutaneous drainage and necrosectomy in the management of pancreatic necrosis. ANZ J Surg 2005; 75(4):204-207
52. Navalho M, Pires F, Duarte A et al. Percutaneous drainage of infected pancreatic fluid collections in critically ill patients: correlation with C-reactive protein values. Clin Imaging 2006; 30(2):114-119
53. Lee JK, Kwak KK, Park JK et al. The efficacy of nonsurgical treatment of infected pancreatic necrosis. Pancreas 2007; 34(4):399-404
54. Bruennler T, Langgartner J, Lang S et al. Outcome of patients with acute, necrotizing pancreatitis requiring drainage-does drainage size matter? World J Gastroenterol 2008; 14(5): 725-730
55. Mortelé KJ, Girshman J, Szejnfeld D et al. CT-guided percutaneous catheter drainage of acute necrotizing pancreatitis: clinical experience and observations in patients with sterile and infected necrosis. AJR Am J Roentgenol 2009; 192(1):110-116
56. Rocha FG, Benoit E, Zinner MJ et al. Impact of radiologic intervention on mortality in necrotizing pancreatitis: the role of organ failure. Arch Surg 2009; 144(3):261-265
57. Phillips B, Ball C, Sackett D et al. Oxford Centre for Evidence-based Medicine Levels of Evidence (May 2001) May 2001
58. Raraty MG, Halloran CM, Dodd S et al. Minimal access retroperitoneal pancreatic necrosectomy: improvement in morbidity and mortality with a less invasive approach. Ann Surg. 2010 May;251(5):787-93.
59 Besselink MG, Verwer TJ, Schoenmaeckers EJ et al. Timing of surgical intervention in necrotizing pancreatitis. Arch Surg 2007; 142(12):1192-1201
60. Howard TJ, Patel JB, Zyromski N et al. Declining morbidity and mortality rates in the surgical management of pancreatic necrosis. J Gastrointest Surg 2007; 11(1):43-49
61. Connor S, Alexakis N, Raraty MG et al. Early and late complications after pancreatic necrosectomy. Surgery 2005; 137(5):499-505
62. Tzovaras G, Parks RW, Diamond T et al. Early and long-term results of surgery for severe necrotising pancreatitis. Dig Surg 2004;21(1):41-46; discussion 46-47
63. Tsiotos GG, Smith CD, Sarr MG. Incidence and management of pancreatic and enteric fistulas after surgical management of severe necrotizing pancreatitis. Arch Surg 1995; 130(1):48-52
64. Harris H, Barcia A, Schell M et al. Necrotizing pancreatitis: a surgical approach independent of documented infection. HPB (Oxford). 2004;6(3):161-168
65. Beger HG, Bittner R, Block S et al. Bacterial contamination of pancreatic necrosis. A prospective clinical study. Gastroenterology 1986; 91(2): 433-438
66. Besselink MG, van Santvoort HC, Bakker OJ et al. Draining sterile fluid collections in acute pancreatitis? Primum non nocere! Surg Endosc 2010, April, Epub ahead of print

67. Mueller PR. Percutaneous drainage of pancreatic necrosis: is it ecstasy or agony? AJR Am J Roentgenol 1998; 170(4): 976-977
68. Moher D, Cook DJ, Eastwood S et al. Improving the quality of reports of meta-analyses of randomised controlled trials: the QUOROM statement. Quality of Reporting of Meta-analyses. Lancet 1999; 354(9193):1896-1900

Chapter 7

Endoscopic transpapillary stenting or conservative treatment for pancreatic fistulas in necrotizing pancreatitis: multicenter series and literature review

Annals of Surgery 2011; 253(5):961-967

Olaf J Bakker[1]
Mark C van Baal[1]
Hjalmar C van Santvoort[1]
Marc G Besselink[1]
Jan-Werner Poley[2]
Joos Heisterkamp[3]
Thomas L Bollen[4]
Hein G Gooszen[1]
Casper H van Eijck[5]
for the Dutch Pancreatitis Study Group

[1] Dept. of Surgery, University Medical Center Utrecht, Utrecht
[2] Dept. of Gastroenterology & Hepatology, Erasmus Medical Center Rotterdam
[3] Dept. of Surgery, St. Elisabeth Hospital, Tilburg
[4] Dept. of Radiology, St. Antonius Hospital, Nieuwegein
[5] Dept. of Surgery, Erasmus Medical Center, Rotterdam
All: the Netherlands

ABSTRACT

Objective: Endoscopic transpapillary stenting (ETS) of the pancreatic duct facilitates ductal outflow and may reduce time to pancreatic fistula closure. However, data on the feasibility of ETS in patients with necrotizing pancreatitis are scarce.

Background: Pancreatic fistulas often occur after intervention in necrotizing pancreatitis and frequently close only after months of conservative treatment.

Methods: From a prospective cohort of patients with acute pancreatitis admitted in 15 hospitals (2004-2007), all patients who underwent ETS or conservative treatment for a pancreatic fistula were identified. Safety, feasibility, and outcome of ETS were evaluated. Furthermore, a literature review was performed for similar studies in necrotizing pancreatitis.

Results: Out of 731 patients with acute pancreatitis, 19 patients were treated with ETS and 16 patients were treated conservatively for a pancreatic fistula. Fistula closure was achieved in 16 of 19 patients (84%) in the ETS group and in 8 of 12 patients (75%) in the conservative group (P=0,175). The median time to fistula closure following ETS was 71 days (IQR 34 – 142) compared to 120 days (IQR 51 – 175 days) in the conservative group (P=0,130). Complications were observed in 6 patients A total of 10 studies reporting the results of 281 patients with stent placement for pancreatic fistulas were included in the literature review. Fistula closure was achieved in 200 patients (71%). Stent-related complications were reported in 9% of patients.

Conclusion: ETS seems a feasible and safe alternative to conservative treatment in patients with pancreatic fistulas after intervention for necrotizing pancreatitis.

INTRODUCTION

Infection of pancreatic necrosis occurs in around 30% of patients with necrotizing pancreatitis and is considered an indication for intervention[1]. Surgical necrosectomy and percutaneous catheter drainage are the most frequently used techniques to debride and drain the infected collections. The drains are generally kept in place until the spontaneous production decreases or the collected fluid becomes clear after postprocedural lavage. A subset of patients will, however, have continued spontaneous production of clear fluids from the drain, indicative of a pancreatico-cutaneous fistula.[2-5] The estimated incidence of persisting pancreatic fistulas varies from 17 to 76% after intervention for necrotizing pancreatitis.[6-9] Pancreatic fistulas are associated with considerable morbidity such as metabolic and nutritional disturbances, prolonged hospitalization and even with mortality.[7]

Pancreatic fistulas most often are treated conservatively, although the time for a pancreatic fistula to resolve spontaneously usually takes more than 3 months and in some cases even over a year.[10] If conservative treatment fails, ultimately, a pancreaticojejunostomy may be indicated.[6,11]

Over the years, several groups of investigators have proposed endoscopic transpapillary stenting (ETS) as an alternative strategy for the management of pancreatic duct (PD) injuries.12-17 ETS decreases intraductal pressure, which facilitates drainage of pancreatic secretions to the duodenum instead of through the fistula. Most series describe the results of ETS in patients with fistulas after pancreatic surgery for suspected malignancy or for pseudocysts complicating chronic pancreatitis. However, performing an endoscopic intervention in a critically ill patient with necrotizing pancreatitis and concomitant papillary edema, pancreatic ductal or duodenal obstruction by pancreatic collections may be technically challenging and may potentially even worsen clinical outcome.

Only limited data are available on ETS and conservative treatment of fistulas in patients with necrotizing pancreatitis and little is known about the safety of ETS in critically ill patients13. We therefore evaluated the feasibility and clinical outcome of ETS and conservative treatment in patients with pancreatic fistulas after intervention for necrotizing pancreatitis from a prospective multicenter database. In addition, we performed a literature review for similar studies reporting the result of ETS and conservative treatment in patients with acute necrotizing pancreatitis.

METHODS

Design

This was a retrospective analysis of a prospective database of 731 patients with a primary episode of acute pancreatitis admitted to the 15 centers of the Dutch Pancreatitis Study Group between March 2004 and March 2007. The ethical review board of each participating hospital approved the protocol

for prospective data collection and all patients or their legal representatives gave written informed consent for inclusion in the database. The baseline characteristics and outcome of this cohort have previous been reported.[18-20]

Patient selection

All patients who underwent percutaneous drainage and/or surgical treatment for (suspected) infected necrotizing pancreatitis and subsequently developed a pancreatic fistula were included in this study. Patients were identified by screening all case record forms, endoscopic retrograde pancreaticography (ERP) and other imaging reports of the patients in the database. No strict criteria existed for the indication to perform ERP in patients with a pancreatic fistula in the participating hospitals. The decision to perform ETS depended on hospital policy and endoscopic skills of the gastroenterologists.

A pancreatico-cutaneous fistula was defined as output via an operatively or percutaneously placed catheter (or drainage canal after removal of drains or from a surgical wound) of any measurable volume with an amylase content greater than 3 times the serum amylase activity. A pancreatico-abdominal fistula was defined as the presence of ascites with amylase content greater than 3 times the serum amylase activity. These definitions were adapted from the International Study Group on Pancreatic Fistula criteria.[2]

Technique of Endoscopic Transpapillary Stenting

ERP was performed to locate the site of PD injury or disruption, as demonstrated by contrast leakage from the pancreatic duct (Fig. 1). In presence of a downstream obstruction or disruption of the PD, a guide wire was inserted and, if possible, a stent was placed. The preferred position was a bridging stent to bypass or cover the PD disruption, and thereby restore the flow of pancreatic secretions. In all patients, bridging of the leakage site was attempted. If bridging was not possible, an internal stent was placed with the proximal tip of the stent in the collection and the distal tip through the papilla (Fig. 2). Finally, if neither a bridging stent nor an internal stent could be placed, a short transpapillary stent was placed to reduce intraductal pressure.

Outcome and data collection

Data on patient demographics and clinical outcome were available from the prospective database. The outcomes of the study were procedure-related complications during ETS, any deterioration in clinical condition after ETS or ERCP, and successful fistula treatment after ETS or after conservative treatment. Successful fistula treatment was defined as total resolution of fistula output and absence of large fluid collections on follow-up computed tomography (CT) after removal of all drains without the need for additional interventions.

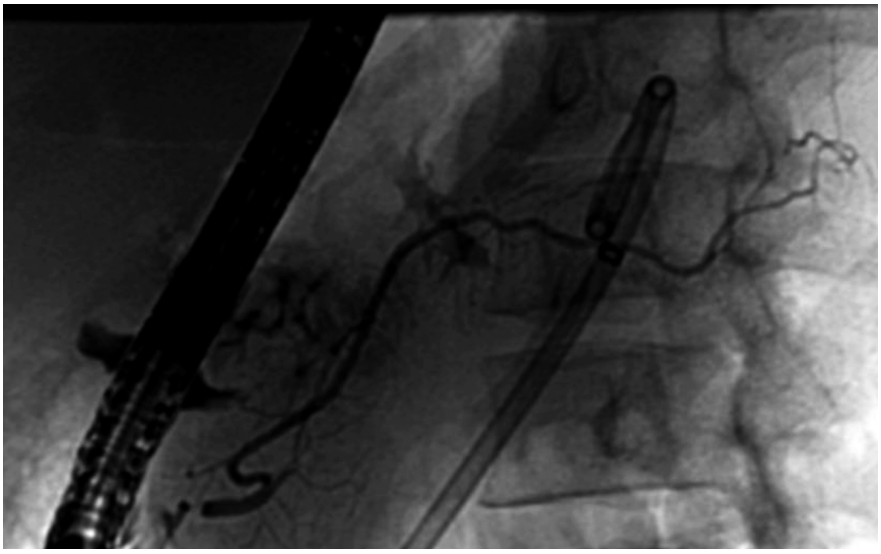

Figure 1: Endoscopic retrograde pancreatography (ERP) of a patient with ductal leakage from the head of the pancreas. A percutaneous catheter drain is visible.

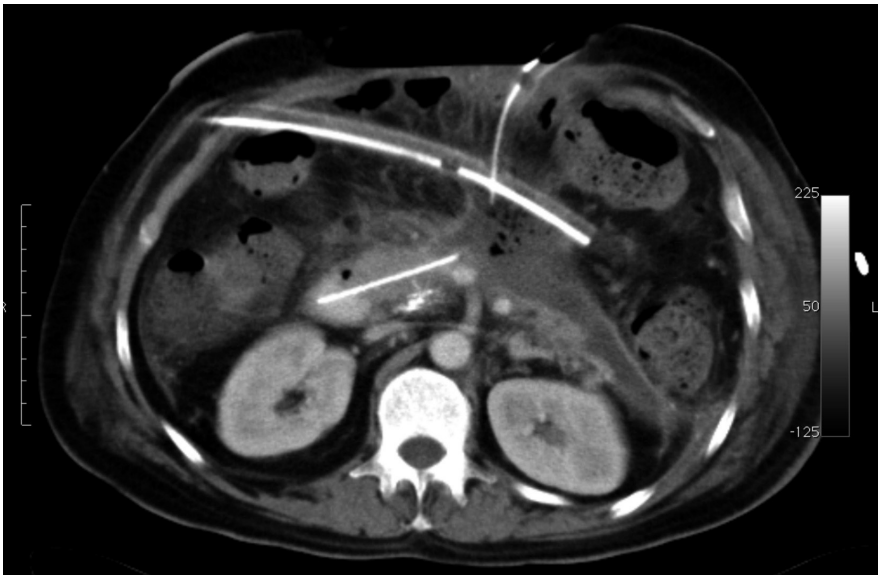

Figure 2: Computed Tomography (CT) of a patient that underwent surgical necrosectomy with postoperative lavage (2 large bore transabdominal drains visible). An internal stent is visible with the proximal tip of the stent in the collection and the distal tip through the papilla.

Follow-up

Peripancreatic fluid collections can develop, persist or even return after initial fistula resolution. Therefore, follow-up imaging (US, CT or MRI) was used for the assessment of long-term outcome of fistula resolution. All available imaging 6 months, 1 year and 2 years after initial fistula resolution were reviewed by a single experienced radiologist (T.L.B.) for the presence of persistent fluid collections (PFCs).

Literature review

A MEDLINE search was performed for similar studies reporting the results of endoscopic stenting or conservative treatment for pancreatic fistulas in patients with acute pancreatitis. Search terms were “pancreatic fistula” OR “duct disruption”. Cross references were searched in the studies found. Only studies published in the English language were included. Studies reporting the results of surgery for pancreatic fistulas were excluded. All studies reporting the result of transgastric or transduodenal endoscopic treatment for pancreatic pseudocysts or pancreatic fluid collections instead of endoscopic transpapillary treatment for pancreatic fistulas were also excluded.

Statistics

Descriptive statistics were performed and data are presented as numbers with subsequent percentages. Non-normally distributed data are presented as median with interquartile range (IQR). Univariate logistic regression analysis was performed to assess potential association of each of the variables with the use of ETS or conservative treatmet. $P<0.05$ was considered statistically significant. Significant variables were explored using a multivariate regression. For outcome, categorical variables were compared using Fisher’s exact test and in case of continuous measures, differences were tested using Mann-Whitney U test. All statistical analyses were performed using SPSS for Windows version 15.0 (SPSS Chicago, Chicago, IL).

RESULTS

Acute pancreatitis cohort

Between March 2004 and March 2007, 731 patients with a first episode of acute pancreatitis were included. From 203 patients with severe pancreatitis, 129 patients (64%) suffered from organ failure and 98 patients (48%) developed infected necrosis. In 115 patients (57%) either percutaneous drainage, necrosectomy or both was performed because of (suspected) infected necrotizing pancreatitis. In 64 patients (56%) percutaneous drainage was performed, 43 of these patients underwent subsequent necrosectomy and 51 patients underwent a primary necrosectomy (without previous drainage).

Pancreatic fistula cohort

From these 115 patients, 35 patients (30%) with a pancreatic fistula after radiologic and/or surgical intervention for necrotizing pancreatitis were identified. Nineteen patients were treated with ETS and 16 patients were treated conservatively (see Table 1 for baseline characteristics). An overview of all patients with ETS and conservative treatment are given in Tables 2 and 3. Univariate logistic regression analysis was performed to assess potential association of each of the variables with ETS or conservative treatment. The patients in the conservative group were older with a median age 61 years compared with 46 years in the ETS group (P=0,028). One patient had a pancreatico-abdominal fistula; the remaining 34 patients had a pancreatico-cutaneous fistula. A PD injury or disruption was confirmed by contrast leakage during ERP in 18 of 19 patients treated with ETS (95%). Concomitant obstruction of the PD was observed in 10 of 19 patients (53%). The median fistula output was approximately 150 (IQR 200-300) mL/day for the ETS group and 250 (IQR 75-338) mL/day for the conservative group. These 19 patients were treated in 6 of 15 participating hospitals. Of 19 patients with a pancreatic fistula treated with ETS, 12 patients (63%) had recovered from (multi)organ failure. Three patients were admitted to the intensive care unit (ICU) due to multiorgan failure at the time of ETS. From 16 patients with conservative treatment, 9 patients (56%) suffered from (multi)organ failure at any moment during admission.

In 24 patients, ETS for a pancreatic fistula was attempted and succeeded in 19 patients (79%). The median duration of conservative treatment before ETS was 34 days (IQR 18-92). The remaining 5 patients, in whom ETS was not possible, were treated conservatively. ETS failed in these patients because cannulation of the PD was not possible due to papillary oedema (n=4) and due to a complete stop of the PD (n=1). In 13 patients (68%) a PD stent could be placed during the initial procedure (Table 4). In 4 patients (21%), a second procedure was required and 2 patients (11%) required 3 procedures. Bridging of the PD disruption was achieved in only 4 of 19 patients (21%). In the remaining 15 patients, 6 patients received an internal stent and 9 patients a short transpapillary stent.

Complications

Complications occurred in 6 patients after stent placement. Migration of the stent occurred in 4 of 19 patients (21%) and clogging in 2 of 19 patients (11%). In 3 of 4 patients with a migrated stent, a new stent was placed. In the fourth patient, the fistula had resolved at the time the stent migration was discovered. The clogged stents were both exchanged during subsequent ERPs. In 1 patient in whom ETS was attempted but failed, a clinical deterioration was observed with an increase in abdominal pain during 1 day and a transient rise in inflammatory markers.

Table 1: Baseline characteristics

Patient characteristics	ETS (N=19)	Conservative (N=16)	*P***
Male gender	11 (37)	8 (50)	0,435
Age (yr)	46 (32-61)	61 (52-70)	0,028
CT severity index	7 (4-9)	8 (6-10)	0,182
Persistent organ failure during admission#	15 (79)	9 (56)	0,156
Pancreatic parenchymal necrosis	14 (74)	15 (94)	0,147
Peripancreatic necrosis/collections only*	5 (26)	1 (6)	0,147
Infected necrosis	13 (68)	12 (75)	0,668
Time from onset of symptoms to intervention for infected necrosis (d)	26 (11-67)	22 (12-35)	0,227
Type of initial intervention			0,067
Surgical necrosectomy	11 (58)	14 (88)	
Percutaneous catheter drainage	8 (42)	2 (13)	
Octreotide therapy	5 (26)	5 (33)	0,656
Sphincterotomy	8 (42)	5 (31)	0,509
Time from intervention to ERP (d)	34 (18-92)	-	
Type of fistula			1,000
Pancreatico-cutaneous	15 (94)	16 (100)	
Pancreatico-abdominal	1 (6)	0 (0)	
Location of PD disruption			0,178
Head	2 (11)	4 (25)	
Body	7 (37)	4 (25)	
Tail	9 (47)	1 (6)	
Normal pancreatic duct	1 (5)	0 (0)	
Not identified	0 (0)	7 (44)	
Pancreatic duct obstruction	10 (53)	-	
Fistula output (mL/d)	150 (200-300)	250 (75-338)	0,350

Data are n (%) or median (interquartile range). CT = computed tomography ERP = endoscopic retrograde pancreatography PD = pancreatic duct ETS = endoscopic transpapillary stenting *No pancreatic parenchymal necrosis. #Organ failure more than 48hrs. **Univariate logistic regression analysis was used to test for differences between groups.

Table 2: Overview of patients with necrotizing pancreatitis undergoing endoscopic transpapillary treatment for pancreatic fistulas.

Patient No.	Age	Gender	CTSI	Type of intervention	Before ETS Organ failure	At time of ETS Organ failure	At time of ETS ICU admission	CRP	Time to ETS (d)	Type of stent	Time to fistula closure (d)	Successful fistula closure	Complication
1				PCD	Circ/Resp/Renal	No	No	52	17	Internal	95	Yes	None
2	51	M	4	SN	None	No	No	-	228	Transpapillary	36	Yes + ETD	None
3	68	M	6	SN	Circ/Resp	No	No	92	30	Internal	36	Yes	Migration
4	44	F	4	PCD	Circ/Resp	Yes	Yes	251	34	Transpapillary	-	Deceased	None
5	45	M	10	SN	Circ/Resp/Renal	Yes	Yes	230	39	Internal	365	Yes	None
6	46	M	10	SN	Circ/Resp	No	No	76	19	Transpapillary	38	Yes	None
7	61	M	6	PCD	Circ/Resp/Renal	Yes	Yes	139	1	Internal	-	Deceased	None
8	74	F	4	PCD	Circ/Resp	No	No	97	28	Transpapillary	91	Yes	None
9	16	F	10	PCD	Circ/Resp	No	No	145	34	Bridging	179	Yes	Migration
10	58	M	10	SN	Circ/Resp/Renal	No	No	104	4	Bridging	151	Yes	occlusion
11	28	M	4	SN	None	No	No	74	12	Internal	186	Yes	occlusion
12	31	F	6	SN	Circ/Resp/Renal	No	No	375	40	Transpapillary	28	Yes	None
13	42	F	4	PCD	None	No	No	17	80	Bridging	7	Yes	None
14	48	M	8	SN	Resp	No	No	82	18	Internal	32	Yes	None
15	50	M	8	SN	Resp	No	No	28	92	Transpapillary	71	Yes	None
16	69	M	8	SN	Circ/Resp/Renal	No	No	7	123	Transpapillary	63	Yes	None
17	32	M	8	SN	Circ/Resp/Renal	No	No	42	151	Transpapillary	104	Yes	Migration
18	45	F	6	PCD	Resp/Renal	No	No	53	97	Transpapillary	133	No + PR	Migration
19	75	M	6	PCD	None	No	No	103	26	Bridging	31	Yes	None

F=female; M=male; PCD=percutaneous drainage; SN=surgical necrosectomy; ETD=endoscopic transgastric drainage; PR=pancreatic resection; CTSI = CT severity index; PD = pancreatic duct; ETS = endoscopic transpapillary stenting.

Table 3: Overview of patients with necrotizing pancreatitis undergoing conservative treatment for a pancreatic fistula.

Patient No.	Age	Gender	CTSI	Type of intervention	Organ failure	ICU admission	ETS attempted	Time to fistula closure (d)	Successful fistula closure	Complication
1	47	F	8	SN	None	No	Yes	349	No + PR	None
2	61	F	6	SN	Circ/Resp	Yes	Yes	128	No + PR	Deterioration*
3	70	F	6	SN	None	No	Yes	47	No + ETD	None
4	51	F	8	SN	Circ/Resp	Yes	Yes	43	Yes	None
5	39	M	10	SN	None	No	Yes	50	No + PR	None
6	70	M	10	SN	Resp	Yes	No	131	Yes	None
7	52	F	8	SN	Circ/Resp	Yes	No	49	Yes	None
8	53	F	10	SN	Circ/Resp	Yes	No	140	Yes	None
9	61	M	8	SN	Circ/Resp/Renal	Yes	No	212	Yes	None
10	72	M	8	SN	Circ/Resp	Yes	No	170	Yes	None
11	63	M	6	SN	None	No	No	90	Yes	None
12	61	M	8	SN	Resp	Yes	No	92	Yes	None
13	54	M	4	PCD	None	No	No	53	Yes	None
14	53	M	5	SN	None	No	No	177	Yes	None
15	81	F	10	SN	Circ/Resp	Yes	No	182	Yes	None
16	75	F	10	PCD	None	No	No	112	Yes	None

F=female; M=male; PCD=percutaneous drainage; SN=surgical necrosectomy; ETD=endoscopic transgastric drainage; PR=pancreatic resection; CTSI = CT severity index; ETS = endoscopic transpapillary stenting. *Clinical deterioration (raise of inflammatory parameters) following unsuccessful stent placement.

Table 4: Characteristics of endoscopic stent placement (ETS) for pancreatic fistulas in patients with necrotizing pancreatitis.

ETS characteristics	N = 19
Number of ETS attempts for stent placement (median)	1 (1-3)
Stent position	
Bridging	4 (21)
Transpapillary	9 (47)
Internal	6 (32)
Diameter stent (Fr)	7 (5-10)
Length stent (cm)	7 (3-15)

Data are n (%) or median (range).

Outcome

Fistula closure (ie, complete resolution of the fistula without need for other interventions) was achieved in 16 of 19 patients (84%) in the ETS group and in 8 of 12 patients (75%) in the conservative group (P=0,175). In the ETS group, 1 patient required a pancreaticojejunostomy for fistula closure 118 days after ETS. Two patients died prior to fistula closure, 18 and 22 days after stent placement. These were 2 patients suffering from severe multiorgan failure that started before any intervention and persisted throughout the disease course. In the conservatively treated patients, 4 patients (25%) underwent an additional intervention to achieve fistula closure. Pancreaticojejunostomy was performed in 3 patients and 1 patient underwent endoscopic transgastric drainage of the collection. The median time to fistula closure after ETS was 71 days (IQR 34 – 142) compared to 120 days (IQR 51 – 175 days)] in the conservative group (P=0,130). The association between age, ETS or conservative treatment and time to fistula resolution was explored using multivariate linear regression. Adjusting for the effect of age did not change outcome of fistula closure or time to fistula resolution.

In the ETS group, the time to fistula closure was not associated with the number of days between intervention for necrotizing pancreatitis and ETS. Neither was it associated with the position of the endoprothesis (transpapillary, bridging or internal) or the presence of a PD stenosis (data not shown).

Follow-up

The presence of PFCs was investigated in all surviving patients that underwent imaging (US, CT or MRI) during follow-up. Six months after initial fistula resolution, PFCs were seen at imaging in 6 of 15 patients (40%) after ETS and in 8 of 13 patients (62%) after conservative treatment (P=0,449). After 1 year, 1 patient in the ETS group had died due to metastatic disease. One year and 2

years after fistula resolution, PFCs were noticed in 3 of 14 patients (21%) after ETS and in 7 of 13 patients (54%) after conservative treatment (P=0,120).

Literature review

After reviewing 58 potential relevant manuscripts, 10 articles with a total of 281 patients (including the present series) with stent placement for pancreatic fistulas were selected.[12-15,17,21-24] An overview is given in Table 5. Only the results for patients with acute pancreatitis are given (as far as these results could be deducted from the articles). Twenty-five complications were reported in the studies, although the pancreatitis severity of most patients was not described in detail. Time to fistula closure ranged from a median of 2 days to 4 months between studies and within the individual studies a wide range of time to closure was observed (similar to our findings). A direct comparison of results between studies is unfortunately seriously hampered due to lack of homogeneity. The study by Boerma and colleagues is most similar to this study. This study, like our study, included only patients with necrotizing pancreatitis and a pancreatic fistula after necrosectomy. In this study, however, only patients with a pancreatic obstruction underwent endoscopic stenting. The overall reported rate of fistula closure after stent placement in the studies retrieved varied between 58 and 100%.

In addition, the literature was searched for studies reporting the result of conservative treatment for pancreatic fistulas in patients with necrotizing pancreatitis. After an extensive MEDLINE and cross-reference search, only a single study was identified.[10] Sikora and colleagues reported on 156 patients with acute severe pancreatitis that underwent percutaneous drainage or surgical intervention. Out of the 81 surviving patients, 43 developed a pancreatic fistula (53%) and were managed conservatively. In 38 patients (88%) fistula closure was achieved after a median of 70 days (28 – 424 days). However, from these 38 patients with spontaneous closure, 3 patients underwent cystogastrostomy (n=3) or cystojejunostomy (n=4) at a later stage for treatment of pseudocysts.

DISCUSSION

This is the largest series on ETS and conservative treatment for pancreatic fistulas after surgical or radiological intervention in patients with necrotizing pancreatitis. Although ETS was performed selectively and not in a prospective manner, our results suggest that ETS is feasible, relatively safe and might form an alternative for conservative treatment in critically ill patients.

Previous studies have described the results of ETS for pancreatic fistulas, as is presented in the literature review. Most studies report a high rate of successful fistula closure after endoscopic stenting. The results, however, are difficult to compare. The studies differ in several aspects. First of all, most studies also included patients with pancreatic fistulas after surgery for

Table 5: Systematic literature review on endoscopic treatment of pancreatic fistulas.

Study	Year	Total No. of patients	No. of patients with endoscopic treatment	No. of patients with acute necrotizing pancreatitis	Position of PD stent+	Successful fistula closure after stent placement	Duration until fistula closure after stent placement* (d)	Number and type of stent related complications
Kozarek	1997	9	9 (100)	2 (22)	TP, B	8 (89)	2 (2-7)	1 stent occlusion; 1 infected pseudocyst
Howard	1998	38	7 (18)	10 (26)	TP	7 (100)	NS	NS
Boerma	2000	16	13 (81)	16 (100)	TP, B	13 (100)	10 (2-64)	1 stent occlusion
Costamagna	2001	16	11 (69)	8 (50)	IN, B	10 (91)	9 (2-33)**	NS
Telford	2002	43	43 (100)	NS	TP, IN, B	25 (58)	NS	4 patients with clinical deterioration
Halttunen	2005	50	43 (86)	9 (18)	TP	36 (84)	122 (16-984)	1 patient with fever; 1 patient with mild post-ERCP pancreatitis
Varadarajulu	2005	97	92 (95)	44 (46)§	TP, IN, B	52 (55)	NS	1 patient died with multiorgan failure 1 week after ETS; 3 stent occlusions; 2 patients with pus from stent
Brennan	2006	30	30 (100)	7 (23)	TP	21 (70)	NS	1 stent occlusion
Cicek	2006	26	14 (54)	NS	TP, IN, B	12 (86)	7 ** ‡	2 stent migrations
Bakker	2009	35	19 (54)	35 (100)	TP, IN, B	16 (84)	71 (34–142)†	2 stent occlusions; 4 stent migrations; 1 (mild) clinical deterioration
Total		**360**	**281 (81)**	**131 (45)**		**200 (71)**	**2–122 (2-984)**	**26 reported complications in 281 patients (9%)**

NS = not specified; ¶N (percentage); + TP = transpapillary stent; IN = internal stent; B = bridging stent; *Data are median (range); ** Data are mean (range); † Median (interquartile range); ‡ Patients were referred to surgery if drain production did not decrease significantly within 10 days after stent insertion.

pancreatic cancer or chronic pancreatitis. Second, the definition of a pancreatic fistula varied between studies. Third, the timing and indication for endoscopic treatment differed or were not reported. Fourth, the patient characteristics of patients with acute pancreatitis (eg, presence of necrosis or organ failure) are not given. Finally, the position of the stent (transpapillary, internal or bridging) was not reported in most studies.

Kozarek et al reported in 1997 that transpapillary stenting can effectively close pancreatic fistulas within a week.[12] In 9 retrospectively identified patients with ductal injury after surgery for chronic and acute pancreatitis, 8 fistulas were closed within a week. One stent occlusion and one stent migration were described. These promising results unfortunately have not been reproduced by other case series. Howard et a. described 7 patients with different types of pancreatic fistulas and treatment with transpapillary stenting.[14] ETS was successful in all patients, although patients with complete ductal disruption were not deemed amenable for ETS and only patients with partial ductal disruption were included. No complications of ETS were reported. Telford et al described results in 43 patients identified with pancreatic ductal disruption of different etiology.[15] Four patients experienced a clinical deterioration after stent placement, although stent occlusion was not documented. The study by Boerma et al is the only study, next to the present series, that solely included patients with necrotizing pancreatitis that had undergone surgical necrosectomy.[13] After ETS, after a median of 10 days, fistula production stopped in all 13 patients. All patients, however, that were treated with endoscopic stenting had a ductal obstruction and all stents were placed through the obstruction. This might have shortened overall time to fistula closure compared with the present series. In the present study, fistula closure was achieved in approximately 80% of patients. The median time to fistula closure, however, was more than 2 months after stent placement. Our results differ from some of the results reported. The observed difference may be explained by the fact that we only included patients that underwent intervention for suspected infected necrosis. In these patients, the ductal anatomy of the pancreas was severely disrupted as is reflected by the high rate of ductal obstruction, ductal disconnection and the median CT severity index (CTSI). CTSI was 8 or higher in 50% of patients. A CTSI of 8 correlates with at least 30% lack of enhancement of pancreatic parenchyma (along with the presence of 2 or more peripancreatic collections).[25] As a result of the disrupted anatomy, in only 4 patients a bridging stent could be positioned. The transpapillary or internal stent placed in the remaining 15 patients lowers the pancreatic intraductal pressure, but probably does not entirely reduce flow of pancreatic juices into the pancreatic fluid collections. As a consequence, complete closure of the fistula will still take considerable time. A bridging stent has been correlated with a more favourable outcome.[15] In this study, outcome for patients with a bridging stent was similar to results after transpapillary or

an internal stent, although patient numbers in the current study probably are too small to formally compare the outcome for the different stent positions.

Despite many challenging factors most often encountered in critically ill patients with necrotizing pancreatitis, no complications such as bleeding or perforation directly related to ETS were noted in this study. Several other complications did occur, such as occlusion and migration of stents. Notably, these events did not result in clinical deterioration.

In this study, we used the adapted definition for pancreatic fistula as proposed by the International Study Group on Pancreatic Fistula (ISGPF) that was designed for pancreatic resections.[2] Fistulas after pancreatic resections represent failure of a healing or sealing of a pancreatic-enteric anastomosis. We defined a pancreatic fistula after intervention for necrotizing pancreatitis as output via a percutaneous drain (or drainage canal after removal of drains or from a surgical wound) of any measurable volume of fluid with an amylase content greater than 3 times the serum amylase activity. We feel this definition is more appropriate for patients with necrotizing pancreatitis.

From the number of patients with a pancreatic fistula in the study cohort, the incidence of pancreatic fistulas after intervention for necrotizing pancreatitis cannot accurately be deducted. The physicians decided to perform ETS at their own discretion and the conservatively treated patients were identified from the ERP reports, imaging reports and case record forms. It is possible that some patients with a pancreatic fistula may have gone unnoticed.

This study has several shortcomings. It was a retrospective analysis and ETS was not performed according to a standardized protocol. Conservative treatment before ETS differed among centers. For example, 4 patients received octreotide and 8 patients underwent a biliary sphincterotomy during early ERCP when signs of an impacted stone or significant cholestasis were present. It is unlikely that these differences in conservative treatment strategies may have influenced outcome of ETS because in most patients conservative treatment was attempted for more than 3 weeks. More importantly, selection of patients for ETS and conservative treatment very likely influenced the time to fistula resolution. It is possible that some of the patients undergoing ETS would have had spontaneous closure of their fistula in the same time period with conservative treatment. On the other hand, it is also very much possible that some conservative treated patients would have had earlier resolution if they were treated with ETS. The overall median time to fistula closure did not differ significantly between groups. If ETS really shortens time to fistula closure can only be answered in a comparative, preferably randomized, design. Such a study is needed, but unfortunately difficult to perform.

In conclusion, this study suggests that endoscopic transpapillary treatment in patients with pancreatic fistulas after intervention for infected necrotizing pancreatitis is a feasible and a safe alternative to conservative treatment.

References

1. Banks PA, Freeman ML. Practice guidelines in acute pancreatitis. Am J Gastroenterol 2006; 101:2379-2400.
2. Bassi C, Dervenis C, Butturini G et al. Postoperative pancreatic fistula: an international study group (ISGPF) definition. Surgery 2005; 138:8-13.
3. Nealon WH, Walser E. Main pancreatic ductal anatomy can direct choice of modality for treating pancreatic pseudocysts (surgery versus percutaneous drainage). Ann Surg 2002; 235:751-758.
4. Nealon WH, Bhutani M, Riall TS et al. A unifying concept: pancreatic ductal anatomy both predicts and determines the major complications resulting from pancreatitis. J Am Coll Surg 2009; 208: 790-799.
5. Werner J, Buchler MW. Management of pancreatic fistulas in acute pancreatitis. In: Beger H, Warshaw A, eds. The Pancreas, an Integrated Textbook of Basic Science, Medicine, and Surgery, 2nd. Wiley-Blackwell 2008. 356-361.
6. Ho HS, Frey CF. Gastrointestinal and pancreatic complications associated with severe pancreatitis. Arch Surg 1995; 130:817-822.
7. Tsiotos GG, Smith CD, Sarr MG. Incidence and management of pancreatic and enteric fistulas after surgical management of severe necrotizing pancreatitis. Arch Surg 1995; 130:48-52.
8. Fotoohi M, D'Agostino HB, Wollman B et al. Persistent pancreatocutaneous fistula after percutaneous drainage of pancreatic fluid collections: role of cause and severity of pancreatitis. Radiology 1999; 213: 573-578.
9. Connor S, Ghaneh P, Raraty M et al. Minimally invasive retroperitoneal pancreatic necrosectomy. Dig Surg 2003; 20:270-277.
10. Sikora SS, Khare R, Srikanth G et al. External pancreatic fistula as a sequel to management of acute severe necrotizing pancreatitis. Dig Surg 2005; 22:446-451.
11. Alexakis N, Sutton R, Neoptolemos JP. Surgical treatment of pancreatic fistula. Dig Surg 2004; 21:262-274.
12. Kozarek RA, Ball TJ, Patterson DJ et al. Transpapillary stenting for pancreaticocutaneous fistulas. J Gastrointest Surg 1997; 1:357-361.
13. Boerma D, Rauws EA, van Gulik TM et al. Endoscopic stent placement for pancreaticocutaneous fistula after surgical drainage of the pancreas. Br J Surg 2000; 87:1506-1509.
14. Howard TJ, Stonerock CE, Sarkar J et al. Contemporary treatment strategies for external pancreatic fistulas. Surgery 1998; 124:627-632.
15. Telford JJ, Farrell JJ, Saltzman JR et al. Pancreatic stent placement for duct disruption. Gastrointest Endosc 2002; 56:18-24.
16. Deviere J, Bueso H, Baize M et al. Complete disruption of the main pancreatic duct: endoscopic management. Gastrointest Endosc 1995; 42:445-451.
17. Varadarajulu S, Noone TC, Tutuian R et al. Predictors of outcome in pancreatic duct disruption managed by endoscopic transpapillary stent placement. Gastrointest Endosc 2005; 61:568-575.

18. van Santvoort HC, Besselink MG, de Vries AC et al. Early endoscopic retrograde cholangiopancreatography in predicted severe acute biliary pancreatitis: a prospective multicenter study. Ann Surg 2009; 250:68-75.
19. Besselink MG, van Santvoort HC, Boermeester MA et al. Timing and impact of infections in acute pancreatitis. Br J Surg 2009; 96:267-273.
20. Besselink MG, van Santvoort HC, Buskens E et al. Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. Lancet 2008; 371:651-659.
21. Costamagna G, Mutignani M, Ingrosso M et al. Endoscopic treatment of postsurgical external pancreatic fistulas. Endoscopy 2001; 33:317-322.
22. Halttunen J, Weckman L, Kemppainen E et al. The endoscopic management of pancreatic fistulas. Surg Endosc 2005; 19:559-562.
23. Brennan PM, Stefaniak T, Palmer KR et al. Endoscopic transpapillary stenting of pancreatic duct disruption. Dig Surg 2006; 23:250-254.
24. Cicek B, Parlak E, Oguz D et al. Endoscopic treatment of pancreatic fistulas. Surg Endosc 2006; 20:1706-1712.
25. Balthazar EJ, Robinson DL, Megibow AJ et al. Acute pancreatitis: value of CT in establishing prognosis. Radiology 1990; 174:331-336.

Chapter 8

Timing of cholecystectomy after mild biliary pancreatitis: a systematic review

Annals of Surgery 2012; 255(5):860-866

Mark C van Baal[1]
Marc G Besselink[2]
Olaf J Bakker[2]
Hjalmar C van Santvoort[2]
Alexander F Schaapherder[3]
Vincent B Nieuwenhuijs[4]
Hein G Gooszen[1]
Bert van Ramshorst[5]
Djamila Boerma[5]
for the Dutch Pancreatitis Study Group

[1] Dept. Operating Room/Evidence Based Surgery, Radboud University Nijmegen Medical Centre, Nijmegen
[2] Dept. of Surgery, University Medical Center, Utrecht
[3] Dept. of Surgery, Leiden University Medical Center, Leiden
[4] Dept. of Surgery, University Medical Center Groningen, Groningen
[5] Dept. of Surgery, St Antonius Hospital, Nieuwegein
All: the Netherlands

ABSTRACT

Objectives: To determine the risk of recurrent biliary events in the period after mild biliary pancreatitis but prior to interval cholecystectomy and to determine the safety of cholecystectomy during the index admission.

Summary of background data: Although current guidelines recommend to perform cholecystectomy early after mild biliary pancreatitis, consensus on the definition of early (i.e. during index admission or within the first weeks after hospital discharge) is lacking.

Methods: We performed a systematic search in Pubmed, Embase and Cochrane for studies published from January 1992 to July 2010. Included were cohort studies of patients with mild biliary pancreatitis reporting on the timing of cholecystectomy, number of re-admissions for recurrent biliary events prior to cholecystectomy, operative complications (e.g. bile duct injury, bleeding) and mortality. Study quality and risks of bias were assessed.

Results: After screening 2413 studies, eight cohort studies and one randomized trial describing 998 patients were included. Cholecystectomy was performed during index admission in 483 patients (48%) without any reported re-admissions. Interval cholecystectomy was performed in 515 patients (52%) after 40 days (median; interquartile range 19-58 days). Prior to interval cholecystectomy, 95 patients (18%) were re-admitted for recurrent biliary events (0% vs. 18%, P<0.0001). These included recurrent biliary pancreatitis (n=43, 8%), acute cholecystitis (n=17) and biliary colics (n=35). Patients who had an endoscopic retrograde cholangiopancreatography had fewer recurrent biliary events (10% vs. 24%, P=0.001), especially less recurrent biliary pancreatitis (1% vs. 9%). There were no differences in operative complications, conversion rate (7%) and mortality (0%) between index and interval cholecystectomy. Because baseline characteristics were only reported in 26% of patients, study populations could not be compared.

Conclusions: Interval cholecystectomy after mild biliary pancreatitis is associated with a high risk of re-admission for recurrent biliary events, especially recurrent biliary pancreatitis. Cholecystectomy during index admission for mild biliary pancreatitis appears safe but selection bias could not be excluded.

INTRODUCTION

The incidence of acute biliary pancreatitis is increasing worldwide, possibly due to an increase in obesity with associated increased risk of gallstone disease.[1,2] In the US alone, the annual costs of acute pancreatitis currently exceed $2.2 billion.[3] Although 20% of patients develops severe pancreatitis, associated with high morbidity and mortality, in 80% of patients the pancreatitis remains mild.[4]

It is generally accepted that patients with severe biliary pancreatitis should undergo cholecystectomy when signs of inflammation have resolved (i.e. interval cholecystectomy).[5] After mild biliary pancreatitis current international guidelines advice 'early' cholecystectomy.[6-8] The definition of 'early', however, varies greatly between guidelines. The International Association of Pancreatology (IAP) recommends that all patients with gallstone pancreatitis should undergo cholecystectomy as soon as the patient has recovered from the attack[8], while the American Gastroenterological Association[7] and the British Society of Gastroenterology6 recommend cholecystectomy within a 2-4 weeks interval after discharge. This lack of consensus is also reflected by several audits from the UK[9-11], Germany[12], Italy[13] and a large database study from the USA[14]. The differences between these guidelines are most likely caused by a lack of randomized controlled studies on this topic. The rationale of early cholecystectomy is to reduce the risk of recurrent biliary events (e.g. recurrent biliary pancreatitis, acute cholecystitis, symptomatic choledocholithiasis, biliary colics). This may be essential, as a recurrent attack of biliary pancreatitis could be severe and thus life threatening.[15]

In case of clinical equipoise, the situation where no clear therapeutic recommendation can be made, many clinicians routinely perform interval cholecystectomy, because this does not stress the usually already busy emergency theatre list, and for reimbursement reasons.[12] Therefore, it is essential to quantify the risks involved with interval cholecystectomy as compared to cholecystectomy during index admission (index cholecystectomy) and to grade the current evidence on this topic.

We performed the first systematic review on timing of cholecystectomy after mild biliary pancreatitis, and focused on: 1) the risk of recurrent biliary events in the period between discharge after mild biliary pancreatitis and interval cholecystectomy, and 2) the safety of index versus interval cholecystectomy after mild biliary pancreatitis.

METHODS

Systematic literature search

A systematic literature search was performed in the Pubmed, Embase and Cochrane Library databases from January 1st, 1992 to July 31st, 2010. We adhered to the 2009 PRISMA statement.[16] The search was limited to this episode, as before 1992 no universally accepted terms were available for acute

pancreatitis and its clinical course. In 1992, the Atlanta symposium provided clear definitions of the disease and its complications.[17] Although the Atlanta classification is currently under revision, the definitions have been widely used in the literature since 1992.

The MeSH headings "cholecystectomy" and "pancreatitis" were used, and the search was restricted to English literature. From the studies identified, all titles and abstracts were screened to select those reporting on the timing of cholecystectomy in patients with mild biliary pancreatitis. Subsequently, full-text papers of the selected studies were independently screened by two authors (MvB and MB) for eligibility. When multiple articles were published by the same study group and no difference in study period was described, only the most recent paper was selected for this systematic review.

Inclusion and exclusion criteria

Inclusion criteria were: 1) cohort of patients undergoing cholecystectomy after mild biliary pancreatitis (i.e. either index or interval cholecystectomy); 2) information on the following essential outcomes: time between recovery from acute pancreatitis and cholecystectomy, number of recurrent biliary events prior to cholecystectomy, complications during the cholecystectomy (e.g. bile duct injury, bleeding) and mortality.

Exclusion criteria were: 1) cohorts with fewer than five patients; 2) cohorts including severe pancreatitis without reporting the results for mild pancreatitis separately; 3) cohorts without reporting on essential outcomes; 4) cohorts in which patients underwent index cholecystectomy during the initial attack of acute pancreatitis (i.e. prior to recovery); the rationale for this being that the IAP guideline advices cholecystectomy only after recovery of biliary pancreatitis.[8]

All references of the included studies were screened for potential relevant studies not identified by the initial literature search. The final decision on eligibility was reached by consensus between the two screening authors.

Data extraction

From the included studies, the following variables were extracted (if available): definition of mild biliary pancreatitis, number of patients undergoing cholecystectomy after mild biliary pancreatitis, number of endoscopic retrograde cholangiography (ERC) and endoscopic sphincterotomy performed, time between first hospital admission and cholecystectomy, reasons for delay of surgery, number of re-admissions during time between first hospital admission and cholecystectomy, total number of recurrent biliary events (i.e. biliary pancreatitis, acute cholecystitis and biliary colics) requiring re-admission during time between first hospital admission and cholecystectomy, conversion to open cholecystectomy, complications and mortality. If reported, follow-up and data of patients with recurrent biliary pancreatitis after cholecystectomy

were extracted. The authors of included studies were contacted if one of these variables could not be extracted from the original article. We defined 'index cholecystectomy' as cholecystectomy during the initial hospital admission for acute biliary pancreatitis. Interval cholecystectomy was defined as cholecystectomy during a new hospital admission for cholecystectomy, usually performed at least one week after discharge.

Assessment of study quality

We performed a quality assessment of the included studies with two previously validated checklists that scored the methodological quality of non-randomized studies.[18,19] Downs *et al* described a checklist with 27 items (one point for each item) which can be used for quality assessment for both randomized and non-randomized studies.[18] The MINORS checklist, described by Slim *et al*, contains eight items for non-comparative studies and 12 items for comparative studies (maximum of two points for each item).[19] In both lists a low score reflects a high risk of bias, whereas a high score reflects a low risk of bias. To facilitate comparison of both lists, each score was converted to a score on a 0-10 scale. Randomized controlled trials were only assessed with the checklist of Downs *et al*. No studies were excluded on the basis of their score. Baseline characteristics were assessed to determine whether selection bias might have played a role in the timing of cholecystectomy (i.e. less sick patients undergoing index cholecystectomy more frequently). Finally, we assessed reasons for delay of cholecystectomy.

Statistical analysis

All data were pooled. Total number of readmissions due to recurrent biliary events was calculated, as well as every recurrent biliary event apart and compared between the patients with early cholecystectomy and interval cholecystectomy. Regarding the number of recurrent biliary events prior to cholecystectomy, comparison was made by patients with or without ERC prior to cholecystectomy. Baseline characteristics were listed, as well as the number of complications occurred. Mortality and conversion rates were calculated and compared between patients with early and interval cholecystectomy.

Non-normally distributed data were presented as median (interquartile range). Proportions were compared by the chi-square test or the Fisher exact test, as appropriate. All statistical analysis were performed using SPSS® for Windows® version 16.0.2 (SPSS, Chicago, Illinois, USA). Two-sided $P<0.05$ was considered statistically significant.

RESULTS

Included studies

The results of the literature search are depicted in Figure 1. The initial search yielded 2413 potentially relevant papers. After screening titles and abstracts for

relevance, 38 remaining papers were further assessed for eligibility. Although all 38 papers reported on the timing of cholecystectomy in biliary pancreatitis, 29 were excluded for the following reasons: cohort of patients not reporting on the incidence of recurrent biliary events prior to cholecystectomy (n=9)[15,20-27], cohorts of patients with mixed severe and mild acute biliary pancreatitis and outcomes not reported separately (n=6)[11,28-32], cohorts of patients without data on the period between index admission and cholecystectomy (n=5)[10,33-36], cohorts where no separate results were described for patients with acute biliary pancreatitis (n=4)[37-40], cohorts in which patients were operated during the initial attack of pancreatitis (n=3)[41-43], cohorts with fewer than five patients per study group (n=1)[44] and cohorts without documentation of essential outcomes (n=1)[45]. Finally, nine studies were included in the current systematic review.[9,46-53] Six studies were retrospective cohort studies[9,48-51,53], two studies were prospective cohort studies[47,52] and one study was a randomized controlled trial[46] (level 4 evidence, level 4 evidence and level 1b evidence, respectively[54]).

In the one randomized trial, Aboulian *et al* randomized between cholecystectomy during the initial attack of pancreatitis versus cholecystectomy after recovery but during index admission.[46] Based on our exclusion criteria we included only the latter arm.

Figure 1: PRISMA flowchart systematic review of timing of cholecystectomy after mild biliary pancreatitis.

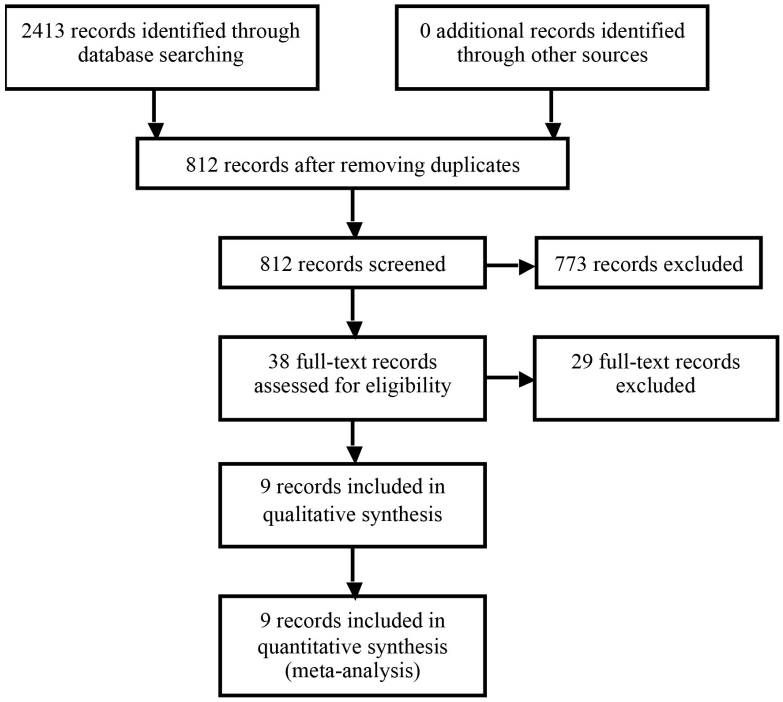

Table 1. Characteristics of the included studies.

Study	Country	Year	Definition of mild acute biliary pancreatitis
Schachter[52]	Israel	2000	Acute abdominal pain with elevated serum and/or urine levels of amylase (>700 IU/L serum, normal 70-220; urine >1500 IU/L, normal <1000). Imaging confirmation of gallstones. Ranson ≤3.
McCullough[50]	Canada	2003	Lipase >400 U/L. Radiographic confirmation of gallstones (US, CT, ERC). No necrosis on CT, no ICU stay.
Cameron[9]	UK	2004	Acute upper abdominal pain, serum amylase >500 IU/l (normal 30-110) Gallstones demonstrated on US or ERC. No description of a severity score.
Griniatsos[48]	UK	2005	Generalized or upper abdominal pain and tenderness, elevation of serum amylase level more than three times the normal. Documented gallstones and absence of other factors known to cause acute pancreatitis. Modified Imrie score <3 within 48h after admission.
Clarke[47]	USA	2008	Elevation of lipase 3 times or more the normal level. Gallstones on US. Ranson ≤3, no required emergent operative intervention for management of the biliopancreatic process.
Ito[49]	USA	2008	Abdominal pain and tenderness, together with elevations in serum amylase and/or lipase concentration (at least 3 times the upper limit of normal). Documentation of gallstones or choledocholithiasis on imaging studies. No necrosis on CT scan.
Nebiker[51]	Switzerland	2008	Acute abdominal pain with a threefold increase of serum amylase activity. Detection of gallstones on US, MRCP or ERC. Modified Ranson score ≤3, no necrosis on CT.
Sinha[53]	India	2008	Serum amylase level more than two times the normal, increase ALT to three or more times the normal. US features of pancreatic edema and cholelithiasis with or without CBD stones. Ranson ≤4.
Aboulian[46]	USA	2010	Upper abdominal pain, nausea, vomiting, epigastric tenderness, absence of ethanol use, elevated amylase level to at least twice the upper limit of normal. Imaging confirmation of gallstones. Ranson ≤3, clinical stability with admission to a non-monitored ward bed, absence of acute cholangitis, low suspicion for a retained CBD stone.

Table 2: Patient outcomes of the included studies.

Study	Number of patients		Time between discharge and cholecystectomy (range/SD) (days)		Re-admissions for recurrent biliary events (%) (N= biliary pancreatitis, cholecystitis, colics)		Conversion to open cholecystectomy (%)		Complications		Reasons for delay in surgery
	Index cholec.	Interval cholec.	Index cholec.	Interval cholec.	Index cholec.	Interval cholec.	Index cholec.	Interval cholec.	Index cholec.	Interval cholec.	
Schachter[52]	-	19	-	>56	-	0	-	2 (11%)	-	0	All study-related
McCullough[50]	74	90	0	mean 40 (+/- 69)	0	18 (20%) (3,5,10)	9 (12%)	8 (9%)	11	16	All hospital-related
Cameron[9]	-	58	-	mean 93 median 68 (5-720)	-	11 (19%) (4,3,4)	-	7 (12%)	-	0	*nr*
Griniatsos*[48]	-	20	-	median 14 (7-14)	-	0	-	0	-	1	All hospital-related
Griniatsos*[48]	-	24	-	median 60 (47-91)	-	1 (4%) (1,0,0)	-	0	-	1	16 hospital-related, 4 delay of clinical improvement, 4 severe comorbidity
Clarke[47]	110	92	0	mean 23 (+/- 10)	0	8 (9%) (7,1,0)	nr	nr	4	5	All study-related
Ito[49]	162	119	0	median 45 (4-436)	0	39 (33%) (16,6,17)	20 (12%)	8 (7%)	37	34	*nr*
Nebiker[51]	32	67	0	>14	0	15 (22%) (9,2,4)	2 (6%)	2 (3%)	2	5	All hospital-related
Sinha[53]	81	26	0	>42	0	3 (12%) (3,0,0)	0	0	0	0	All patient-related
Aboulian[46]	24	-	0	-	0	-	0	-	0	-	-
TOTAL	**483**	**515**	**0**	**Median 40**	**0**	**95 (18%) (43,17,35)**	**31 (9%)#**	**27 (6%)**	**17 (4%)**	**29 (6%)**	**-**

*In one study two different groups of interval cholecystectomy were described. #Percentages only calculated for the studies that reported this endpoint. SD: Standard deviation, *nr:* Not reported

Baseline characteristics

The pooled data comprised of 998 patients undergoing cholecystectomy after mild biliary pancreatitis (range per study: 19-281 patients). The definitions of mild biliary pancreatitis per study are shown in Table 1. In the nine studies, 15 cohorts with different timing of cholecystectomy were described. One study described two different cohorts of patients undergoing interval cholecystectomy (Table 2).[48] Relevant baseline characteristics (i.e. age and ASA classification) were only reported both in two studies, including 263 patients (26%). A total number of 483 of 998 patients (48%; described in six different cohorts), underwent cholecystectomy during index admission. In the remaining nine cohorts, 515 of 998 patients (52%) underwent interval cholecystectomy at a median of 40 days (interquartile range 19-58 days) after discharge. Six studies (645 patients) reported on gender and age. The male:female ratio was 1:2, with a median age of 56 years (interquartile range 53-60 years). Eight studies, including 13 different cohorts and 796 patients, reported on the number of patients who underwent pre-operative ERC: 308 patients (39%). Not all eight studies reported numbers on the use of endoscopic sphincterotomy implicitly. Four studies described the use of intra-operative cholangiography.[9,46,48,50]

Re-admission prior to cholecystectomy

Table 2 shows outcomes as reported in the included studies. The re-admission rate between discharge and interval cholecystectomy was 95/515 (18%). Recurrent biliary pancreatitis occurred in 43/515 patients (8%), acute cholecystitis in 17/515 patients (3%) and biliary colics requiring re-admission in 35/515 patients (7%). No new episodes of biliary events prior to cholecystectomy were reported in the patients undergoing cholecystectomy during index admission (18% vs. 0%, $P<0.0001$). Details about the severity of recurrent biliary pancreatitis could only be retrieved for 3/43 patients: two patients suffered from severe recurrent biliary pancreatitis[49] and one patient suffered from a mild recurrent biliary pancreatitis[48].

Outcome of cholecystectomy

Eight studies, including 796 patients, reported on the conversion rate. Overall, conversion to open cholecystectomy occurred in 58 patients (7%), without differences between index and interval cholecystectomy. Major reasons for conversion were intra-abdominal adhesions, however, no exact data about the distribution of the conversions among the two groups could be retrieved. One study did not distinguish between laparoscopic and conventional cholecystectomy.[47] Although complications were described in all timing cohorts, not all studies described the number of patients with complications, but only the number of complications. For this reason, no overall complication rate could be calculated. A total number of 116 different complications were described, including three common bile duct injuries, without mortality. Again,

Table 3: Patient outcomes in patients with or without ERC undergoing delayed cholecystectomy.

Study	N	ERC/ES (%)	Re-admissions after previous ERC/ES (biliary pancreatitis, cholecystitis, colics)	Re-admissions without previous ERC/ES (biliary pancreatitis, cholecystitis, colics)
Schachter[52]	19	100%	0	0
McCullough[50]	90	63%	*nr*	*nr*
Cameron[9]	58	64%	(0,*nr*,*nr*)	≥4 (4,*nr*,*nr*)
Griniatsos*[48]	20	0%	0	0
Griniatsos*[48]	24	0%	0	1 (4%) (1,0,0)
Clarke[47]	92	*nr*	*nr*	*nr*
Ito[49]	119	47%	14 (12%) (2,5,7)	25 (21%) (4,1,10)
Nebiker[51]	67	36%	0	15 (22%) (9,2,4)
Sinha[53]	26	0%	0	3 (12%) (3,0,0)
TOTAL	**515**	**40%**	**14 (10%) (2,5,7)**	**≥48 (24%) (31,3,14)**

*In one study two different groups of interval cholecystectomy were described. *nr:* Not reported. ERC: Endoscopic retrograde cholangiography. ES: Endoscopic sphincterotomy.

Table 4: Quality assessment of the included studies with two different scoring lists.

Study	MINORS checklist*	Checklist for (non-) randomized trials*
Schachter[52]	3.8	3.7
McCullough[50]	6.7	5.9
Cameron[9]	5.6	5.9
Griniatsos*[48]	6.7	6.7
Clarke[47]	6.7	6.7
Ito[49]	7.5	6.3
Nebiker[51]	7.5	6.7
Sinha[53]	6.3	5.2
Aboulian[46]	-	8.9

*All scores are 0-10, with 10 reflecting the highest methodological score.

the exact type of complications and distribution among the two groups could not be extracted from the included studies.

Role of endoscopic sphincterotomy / ERC

Table 3 provides an overview of re-admissions for biliary events in relation to the use of ERC. Re-admission after previous ERC occurred in 14/136 patients (10%), due to recurrent biliary pancreatitis in two patients, acute cholecystitis in five patients and biliary colics in seven patients. Notably, of 197 patients without previous ERC, 48 (24%) were re-admitted. These re-admissions were due to recurrent biliary pancreatitis (n=31), acute cholecystitis (n=3) and biliary colics (n=14). ERC protected against re-admissions (10% vs. 24%, P=0.001).

Recurrent pancreatitis after cholecystectomy

Only two out of nine studies (n=157) reported follow-up after cholecystectomy.[9,51] Cameron *et al*[9] reported one case of recurrent biliary pancreatitis (2%), 223 days after cholecystectomy and Nebiker *et al*[51] reported one case (1%) of recurrent biliary pancreatitis, five years after cholecystectomy. In both patients a common bile duct stone was found. These patients suffered from mild pancreatitis and recovered uneventfully. It is unclear whether these patients had undergone index or interval cholecystectomy. The overall risk of recurrent pancreatitis after cholecystectomy in the pooled data was therefore 2/157 (1%).

Assessment of study quality

Table 4 shows the converted quality scores on a 0-10 scale. The randomized trial scored high[46], five studies scored moderate[47-51], two studies scored moderate to low[9,53] and one study scored low[52]. Reasons for delay of surgery were reported in 338/515 patients (66%) and were due to patient-related affairs (both patient-preferred delay and comorbidity, 34 patients, 10%), hospital-logistics (193 patients, 57%) and study design (111 patients, 33%). Two prospective cohort studies stated explicitly that cholecystectomy had intentionally been postponed because of the study design.[47,52]

DISCUSSION

This first systematic review on the timing of cholecystectomy after mild biliary pancreatitis found high re-admission rates (18%) for interval cholecystectomy. As morbidity was comparable between index and interval cholecystectomy, it seems that cholecystectomy during index admission should be the preferred strategy for patients with mild pancreatitis. However, as baseline characteristics were often not provided we cannot be sure whether the two groups are truly comparable. Selection bias might have played a role, e.g. patients with more co-morbidity might have undergone interval cholecystectomy.

Why do clinicians perform interval cholecystectomy so often? Lankisch *et al* sent a questionnaire to 190 German gastroenterologists and found that

lack of operation room availability and budgetary restraints were the reason that only 23% of patients had undergone cholecystectomy during the initial hospital admission for mild biliary pancreatitis.[12] These arguments have been challenged by a recent paper, concluding that cholecystectomy during index admission is both feasible and cost neutral.[10]

For long, surgeons have legitimated the choice for interval cholecystectomy by the belief that cholecystectomy during index admission would be associated with difficult dissection due to edema caused by pancreatitis, which could lead to more surgical complications and 'unnecessary' conversions. In contrast to this belief, in 107 patients with mild biliary pancreatitis, Sinha *et al* found that difficult dissection of Calot's triangle occurred more frequently in interval cholecystectomy as compared to index cholecystectomy (42% vs. 12%, P<0.001).[53]

Since the majority of patients apparently do not suffer from recurrent biliary events necessitating re-admission, one might ask "How detrimental are these recurrent biliary events? Why not only perform cholecystectomy in case of re-admission?" Furthermore, although cholecystectomy is considered definitive treatment, still 1% to 8.7% of patients suffer from recurrent biliary pancreatitis after cholecystectomy.[13] Nevertheless, 4-50% of cases of recurrent biliary pancreatitis are severe which might lead to mortality, although fortunately not reported in this review.[15,55] Since recurrent biliary pancreatitis occurred in 8% of patients in the interval cholecystectomy group, we feel this is a strong argument in favor of cholecystectomy during index admission.

It is generally accepted that patients with mild biliary pancreatitis without signs of (potential) cholangitis do not benefit from endoscopic sphinterotomy.[4] In this review, however, ERC was performed in 39% of all patients, but no explicit data on the number of endoscopic sphincterotomies performed during ERC or the presence of cholangitis could be extracted from the included studies. Recurrent biliary complications occurred in 10% of patients with and in 24% of patients without ERC. This difference was mainly due to a difference in recurrent biliary pancreatitis (2% of patients with ERC vs. 16% of patients without ERC). So, although ERC prevents recurrent pancreatitis to a large extent, it does not prevent against acute cholecystitis and biliary colics (8% with ERC vs. 9% without ERC). These numbers support the finding of a recent non-systematic review; ERC decreases the incidence of common bile duct stones-related complications, but will not prevent gallbladder stones-related complications, like biliary colics and acute cholecystitis.[56] Although in this review the percentage of patients with pre-operative ERC seems to be very high, it is important to realize that with lower incidence of the protective ERC the incidence of recurrent biliary events, especially biliary pancreatitis, could well have been even higher.

The results of the included studies are possibly flawed by selection bias, because the choice for index or interval cholecystectomy was to a large extent

based on clinical arguments. There may also have been publication bias, as a result of underreporting by doctor or patient of biliary colics in general or as a reason for readmission. Another limit of this systematic review is the fact that most included studies were of moderate to low methodological quality. Furthermore, no adequate follow-up after cholecystectomy was reported in most studies. Although follow-up is not necessarily needed in a comparative study of pre-operative readmissions for biliary events, it would give us a better insight of postoperative complications and recurrent biliary events after removal of the gallbladder.

Only one randomized trial, by Aboulian *et al*, was included in this review.[46] The primary endpoint of this trial was length of hospital stay. The study was stopped at interim analysis for a one day shorter hospital stay after early cholecystectomy with no difference in secondary endpoints (e.g. conversion rate, complication rate and mortality). We did not include the early group of this RCT, because we did not study cholecystectomy *during* pancreatitis but cholecystectomy *after* pancreatitis. There may be risks involved with performing cholecystectomy in the first 48-72 hours of pancreatitis, regardless of the clinical condition of the patient.[57] Of all patients with predicted mild pancreatitis, some 15% of patients will progress to severe pancreatitis.[58,59] Performing a cholecystectomy in patients with severe pancreatitis may be unsafe.[5] As in other studies[41-43], no life-threatening complications or mortality were noted, but with only 25 patients in the early group these numbers might have been too small to detect these complications.[46] Furthermore, the authors mainly included young Hispanic females, a population that may be of lower risk of complications than many populations with mild pancreatitis worldwide.[60] Although interval cholecystectomy is clearly associated with an undesirable high rate of readmissions, the included studies were of insufficient quality to exclude selection bias. Therefore, randomized controlled studies should confirm the efficacy and safety of cholecystectomy during index admission for mild biliary pancreatitis.

Acknowledgements

The authors wish to thank the following persons for providing additional data on their studies: Rodney Mason, Rajeev Sinha, John Griniatsos, Daniel Frey and Pinhas Schachter.

References

1. Yadav D, Lowenfels AB. Trends in the epidemiology of the first attack of acute pancreatitis: a systematic review. Pancreas 2006; 33:323-330.
2. Torgerson JS, Lindroos AK, Naslund I et al. Gallstones, gallbladder disease, and pancreatitis: cross-sectional and 2-year data from the Swedish Obese Subjects (SOS) and SOS reference studies. Am J Gastroenterol 2003; 98:1032-1041.
3. Fagenholz PJ, Fernandez-del CC, Harris NS et al. Direct medical costs of acute pancreatitis hospitalizations in the United States. Pancreas 2007; 35:302-307.
4. Banks PA, Freeman ML. Practice guidelines in acute pancreatitis. Am J Gastroenterol 2006; 101:2379-2400.
5. Nealon WH, Bawduniak J, Walser EM. Appropriate timing of cholecystectomy in patients who present with moderate to severe gallstone-associated acute pancreatitis with peripancreatic fluid collections. Ann Surg 2004; 239: 741-749.
6. UK guidelines for the management of acute pancreatitis. Gut 2005; 54 Suppl 3:iii1-iii9.
7. Forsmark CE, Baillie J. AGA Institute technical review on acute pancreatitis. Gastroenterology 2007; 132:2022-2044.
8. Uhl W, Warshaw A, Imrie C et al. IAP Guidelines for the Surgical Management of Acute Pancreatitis. Pancreatology 2002; 2:565-573.
9. Cameron DR, Goodman AJ. Delayed cholecystectomy for gallstone pancreatitis: re-admissions and outcomes. Ann R Coll Surg Engl 2004; 86:358-362.
10. Monkhouse SJ, Court EL, Dash I et al. Two-week target for laparoscopic cholecystectomy following gallstone pancreatitis is achievable and cost neutral. Br J Surg 2009; 96:751-755.
11. Sargen K, Kingsnorth AN. Management of gallstone pancreatitis: effects of deviation from clinical guidelines. JOP 2001; 2:317-322.
12. Lankisch PG, Weber-Dany B, Lerch MM. Clinical perspectives in pancreatology: compliance with acute pancreatitis guidelines in Germany. Pancreatology 2005; 5:591-593.
13. Pezzilli R, Uomo G, Gabbrielli A et al. A prospective multicentre survey on the treatment of acute pancreatitis in Italy. Dig Liver Dis 2007; 39:838-846.
14. Nguyen GC, Boudreau H, Jagannath SB. Hospital volume as a predictor for undergoing cholecystectomy after admission for acute biliary pancreatitis. Pancreas 2010; 39:e42-e47.
15. Hernandez V, Pascual I, Almela P et al. Recurrence of acute gallstone pancreatitis and relationship with cholecystectomy or endoscopic sphincterotomy. Am J Gastroenterol 2004; 99:2417-2423.
16. Liberati A, Altman DG, Tetzlaff J et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. PLoS Med 2009; 6:e1000100.
17. Bradley EL, III. A clinically based classification system for acute pancreatitis. Summary of the International Symposium on Acute Pancreatitis, Atlanta, Ga, September 11 through 13, 1992. Arch Surg 1993; 128:586-590.

18. Downs SH, Black N. The feasibility of creating a checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care interventions. J Epidemiol Community Health 1998; 52:377-384.
19. Slim K, Nini E, Forestier D et al. Methodological index for non-randomized studies (minors): development and validation of a new instrument. ANZ J Surg 2003; 73:712-716.
20. Bedirli A, Sozuer EM, Sakrak O et al. Comparison of the results of early, delayed and elective surgery in biliary pancreatitis. Turk J Gastroenterol 2003; 14:97-101.
21. Hui CK, Lai KC, Yuen MF et al. The role of cholecystectomy in reducing recurrent gallstone pancreatitis. Endoscopy 2004; 36:206-211.
22. Isla A, Griniatsos J, Rodway A. Single-stage definitive laparoscopic management in mild acute biliary pancreatitis. J Laparoendosc Adv Surg Tech A 2003; 13:77-81.
23. Kaw M, Al-Antably Y, Kaw P. Management of gallstone pancreatitis: cholecystectomy or ERCP and endoscopic sphincterotomy. Gastrointest Endosc 2002; 56:61-65.
24. Lee JK, Ryu JK, Park JK et al. Roles of endoscopic sphincterotomy and cholecystectomy in acute biliary pancreatitis. Hepatogastroenterology 2008; 55:1981-1985.
25. Liu CL, Lo CM, Fan ST. Acute biliary pancreatitis: diagnosis and management. World J Surg 1997; 21:149-154.
26. Tang E, Stain SC, Tang G et al. Timing of laparoscopic surgery in gallstone pancreatitis. Arch Surg 1995; 130:496-499.
27. Uhl W, Muller CA, Krahenbuhl L et al. Acute gallstone pancreatitis: timing of laparoscopic cholecystectomy in mild and severe disease. Surg Endosc 1999; 13:1070-1076.
28. DeIorio AV, Jr., Vitale GC, Reynolds M et al. Acute biliary pancreatitis. The roles of laparoscopic cholecystectomy and endoscopic retrograde cholangiopancreatography. Surg Endosc 1995; 9:392-396.
29. Lawrentschuk N, Hewitt PM, Pritchard MG. Elective laparoscopic cholecystectomy: implications of prolonged waiting times for surgery. ANZ J Surg 2003; 73:890-893.
30. Rosing DK, de VC, Yaghoubian A et al. Early cholecystectomy for mild to moderate gallstone pancreatitis shortens hospital stay. J Am Coll Surg 2007; 205:762-766.
31. Sanjay P, Yeeting S, Whigham C et al. Management guidelines for gallstone pancreatitis. Are the targets achievable? JOP 2009; 10:43-47.
32. Soper NJ, Brunt LM, Callery MP et al. Role of laparoscopic cholecystectomy in the management of acute gallstone pancreatitis. Am J Surg 1994; 167: 42-50.
33. Alimoglu O, Ozkan OV, Sahin M et al. Timing of cholecystectomy for acute biliary pancreatitis: outcomes of cholecystectomy on first admission and after recurrent biliary pancreatitis. World J Surg 2003; 27:256-259.
34. Archibald JD, Love JR, McAlister VC. The role of prophylactic cholecystectomy versus deferral in the care of patients after endoscopic sphincterotomy. Can J Surg 2007; 50:19-23.

35. Hammarstrom LE, Stridbeck H, Ihse I. Effect of endoscopic sphincterotomy and interval cholecystectomy on late outcome after gallstone pancreatitis. Br J Surg 1998; 85:333-336.
36. Ricci F, Castaldini G, de MG et al. Treatment of gallstone pancreatitis: six-year experience in a single center. World J Surg 2002; 26:85-90.
37. Schiphorst AH, Besselink MG, Boerma D et al. Timing of cholecystectomy after endoscopic sphincterotomy for common bile duct stones. Surg Endosc 2008; 22:2046-2050.
38. Srinathan SK, Barkun JS, Mehta SN et al. Evolving management of mild-to-moderate gallstone pancreatitis. J Gastrointest Surg 1998; 2:385-390.
39. Teoh AY, Poon MC, Leong HT. Role of prophylactic endoscopic sphincterotomy in patients with acute biliary pancreatitis due to transient common bile duct obstruction.J Gastroenterol Hepatol 2007; 22:1415-1418.
40. Thornton DJ, Robertson A, Alexander DJ. Patients awaiting laparoscopic cholecystectomy--can preoperative complications be predicted? Ann R Coll Surg Engl 2004; 86:87-90.
41. Ballestra-Lopez C, Bastida-Vila X, Bettonica-Larranaga C et al. Laparoscopic management of acute biliary pancreatitis. Surg Endosc 1997; 11:718-721.
42. Borzellino G, Lombardo F, Minicozzi AM et al. Early laparoendoscopic rendezvous for acute biliary pancreatitis: preliminary results. Surg Endosc 2010; 24:371-376.
43. Taylor E, Wong C. The optimal timing of laparoscopic cholecystectomy in mild gallstone pancreatitis. Am Surg 2004; 70:971-975.
44. Prabhu RY, Irpatgire R, Naranje B et al. Influence of timing on performance of laparoscopic cholecystectomy for acute biliary pancreatitis. Trop Gastroenterol 2009; 30:113-115.
45. Sandzen B, Haapamaki MM, Nilsson E et al. Cholecystectomy and sphincterotomy in patients with mild acute biliary pancreatitis in Sweden 1. BMC Gastroenterol 2009; 9:80.
46. Aboulian A, Chan T, Yaghoubian A et al. Early cholecystectomy safely decreases hospital stay in patients with mild gallstone pancreatitis: a randomized prospective study. Ann Surg 2010; 251:615-619.
47. Clarke T, Sohn H, Kelso R et al. Planned early discharge-elective surgical readmission pathway for patients with gallstone pancreatitis. Arch Surg 2008; 143:901-905.
48. Griniatsos J, Karvounis E, Isla A. Early versus delayed single-stage laparoscopic eradication for both gallstones and common bile duct stones in mild acute biliary pancreatitis. Am Surg 2005; 71: 682-686.
49. Ito K, Ito H, Whang EE. Timing of cholecystectomy for biliary pancreatitis: do the data support current guidelines? J Gastrointest Surg 2008; 12:2164-2170.
50. McCullough LK, Sutherland FR, Preshaw R et al. Gallstone pancreatitis: does discharge and readmission for cholecystectomy affect outcome? HPB (Oxford) 2003; 5:96-99.
51. Nebiker CA, Frey DM, Hamel CT et al. Early versus delayed cholecystectomy in patients with biliary acute pancreatitis. Surgery 2009; 145:260-264.

52. Schachter P, Peleg T, Cohen O. Interval laparoscopic cholecystectomy in the management of acute biliary pancreatitis. HPB Surg 2000; 11:319-322.
53. Sinha R. Early laparoscopic cholecystectomy in acute biliary pancreatitis: the optimal choice? HPB (Oxford) 2008; 10:332-335.
54. Howick J. Oxford Centre for Evidence-based Medicine - Levels of Evidence. Oxford Centre for Evidence-based Medicine March 2009.
55. Lankisch PG, Bruns A, Doobe C et al. The second attack of acute pancreatitis is not harmless. Pancreas 2008; 36:207-208.
56. van Geenen EJ, van der Peet DL, Mulder CJ et al. Recurrent acute biliary pancreatitis: the protective role of cholecystectomy and endoscopic sphincterotomy. Surg Endosc 2009; 23:950-956.
57. Bouwense SA, Bakker OJ, van Santvoort HC et al; Dutch Pancreatitis Study Group. Safety of cholecystectomy in the first 48 hours after admission for gallstone pancreatitis not yet proven. Ann Surg 2011 May;253(5):1053-4.
58. Dambrauskas Z, Gulbinas A, Pundzius J et al. Value of the different prognostic systems and biological markers for predicting severity and progression of acute pancreatitis. Scand J Gastroenterol 2010; 45:959-970.
59. Papachristou GI, Muddana V, Yadav D et al. Comparison of BISAP, Ranson's, APACHE-II, and CTSI scores in predicting organ failure, complications, and mortality in acute pancreatitis. Am J Gastroenterol 2010; 105:435-441.
60. Aboulian A, de Virgilio C. Safety of Cholecystectomy in the First 48 hours After Admission for Gallstone Pancreatitis not yet Proven. Ann Surg. 2011 May; 253(5): 1054-5.

Chapter 9

Summary and general discussion

This thesis has focused on questions remaining after two pivotal multicenter randomized controlled trials, (RCT) conducted by the Dutch Pancreatitis Study Group (DPSG). Both trials had a major impact on clinical care for patients with severe forms of acute pancreatitis. The first trial, PROPATRIA (probiotics in pancreatitis trial, 2004-2007), has given rise to an intensive discussion on ethical, procedural and methodological aspects of randomized trials in the Netherlands.[1] The outcome of the study was dramatic and fully unexpected, since mortality was higher in the patients prophylactically treated with probiotics compared to the placebo group, whereas probiotics did not reduce infectious complications. Even more, bowel ischemia was diagnosed in 6% of patient in the probiotic group vs. 0% in the placebo group. From that moment on, probiotics were not considered harmless anymore, especially not when administrated in critically ill patients. However, except for several speculations, the underlying mechanism for mortality and bowel ischemia remained unclear. The second multicenter RCT of the DPSG was the PANTER trial (pancreatitis, maximal necrosectomy versus minimally invasive step-up approach, 2005-2008) and showed that the step-up approach (percutaneous catheter drainage (PCD), if needed, followed by minimally invasive necrosectomy) was superior to open surgical necrosectomy in terms of major and minor complications and cost efficiency.[2] Furthermore, it showed that a third of patients with (suspected) infected necrosis could be successfully treated by PCD alone, without the need for additional surgical necrosectomy.

Crucial questions remained after finishing the PROPATRA and PANTER trials. The first part of this thesis focuses on the interaction between probiotics and enteral nutrition in order to unravel the mechanism responsible for the increased mortality and bowel ischemia in patients treated with probiotics in the PROPATRIA trial. The second part of this thesis focuses on how to accurately diagnose infection and how to design further interventional treatment strategies in acute pancreatitis, having found that after PANTER, “drainage first” is the new paradigm in treatment of infected necrosis in severe acute pancreatitis.

Part I – Interaction between probiotics and enteral nutrition in acute pancreatitis

For long, probiotics were thought to be harmless food supplements. The PROPATRIA trial, however, showed that a specific mixture of six probiotic strains (Ecologic 641) could be harmful to critically ill patients with severe pancreatitis.[1] A causal relation between administration of probiotics and non-occlusive bowel ischemia (NOMI) has not been established yet. It has been hypothesized, however, that non-occlusive bowel ischemia could have been induced by probiotics by generating potentially cytotoxic fermentation products.

In **Chapter 2**, we used a dynamic, computer-controlled model for simulating *in vivo* conditions of the stomach and small bowel (TIM-1 model). We were particularly interested in whether the composition of enteral nutrition could have any influence on the intraluminal metabolic activity of Ecologic 641. Each experiment with TIM-1 lasted six hours, reflecting the slow intestinal transit time clinically observed in very ill patients. Enteral nutrition was continuously administered directly into the duodenum. Probiotics and placebo both were also administered into the duodenum, but as in a single shot. Bile salts and pancreatic enzymes were added or left out, depending on the experimental design. We found that in the presence of pancreatic enzymes, significantly more acid was produced. In the presence of pancreatic enzymes, lactic acid production is increased, whereas in the addition of bile salts lactic acid production is decreased. Addition of fibre-enriched enteral nutrition resulted in a higher lactic acid production compared to protein-enriched enteral nutrition. In the presence of fibre-enriched enteral nutrition, short-chain fatty acid production was increased compared to protein-enriched enteral nutrition. It was concluded that the metabolic activity of the probiotic mixture is maximal in the presence of both pancreatic enzymes and fibre-enriched enteral nutrition in the duodenum.

In 2008, van Minnen et al. described the effects of pretreatment with Ecologic 641, a probiotic mixture of six different probiotic strains, on bacterial translocation and mortality in an experimental rat model.[3] Pretreatment with probiotics led to a better survival and lower bacterial translocation than observed in rats treated with placebo. A major difference with the PROPATRIA trial was the timing of administration of probiotics. In patients, the pancreatitis was already ongoing, whereas in rats administration was given prophylactically in the true sense, so prior to onset of the pancreatitis. In this series of experiments described in **Chapter 3**, the same experimental rat model for severe necrotizing pancreatitis as described by van Minnen et al. was used and the clinical situation was mimicked as closely as possible.[2,3] Animals were allocated to four experimental groups: 1) acute pancreatitis with administration of saline and placebo, 2) acute pancreatitis with administration of saline and probiotics, 3) acute pancreatitis with administration of fibre-enriched enteral nutrition and placebo, and 4) acute pancreatitis with administration of fibre-enriched enteral nutrition and probiotics. Based on clinical, biochemical, histological and bacteriological findings, no causal relationship between NOMI and the combination of administrated fibre-enriched enteral nutrition and probiotics and no reduction of bacterial translocation to the pancreas in animals treated with probiotics could be found.

Since 2008, after the outcome of the PROPATRIA trial, no new clinical studies have been published about probiotic prophylaxis in patients with acute pancreatitis. In 2010, we became aware of a hospital, the Faculty Thomayer´s Hospital, Prague, Czech Republic, where administration of

probiotics in combination with enteral nutrition was continued in patients with acute pancreatitis, without organ failure. In **Chapter 4**, we describe a retrospective analysis of all these patients and concluded that in this study, no negative impact of probiotic prophylaxis, bowel perfusion and/or mortality was observed in this group of patients with predicted severe pancreatitis without organ failure. Furthermore, no reduction of infectious complications was observed when compared to the literature.

In summary, the unfavorable outcome of the PROPATRIA trial was investigated with focus on a potential negative interaction between probiotics and enteral nutrition in acute pancreatitis as the driving force behind the excess mortality. Neither *in vitro*, nor *in vivo* studies in rats could detect a negative interference between probiotics, intraluminal conditions and enteral feeding. As a consequence, no support could be lent to the hypothesis that the combination of the three above mentioned factors leads to excessive oxygen consumption, mucosal hypoxemia, mucosal damage and bacterial translocation. This series of experiments, therefore, provide no clue as to whether the experimental prophylactic administration of probiotics has started a cascade of events leading to local mucosal hypoxia and NOMI. The *in vitro* experiments and the studies in rats have not brought us any closer to an explanation for the higher death rate in the PROPATRIA trial for patients who received probiotics to lower the infection rate. The analysis of the data from Prague, although performed in patients who did not suffer from early organ failure, showed no positive or negative effect of probiotics on the number of infectious complications. Furthermore, no increased mortality and bowel ischemia were found. These findings are comparable to another study by Besselink et al, in which negative effects of probiotics were only observed in patients with (multiple) organ failure. In PROPATRIA, it is important to note that NOMI was a post-hoc outcome measure and has not been part of the study hypothesis. As a consequence we have not systematically looked for the presence or absence of NOMI in both groups. Since NOMI is not necessarily a lethal complication and can escape to detection when not systematically searched for, a clear association between probiotics, NOMI and death cannot be confirmed based on the combined findings of the PROPATRIA trial and the studies that have been described in this thesis.

Part II – Diagnosis of infection and interventional strategies in acute pancreatitis

In the **Chapters 5, 6, 7 and 8** several aspects of (surgical) intervention were studied to investigate the role of PCD only in the treatment of infected necrosis. We performed these additional studies, since accurate diagnosis of infection remained a key issue in our trial and in intervention studies of others. The potential of percutaneous drainage turned out to be the most prominent finding of the PANTER trial, fistula formation was a frequently

observed complication and timing of cholecystectomy was an important topic, once pancreatitis had been survived.

About 20% of patients with acute pancreatitis develop pancreatic or peripancreatic necrosis (i.e. necrotizing pancreatitis). In a third of these patients infection of the necrosis occurs, and in the vast majority of these patients an intervention (i.e., catheter drainage, endoscopic or surgical necrosectomy) is required.[4] Even with an intervention, morbidity and mortality still ranges between 50-100% and 15-25%, respectively.[6-9] In the last decades, many studies have focused on prevention of infection of the necrosis, however only a few studies addressed the topic of diagnosing infection of the necrosis. In general, infection of the necrosis is suspected based on positive tissue cultures (e.g., obtained by fine-needle aspiration, FNA), gas bubbles in fluid collections on contrast-enhanced computer tomography (CECT), and by clinical signs (e.g. fever, leucocytosis, organ failure). In **Chapter 5**, we have tried to study the role of each of these methods for diagnosing infection in a post-hoc analysis of a prospective cohort of 639 patients with necrotizing pancreatitis.[5] We selected all 208 patients who underwent an intervention for infected necrotizing pancreatitis to study the meaning of strictly clinical parameters, imaging modalities and FNA for the confirmation of infection. In general, interventions were postponed as long as possible, preferably more than four weeks after onset of symptoms, allowing demarcation of the necrotic cavity and ultimately reducing the complication rate. Even in case of proven infection, interventions were postponed, whenever possible. It was concluded that in the majority of patients infected necrosis can be diagnosed based on clinical and imaging signs and that FNA may be reserved for patients with unclear clinical signs with absence of imaging signs of infection.

For decades, the only interventional strategy for patients with (suspected) infected necrosis was open surgical necrosectomy. However, this procedure is associated with a mortality of 30% and a morbidity of up to 95%. Furthermore, a high incidence of long term pancreatic insufficiency is reported in patients who undergo open necrosectomy.[10-12] Since the late eighties, less invasive interventions have gained popularity in the treatment of infected necrotizing pancreatitis. The breakthrough occurred in the late nineties, when PCD as primary treatment of infected necrotizing pancreatitis was first described.[13] Since then, many studies reported on minimally invasive strategies for infected necrotizing pancreatitis. The hypothesis of PCD is to treat infected necrosis as an abscess and drain the infected fluid (i.e., pus) under pressure to enable the patient's immune system to cope with the infected focus and to leave the necrotic material for the patient to deal with. Successful drainage of the infected fluid under pressure will ameliorate sepsis and improve the patient's clinical condition. If necrosectomy is still needed after PCD, PCD may have postponed the need of surgical intervention, allowing for further encapsulation of the necrotic collections and improvement of the patient's

clinical condition. In **Chapter 6**, we described the results of the first systematic review on the role of PCD in the treatment of (suspected) infected necrotizing pancreatitis and concluded that a considerable number (percentages ranging from 18 to 90%) of patients could be treated with PCD only, without the need for additional surgical necrosectomy. How to select patients for PCD and how predict which (group of) patients will react favorably to PCD as the first step, could not be concluded from the systematic review.

A well-known complication after surgical necrosectomy or PCD in patients with (suspected) infected necrotizing pancreatitis is a pancreatico-cutaneous fistula. Incidences of persisting pancreatic fistulas range from 17% to 76% and are associated with metabolic and nutritional disturbances, prolonged hospitalization and even with mortality.[14-17] Usually, these fistulas are treated conservatively, however, endoscopic transpapillary stent placement (ETS) is suggested to be a safe and feasible alternative.[18-20] The rationale of ETS is to reduce intraductal pressure, facilitating drainage of pancreatic secretions to the duodenum instead of through the fistula. In **Chapter 7**, we compared the outcome of 35 patients with persisting pancreatic fistula after intervention for (suspected) infected necrotizing pancreatitis who were treated conservatively or with ETS. In 5 patients who were ultimately treated conservatively, ETS was not successful. In the majority of patients (84%) with persisting fistula, complete resolution of the fistula was achieved with ETS only. In the patients who were treated conservatively, 25% needed additional intervention for fistula closure. Median time to fistula closure was 71 days in the ETS group and 120 days in the conservative group. It was concluded that ETS may be a safe and feasible alternative to conservative treatment.

Current guidelines advocate early cholecystectomy after an episode of mild biliary pancreatitis. However, there is no consensus on the definition of "early": within two or four weeks after hospital discharge or even during index admission.[4,21-23] The DPSG analyzed all patients with mild biliary pancreatitis from the PROPATRIA trial and found that 34/249 patients (14%) suffered from recurrent biliary events prior to cholecystectomy.[24] In **Chapter 8** we describe a systematic review of the literature and focused primarily on the risk of recurrent biliary events in the period between discharge after mild biliary pancreatitis and interval cholecystectomy, and secondary on the safety of index versus interval cholecystectomy after mild biliary pancreatitis. Overall, 39% of patients underwent a pre-operative endoscopic retrograde cholangiography (ERC). Re-admission rate in the group of patients who underwent delayed cholecystectomy was 18%, of whom around 40% suffered from recurrent biliary pancreatitis. None of the patients who underwent cholecystectomy during index admission suffered from recurrent biliary complications prior to cholecystectomy. No differences in conversion rate and complications were found between groups. To reduce recurrent biliary complications after an episode of mild biliary

pancreatitis, it is suggested to minimize the period between cholecystectomy and hospital discharge from the index admission.

Conclusions and ways to proceed

Part I – Interaction between probiotics and enteral nutrition in acute pancreatitis

The first part of this thesis was dedicated to the question: how to explain the unexpected findings of the PROPATRIA trial? A question that was frequently brought up was: *"have we challenged the small bowel in these very sick patients too much by administrating the combination of enteral feeding and probiotics in the lumen of the proximal small bowel and could this have induced high oxygen consumption leading to local hypoxia with non-occlusive small bowel ischemia?"*. If this would have been the case, can the observation of bowel ischemia in 9 patients serve as an explanation for the excess mortality in the experimental group in the PROPATRIA trial?[1] This concept was the focus of several studies in this thesis and has also been addressed in part in the work of Paul van Minnen, Harro Timmermans, Marc Besselink and Femke Lutgendorff. Besselink et al. showed that probiotics may increase mucosal damage in the subgroup of patients with predicted severe acute pancreatitis and organ failure, whereas no increased mucosal damage was found in patients without organ failure.[25] Although this analyses was performed in a small subgroup of patients of the PROPATRIA trial (115 patients without and 26 with organ failure), these results point out that the presence of organ failure might be the key factor in the search for answers after the PROPATRIA trial. Unfortunately, the studies described in this thesis did not have given us real new clues to how the excess mortality has been brought about. Earlier experimental studies by our group, specifically those conducted by Femke Lutgendorff, have shown that probiotics, when administered prior to onset of acute pancreatitis, have a positive impact on small bowel mucosa, in line with the theoretical concept of how probiotics are supposed to improve small bowel function and transport.[26,27] In addition, the available clinical data do not provide us with a mechanism to hold the probiotics, the enteral feeding or the combination responsible for the unexpected findings in the PROPATRIA trial. A comment has to be made here that the results of neither *in vitro* studies, nor experimental studies in animals can be extrapolated to the situation in human, and as a consequence, our findings are all indirect data on pathophysiological mechanisms playing a role in patients with acute pancreatitis.

As shown in **Chapter 2**, In TIM-1 some potential harmful metabolites for the intestinal mucosa are produced in the presence of fibre-enriched enteral nutrition and pancreatic enzymes, however, the collected samples should be further used in Caco-2 cell culture experiments to focus on the cytotoxicity of these samples on bowel mucosa. Furthermore, each single probiotic strain of Ecologic 641 should be investigated in TIM-1 to find out what strain is

responsible for what metabolite. For better understanding of the findings in TIM-1, future studies should also focus on whether the intraluminal concentration of pancreatic enzymes is increased or decreased in patients with (severe) acute pancreatitis, since only a few small studies showed conflicting results.[28,29] However, since no major differences in acid production were found between experiments with different intraluminal conditions in TIM-1, it is unlikely that in future *in vitro* experiments the mechanism responsible for the increased mortality and bowel ischemia as shown in the PROPATRIA trial will be found. When focusing on experimental studies in rats, none of the studies performed after the PROPATRIA trial have shown negative effects of probiotics and the results described in **Chapter 3** did not show any negative effect of probiotics and enteral nutrition. Therefore, it is unlikely that future experimental animal studies will provide knowledge to understand the potential negative effects of probiotics.

As shown in **Chapter 4**, in patients in Prague with predicted severe pancreatitis without organ failure at the time the first dose of multispecies probiotic prophylaxis and enteral nutrition was administered, no negative effects of probiotics were found. However, as in PROPATRIA, no positive impact on the number of infectious complications was observed. Although this monocenter study suggests that this specific probiotic mixture was probably not harmful to patients with pancreatitis without organ failure, one should notice that a relatively small cohort of patients was described in a retrospective manner using a different multispecies probiotic product as used in PROPATRIA. Because of the shortcomings, a direct comparison with PROPATRIA is difficult to make.

So we are left with the following conclusions. In this thesis, no clear new insights have been gained to unravel the puzzling findings of the PROPATRIA trial. We have to accept that current knowledge and current techniques to study phenomena at the level of the small bowel are potentially suboptimal. It will be very difficult to design further studies to unravel the mechanism of NOMI and its local and systemic consequences. This leaves us with the dilemma: do we need to proceed and design further studies or do we accept the fact that we have not been able to find the answer to the questions remaining after the PROPATRIA trial?

Scientifically, the next logical step would be to repeat the PROPATRIA trial investing in the same group of patients as participated in the PROPATRIA trial, in order to determine the reproducibility of the initial findings. Apart from strong ethical objections and reluctance to embark on such a study, a clinically meaningful study hypothesis would be lacking, since reduction of infectious complications in predicted severe acute pancreatitis was the primary outcome measure in the PROPATRIA study. In that respect, the outcome of the study was negative and therefore there is no scientific basis for repeating the study.

Is there still a (minute) possibility that there is no causal relation between the

excess mortality and the administration of probiotics in predicted severe acute pancreatitis? Probably not. We only found confirmation that (certain) probiotics are probably not harmful in the subgroup of patients with acute pancreatitis without organ failure, but we were unable to construct the mechanism by which probiotics, in extreme conditions of organ failure, can potentially induce NOMI. The fact that the mechanism was not found does obviously not necessarily mean that such a mechanism does not exist. The PROPATRIA trial was powered for infectious complications as the primary outcome measure and underpowered for mortality, but, again, this should not be a reason to dismiss the increased mortality in the study as coincidence.

Since it is not likely that new *in vitro* and *in vivo* experimental studies will bring us closer to an explanation of the still unexplained findings in the PROPATRIA trial, and that no new clinical randomized controlled trial will be performed in the near future, we can only accept that with the current knowledge and techniques no explanation could be found for the increased mortality and incidence of bowel ischemia in enterally fed patients with predicted severe pancreatitis and prophylactically treated with probiotics in the PROPATRIA trial.

Part II – Diagnosis of infection and interventional strategies in acute pancreatitis

In the last decades, minimally invasive procedures have become the new standard practice of care. For long, open necrosectomy has been the gold standard for treatment of (suspected) infected necrosis, however, the PANTER trial showed that even in a complicated disease as severe acute pancreatitis, minimally invasive intervention strategies are superior to conventional strategies.[2] Furthermore, the need for invasive diagnostic procedures to determine infection of necrosis could be disputed. With the policy of the PANTER trial to postpone surgical intervention for as long as possible, the second part of this thesis gives answers on: how to accurately diagnose infection in patient with suspected infected necrosis; if infection is suspected, what is the role of PCD compared to open surgical necrosectomy; and how to treat long lasting pancreatic fistulas after drainage procedures in acute pancreatitis? Furthermore, we focused on the optimal timing of cholecystectomy after mild biliary pancreatitis. These questions remained after finishing the PANTER and PROPATRIA trials. With a combined database of both studies, patients were selected to answer these questions, together with systematic reviews of the literature. The following conclusions can be drawn from this thesis:

1) With the policy of postponing intervention in patients with (suspected) infected necrotizing pancreatitis, FNA is of limited value and should only be used in patients with unclear clinical signs without radiological signs of infection.
2) In patients with infected necrotizing pancreatitis, a considerable number of patients can be treated with PCD only, without the need for surgical

necrosectomy. Therefore, PCD should be the first step as interventional treatment strategy.

3) Persistent pancreatic fistulas after interventional treatment of (infected) necrotizing pancreatitis could be safely treated by ETS as alternative to conservative treatment.
4) In patients with mild biliary pancreatitis, the period between hospital discharge from the index admission and the cholecystectomy should be minimized to lower recurrent biliary complications prior to cholecystectomy.

As identified in **Chapter 5** and **6**, with the policy of postponing intervention for as long as possible, FNA is of limited value. However, there may be a subgroup of patients with early signs of infected necrotizing pancreatitis that may benefit from an early intervention, and in particularly PCD because of the minimally induced immunological response compared to surgical necrosectomy. In this subgroup of patients, PCD should only be performed when the pancreatic necrosis is infected. Without radiological signs and unclear clinical signs, FNA may play a more prominent role in the diagnostic work-up. In the near future, the POINTER trial (postponed or immediate drainage of infected necrotizing pancreatitis) will investigate what the optimal timing for intervention will be in patients with (suspected) infected necrotizing pancreatitis and what the role of FNA will be in early diagnosing infection.

As identified in **Chapter 7**, a well-known complication of surgical or radiological intervention in infected necrotizing pancreatitis is a persisting pancreatic fistula. It is shown that ETS is a safe and alternative treatment to conservative treatment. Future randomized studies should investigate whether ETS must be considered to be a useful alternative to conservative treatment or must be the gold standard for persisting pancreatic fistulas. Besides duration of closure of the fistula, quality of life and cost-efficacy should be investigated.

As identified in **Chapter 8**, cholecystectomy after an episode of mild biliary pancreatitis should be performed as soon as possible after recovery, since a prolonged period between the initial episode of biliary pancreatitis and cholecystectomy is associated with a higher recurrence of biliary complications. Studies included in the systematic review were of medium methodological quality, therefore the PONCHO trial was conducted to investigate whether early cholecystectomy should be preferred above delayed cholecystectomy. The results of the PONCHO trial (trial number ISRCTN72764151) are expected in 2014. The optimal timing of cholecystectomy in patients with severe biliary pancreatitis remains unclear, however, scarce evidence suggests that early cholecystectomy is associated with an increased number of infectious complications.[30,31] After determination of the optimal timing of cholecystectomy in patients with mild biliary pancreatitis, future studies should focus on the optimal timing of cholecystectomy in patients with severe biliary pancreatitis.

References

1. Besselink MG, van Santvoort HC, Buskens E, et al. Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. Lancet 2008; 371:651-659.
2. van Santvoort HC, Besselink MG, Bakker OJ, et al. A step-up approach or open necrosectomy for necrotizing pancreatitis. N Engl J Med 2010; 362:1491-1502.
3. van Minnen LP, Timmerman HM, Lutgendorff F, et al. Modification of intestinal flora with multispecies probiotics reduces bacterial translocation and improves clinical course in a rat model of acute pancreatitis. Surgery 2007; 141: 470-80.
4. Banks PA, Freeman ML; Practice Parameters Committee of the American College of Gastroenterology. Practice guidelines in acute pancreatitis. Am J Gastroenterol 2006; 101: 2379-2400.
5. van Santvoort HC, Bakker OJ, Bollen TL, et al. A conservative and minimally invasive approach to necrotizing pancreatitis improves outcome. Gastroenterology 2011; 141:1254-1263.
6. Rau B, Bothe A, Beger HG. Surgical treatment of necrotizing pancreatitis by necrosectomy and closed lavage: changing patient characteristics and outcome in a 19-year, single-center series. Surgery 2005; 138:28-39.
7. Rodriguez JR, Razo AO, Targarona J, et al. Debridement and closed packing for sterile or infected necrotizing pancreatitis: insights into indications and outcomes in 167 patients. Ann Surg 2008; 247:294-299.
8. Bakker OJ, van Santvoort HC, van Brunschot S, et al. Endoscopic transgastric vs surgical necrosectomy for infected necrotizing pancreatitis: a randomized trial. JAMA 2012; 307:1053-1061.
9. van Baal MC, van Santvoort HC, Bollen TL, et al. Systematic review of percutaneous catheter drainage as primary treatment for necrotizing pancreatitis. Br J Surg 2011; 98:18-27.
10. Rau B, Bothe A, Beger HG. Surgical treatment of necrotizing pancreatitis by necrosectomy and closed lavage: changing patient characteristics and outcome in a 19-year, single-center series. Surgery 2005; 138:28-39.
11. Büchler MW, Gloor B, Müller CA, et al. Acute necrotizing pancreatitis: treatment strategy according to the status of infection. Ann Surg 2000; 232:619-626.
12. Rodriguez JR, Razo AO, Targarona J, et al. Debridement and closed packing for sterile or infected necrotizing pancreatitis: insights into indications and outcomes in 167 patients. Ann Surg 2008; 247:294-299.
13. Freeny PC, Hauptmann E, Althaus SJ, et al. Percutaneous CT-guided catheter drainage of infected acute necrotizing pancreatitis: techniques and results. AJR Am J Roentgenol 1998; 170:969-975.
14. Ho HS, Frey CF. Gastrointestinal and pancreatic complications associated with severe pancreatitis. Arch Surg 1995; 130:817-822.
15. Tsiotos GG, Smith CD, Sarr MG. Incidence and management of pancreatic and enteric fistulas after surgical management of severe necrotizing pancreatitis. Arch Surg 1995; 130:48-52.
16. Fotoohi M, D'Agostino HB, Wollman B, et al. Persistent pancreatocutaneous fistula after percutaneous drainage of pancreatic fluid collections: role of cause and severity of pancreatitis. Radiology 1999; 213: 573-578.

17. Connor S, Ghaneh P, Raraty M, et al. Minimally invasive retroperitoneal pancreatic necrosectomy. Dig Surg 2003; 20:270-277.
18. Boerma D, Rauws EA, van Gulik TM, et al. Endoscopic stent placement for pancreaticocutaneous fistula after surgical drainage of the pancreas. Br J Surg 2000; 87:1506-1509.
19. Telford JJ, Farrell JJ, Saltzman JR, et al. Pancreatic stent placement for duct disruption. Gastrointest Endosc 2002; 56:18-24.
20. Varadarajulu S, Noone TC, Tutuian R, et al. Predictors of outcome in pancreatic duct disruption managed by endoscopic transpapillary stent placement. Gastrointest Endosc 2005; 61:568-575.
21. UK guidelines for the management of acute pancreatitis. Gut 2005; 54 Suppl 3:iii1-9.
22. Forsmark CE, Baillie J. AGA institute technical review on acute pancreatitis. Gastroenterology 2007; 132:2022-2044.
23. Uhl W, Warshaw A, Imrie C et al. IAP Guidelines for the surgical management of acute pancreatitis. Pancreatology 2002; 2:565-573.
24. Bakker OJ, van Santvoort HC, Hagenaars JC, et al. Timing of cholecystectomy after mild biliary pancreatitis. Br J Surg 2011; 98:1446-1454.
25. Besselink MG, van Santvoort HC, Renooij W et al. Intestinal barrier dysfunction in a randomized trial of a specific probiotic composition in acute pancreatitis. Ann Surg 2009; 250:712-719.
26. Lutgendorff F, Nijmeijer RM, Sandström PA et al. Probiotics prevent intestinal barrier dysfunction in acute pancreatitis in rats via induction of ileal mucosal glutathione biosynthesis. PLoS One 2009; 4:e4512
27. Lutgendorff F, Trulsson LM, van Minnen LP et al. Probiotics enhance pancreatic glutathione biosynthesis and reduce oxidative stress in experimental acute pancreatitis. Am J Physiol Gastrointest Liver Physiol 2008; 295:G1111-1121
28. O'Keefe SJ, Lee RB, Li J et al. Trypsin secretion and turnover in patients with acute pancreatitis. Am J Physiol Gastrointest Liver Physiol 2005; 289: G181-187.
29. Czakó L, Yamamoto M, Otsuki M. Exocrine pancreatic function in rats after acute pancreatitis. Pancreas 1997; 15:83-90.
30. Nealon WH, Bawduniak J, Walser EM. Appropriate timing of cholecystectomy in patients who present with moderate to severe gallstone-associated acute pancreatitis with peripancreatic fluid collections. Ann Surg 2004; 239: 741-751.
31. Heider RT, Brown A, Grimm IS et al. Endoscopic sphincterotomy permits interval laparoscopic cholecystectomy in patients with moderately severe gallstone pancreatitis. J Gastrointest Surg 2006; 10:1-5.

Chapter 10

Nederlandse samenvatting

In dit proefschrift ligt de focus op vraagstukken die zijn overgebleven na het uitvoeren van twee belangrijke, multicentrische, gerandomiseerde studies door de Pancreatitis Werkgroep Nederland. Beide studies hebben een grote invloed gehad op de behandeling van patiënten met een ernstige vorm van acute pancreatitis. De eerste studie, PROPATRIA (probiotica en pancreatitis trial, 2004-2007), heeft geleid tot een uitgebreide disussie over de ethische, procedurele en methodologische aspecten van gerandomiseerde studies in Nederland.[1] De resultaten van PROPATRIA waren dramatisch en volledig onverwacht, aangezien het toedienen van probiotica niet resulteerde in een verlaging van het aantal infectieuze complicaties, maar wel in een verhoogde mortaliteit onder patiënten die profylactisch probiotica kregen toegediend. Daarnaast werd bij 6% van de patiënten die probiotica kreeg toegediend darmischemie gediagnosticeerd, tegenover 0% in de groep patiënten met placebo. Vanaf toen bleek dat probiotica ook schadelijk kunnen zijn, zeker wanneer deze worden toegediend aan ernstig zieke patiënten. Echter, ondanks de vele speculaties is het onderliggende mechanisme voor de verhoogde mortaliteit en darmischemie vooralsnog onbekend.

De tweede multicentrische, gerandomiseerde studie van de Pancreatitis Werkgroep Nederland was de PANTER studie (pancreatitis, maximale necrosectomie tegenover minimaal invasieve step-up benadering, 2005-2008).[2] Deze studie liet zien dat de step-up benadering (percutane catheter drainage (PCD), eventueel gevolgd door een minimaal invasieve necrosectomie) superior was ten opzichte van de open chirurgische necrosectomie op het gebied van majeure en mineure complicaties en kosteneffectiviteit. Daarnaast werd duidelijk dat een derde van alle patiënten met verdenking op geïnfecteerde necrose succesvol behandeld kan worden met alleen PCD, zonder dat aanvullende chirurgische necrosectomie nodig is.

Na afloop van beide studies bleven nog een aantal cruciale vragen onbeantwoord. Het eerste deel van dit proefschrift richt zich met name op de interactie tussen probiotica en enterale voeding. In dit deel wordt getracht het mechanisme te ontdekken dat verantwoordelijk is voor de verhoogde mortaliteit en incidentie van darmischemie bij patiënten die probiotica kregen toegediend tijdens de PROPATRIA studie. Het tweede deel van dit proefschrift richt zich op hoe de diagnose “geïnfecteerde necrose” gesteld kan worden en welke verdere interventiestrategieën er zijn in de behandeling van acute pancreatitis. Dit alles met in het achterhoofd dat na de PANTER studie PCD de eerste keus van interventie is in de behandeling van geïnfecteerde pancreasnecrose.

Deel I – Interactie tussen probiotica en enterale voeding tijdens acute pancreatitis

Van oudsher worden probiotica gezien als ongevaarlijke voedingssuplementen. Echter, de PROPATRIA studie liet zien dat een speciale mix van zes probio-

ticsstammen (Ecologic 641) wel degelijk schadelijk kan zijn wanneer dit wordt toegediend aan ernstig zieke patiënten met acute pancreatitis.[1] Het bestaan van een causaal verband tussen de toediening van probiotica en het ontstaan van niet-obstructieve darmischemie is vooralsnog niet bewezen. Wel bestaan er verschillende theorieën, zoals dat de darmischemie veroorzaakt zou zijn door het vrijkomen van fermentatieproducten die mogelijk cytotoxisch zijn.

In **Hoofdstuk 2** wordt een dynamisch, computer-gestuurd model beschreven voor het simuleren van de verschillende condities van de maag en de dunne darm (TIM-1 model). Wij stelden ons de vraag of de samenstelling van de gebruikte sondevoeding invloed heeft gehad op de intraluminale activiteit van de probioticastammen in Ecologic 641. Elk experiment met TIM-1 duurde 6 uur, aangezien deze duur het meest overeenkomt met de langzame passagetijd van het maagdarmstelsel bij ernstig zieke patiënten. De sondevoeding werd met een constante snelheid geïnjecteerd in het duodenum. De probiotica en het placebo werden in een eenmalige dosis ook geïnjecteerd in het duodenum. Afhankelijk van het experiment werden galzouten en pancreasenzymen toegevoegd of weggelaten. We vonden dat de aanwezigheid van pancreasenzymen resulteerde in een hogere interluminale productie van zuur. Door aanwezigheid van pancreasenzymen werd de lactaatproductie vergroot, maar de toevoeging van galzouten zorgde voor een daling hiervan. Het gebruik van vezelrijke voeding zorgde ook voor een hogere lactaatproductie vergeleken met eiwitrijke voeding. Er werden meer korte-keten vetzuren geproduceerd wanneer vezelrijke voeding werd gebruikt ten opzichte van eiwitrijke voeding. Er werd geconcludeerd dat de metabole activiteit van de probiotica maximaal is wanneer zowel pancreasenzymen als vezelrijke voeding aanwezig zijn in het duodenum.

In 2008 schreven van Minnen en collega's over de effecten van voorbehandeling met Ecologic 641 op bacteriële translocatie en mortaliteit in een ratmodel voor acute pancreatitis.[3] Voorbehandeling met probiotica resulteerde in een vermindere mortaliteit en minder bacteriële translocatie ten opzichte van ratten die placebo kregen toegediend. Echter, een groot verschil met de PROPATRIA studie was de timing van toediening van de probiotica. Bij patiënten was de pancreatitis al aan de gang, terwijl bij ratten de probiotica een aantal dagen voorafgaand aan de pancreatitis werden toegediend. In **Hoofdstuk 3** wordt hetzelfde experimentele ratmodel beschreven zoals van Minnen en collega's gebruikt hebben, waarbij getracht is de klinische situatie zo goed mogelijk na te bootsen.[1,3] De dieren werden in vier experimentele groepen ingedeeld: 1) acute pancreatitis met toediening van fysiologisch zout en placebo, 2) acute pancreatitis met toediening van fysiologisch zout en probiotica, 3) acute pancreatitis met toediening van vezelrijke sondevoeding en placebo, 4) acute pancreatitis met toediening van vezelrijke sondevoeding en probiotica. Er werd gescoord op klinische, biochemische, histologische en bacteriologische parameters, waarbij geen causaal verband kon worden

aangetoond tussen niet-obstructieve darmischemie en de toegediende combinatie van vezelrijke sondevoeding met probiotica. Daarnaast werd geen vermindering van bacteriële translocatie gezien bij de dieren die werden voorbehandeld met probiotica.

Nadat de resultaten van de PROPATRIA studie in 2008 bekend werden gemaakt, is er sindsdien geen klinische studie meer gepubliceerd over het gebruik van probiotica bij patiënten met acute pancreatitis. In 2010 ontdekten wij dat er in een ziekenhuis in Praag (Faculty Thomayer's Hospital, Tsjechië) nog steeds patiënten met acute pancreatitis zonder orgaanfalen probiotica en enterale voeding kregen toegediend. In **Hoofdstuk 4** beschrijven we een retrospectieve analyse van al deze patiënten en kwamen tot de conclusie dat in deze studie geen negatieve effecten van probiotica werden gezien op de darmperfusie en mortaliteit bij patiënten met voorspeld ernstige pancreatitis zonder orgaanfalen. Daarnaast werd wederom geen daling gezien van het aantal infectieuze complicaties ten opzichte van de bestaande literatuur.

Samenvattend kan gesteld worden dat in dit eerste deel van het proefschrift de focus ligt op het vinden van het mechanisme dat mogelijk heeft gezorgd voor de verhoogde mortaliteit bij patiënten met acute pancreatitis die behandeld zijn met probiotica en enterale voeding. Zowel *in vitro* als *in vivo* studies hebben geen negatieve interactie tussen probiotica, intraluminale condities en enterale voeding kunnen aantonen. Daarom kan de theorie dat de combinatie van de drie hierboven genoemde factoren heeft geleid tot een toegenomen zuurstofverbruik, mucosale hypoxie, mucosale schade en bacteriële translocatie niet worden ondersteund.

Deze serie experimenten geven geen nieuwe inzichten voor de theorie dat de toediening van probiotica heeft geleid tot een cascade van events, resulterend in locale mucosale ischemie en niet-occlusieve darmischemie. De *in vitro* experimenten en de studie met ratten heeft ons niet dichterbij een verklaring gebracht voor de verhoogde mortaliteit in de PROPATRIA studie bij patiënten die probiotica kregen toegediend om het aantal infectieuze complicaties te verminderen. De analyse van de data uit Praag liet geen positief of negatief effect zien op het percentage infectieuze complicaties bij patiënten met acute pancreatitis zonder orgaanfalen. Daarnaast werd geen verhoging van mortaliteit of percentage darmischemie gezien. Deze bevindingen komen overeen met een andere studie van Besselink en collega's, waarin de negatieve effecten van probiotica alleen werden gezien bij patiënten met (multi-)orgaanfalen. Hierbij moet worden opgemerkt dat in PROPATRIA niet-occlusieve darmischemie een post-hoc eindpunt was en niet een onderdeel van de studiehypothese. Om deze reden is tijdens de studie niet systematisch gekeken naar de aan- of afwezigheid van non-occlusieve darmischemie. Hierbij is het ook belangrijk te vermelden dat non-occlusieve darmischemie geen letale complicaties is en daarom onopgemerkt kan blijven wanneer er niet systematisch naar gezocht wordt. Gebaseerd op bovenstaande

feiten, de resultaten van de PROPATRIA studie en de studies beschreven in dit proefschrift kan er daarom niet bevestigd worden dat er een associatie is tussen de toediening van probiotica, non-occlusieve darmischemie en sterfte.

Deel II – Diagnose van infectie en interventie strategieën bij acute pancreatitis

In de **Hoofdstukken 5, 6, 7 en 8** worden een aantal aspecten van (chirurgische) interventies bestudeerd bij patiënten met acute pancreatitis. Deze studies zijn verricht omdat een accurate diagnose van geïnfecteerde necrose een zeer belangrijke factor is in de PANTER studie en in verschillende interventiestudies, zoals beschreven in de literatuur. Daarnaast wilden we de precieze waarde van PCD bepalen bij de behandeling van geïnfecteerde necrose. De PANTER studie liet al de potentie van PCD zien, maar een veel voorkomende complicatie na interventie is het ontstaan van een pancreasfistel. Als laatste is de timing van cholecystectomie na biliaire pancreatitis een belangrijk onderwerp bij patiënten die een episode van acute pancreatitis overleefd hebben.

Ongeveer 20% van de patiënten met acute pancreatitis ontwikkelt (peri-) pancreatische necrose (necrotiserende pancreatitis). In een derde van deze patiënten treedt infectie van de necrose op, waarna het overgrote deel van deze patiënten een interventie moet ondergaan (bijv. PCD en/of endoscopische of chirurgische necrosectomie).[4] Zelfs als deze groep patiënten een interventie ondergaat, wordt er een morbiditeit van 50-100% gevonden en een mortaliteit van 15-25%.[5-9] In de laatste jaren is er veel onderzoek verricht naar de preventie van infectie van de necrose. Echter, er is weinig onderzoek bekend over de diagnostiek bij verdenking op geïnfecteerde necrose. Over het algemeen wordt aan geïnfecteerde necrose gedacht wanneer een weefselkweek (bijv. verkregen door fijne-naald aspiratie (FNA)) positief is, bij de aanwezigheid van gasbellen in vochtcollecties op een CT-scan met contrast, en bij verschillende klinische kenmerken (bijv. koorts, leucocytose of orgaanfalen). In **Hoofdstuk 5** hebben we getracht om middels een post-hoc analyse van een prospectief cohort van 639 patiënten met necrotiserende pancreatitis de waarde van elk van bovenstaande methodes te onderzoeken bij het diagnosticeren van geïnfecteerde necrose.5 In totaal werden 208 patiënten geanalyseerd welke allen een interventie hebben ondergaan voor verdenking op geïnfecteerde necrotiserende pancreatitis, om zo de waarde van puur alleen de klinische parameters te bestuderen in de bevestiging van de diagnose “infectie”, evenals de waarde van beeldvorming en FNA. Over het algemeen werden de interventies voor verdenking op geïnfecteerde necrose zo lang mogelijk uitgesteld, waarbij de voorkeur uitging naar meer dan vier weken na ontstaan van de symptomen van acute pancreatitis, om zo de necrotische holte beter te laten demarceren, met het uiteindelijke doel om het aantal complicaties te verlagen. Zelfs al was er een zeer sterke verdenking op infectie, of zelfs bewezen geïnfecteerde pancreasnecrose, dan nog werd

geprobeerd de interventie zo lang mogelijk uit te stellen. Deze studie laat zien dat in het merendeel van de patiënten infectie van de (peri-)pancreatische necrose gediagnosticeerd kan worden op basis van de klinische beoordeling van de patient, in combinatie met goede beeldvorming. FNA hoeft alleen gebruikt te worden bij patiënten zonder duidelijke klinische en radiologische kenmerken van infectie.

Jarenlang was open chirurgische necrosectomie de enige toegepaste interventie bij patiënten met verdenking op geïnfecteerde necrose. Echter, deze procedure is geassocieerd met een mortaliteit die kan oplopen tot 35% en een morbiditeit tot 95%. Daarnaast werd er op de lange termijn na open necrosectomie een hoge incidentie van pancreasinsufficiëntie gevonden.[10-12] Sinds eind jaren tachtig wonnen minder invastieve interventies steeds meer populariteit bij de behandeling van geïnfecteerde necrotiserende pancreatitis. De doorbraak kwam pas eind jaren negentig, toen PCD als primaire behandeling voor patiënten met geïnfecteerde necrotiserende pancreatitis werd beschreven.[13] Vanaf dat moment zijn er veel studies beschreven over minimaal invastieve behandelingen bij patiënten met verdenking op geïnfecteerde necrose. De gedachte geïnfecteerde PCD is om de geïnfecteerde necrose te behandelen als een abces en om het geinfecteerde vocht (pus) te draineren. Op deze manier wordt het immuunsysteem van de patiënt geholpen met het infectieuze focus, waarna het overgebleven necrotische materiaal door het lichaam zelf opgeruimd kan worden. Een succesvolle drainage van het geïnfecteerde vocht zal de sepsis verminderen en daarmee de klinische conditie van de patiënt verbeteren. Wanneer daarna onverhoopt necrosectomie alsnog nodig blijkt te zijn, dan heeft PCD de chirurgische interventie in ieder geval deels uitgesteld, waardoor er een betere afkapseling van de necrotische collectie heeft kunnen plaatsvinden. In **Hoofdstuk 6** worden de resultaten van de eerste systematische review naar de rol van PCD bij behandeling van (verdenking op) geïnfecteerde necrotiserende pancreatitis beschreven. Er werd geconcludeerd dat een aanzienlijk aantal patiënten (percentages varierend tussen 18-90%) behandeld kan worden met alleen PCD, zonder dat er een aanvullende chirurgische necrosectomie nodig is. Uit deze systematische review is helaas niet gebleken welke patiënten vooraf geselecteerd moeten worden voor PCD en welke groep patiënten na PCD alsnog necrosectomie moet ondergaan.

Een beruchte complicatie na chirurgische necrosectomie en/of PCD bij patiënten met (verdenking op) geïnfecteerde necrotiserende pancreatitis is een pancreatico-cutane fistel. De incidentie van deze complicatie varieert tussen de 17-67% en is geassocieerd met een verstoring in het metabolisme en de voedingsstatus, een toegenomen lengte van de ziekenhuisopname, en zelfs met sterfte.[14-17] Normaal gesproken worden deze fistels conservatief behandeld, echter, van endoscopische transpapillaire stentplaatsing (ETS) wordt gedacht dat het een veilig en goed uitvoerbaar alternatief is voor

conservatieve behandeling.[18-20] De gedachte achter ETS is dat het de intraductale druk vermindert, waardoor het de drainage van pancreassecreties richting het duodenum fasciliteert in plaats van drainage door de fistel. In Hoofdstuk 7 worden de resulaten beschreven van 35 patiënten die conservatief en/of met ETS zijn behandeld voor een persisterende pancreasfistel, ontstaan na interventie voor (verdenking op) geïnfecteerde necrose. In de meerderheid van de patiënten met een persisterende pancreasfistel (84%) trad complete verdwijning van de fistel op met alleen ETS. Vijf patiënten, bij wie ETS niet succesvol was, werden uiteindelijk conservatief behandeld. Van de patiënten die conservatief werden behandeld, onderging uiteindelijk 25% een aanvullende interventie om de fistel op te heffen. De mediane duur tot het droogvallen van de fistel was 71 dagen in de groep patiënten die ETS onderging en 120 dagen in de groep patiënten die conservatief werd behandeld. Er werd geconcludeerd dat ETS een veilig en goed uitvoerbaar alternatief is voor conservatieve therapie.

Huidige richtlijnen adviseren vroege cholecystectomie na een episode van milde biliaire pancreatitis. Echter, in de literatuur bestaat geen consensus over de definitie van “vroeg”: binnen 2 tot 4 weken na ziekenhuisontslag óf tijdens dezelfde opname (indexopname).[4,21-23] De Pancreatitis Werkgroep Nederland analyseerde alle patiënten met milde biliaire pancreatitis uit de PROPATRIA studie en vond dat 34/249 patiënten (14%) recidiverende klachten van biliaire aard ondervonden voordat een cholecystectomie was uitgevoerd.[24] In **Hoofdstuk 8** beschrijven we een systematische review van de literatuur, waarbij we onderzochten wat het risico is op recidiverende klachten van biliaire aard in de wachttijd tussen ziekenhuisontslag na een episode van milde biliaire pancreatitis en een uitgestelde cholecystectomie. Tevens onderzochten we of vroege cholecystectomie even veilig is als een uitgestelde cholecystectomie. In totaal onderging 39% van de patiënten een pre-operatief endoscopisch retrograad cholanchiogram. Het percentage heropname in de groep patiënten die een uitgestelde cholecystectomie onderging was 18%, waarbij 40% van deze patiënten een recidief biliaire pancreatitis kreeg. Geen van de patiënten die tijdens de indexopname een cholecystectomie onderging had recidiverende klachten van biliaire aard voorafgaand aan de cholecystectomie. Er werd geen verschil gezien in het aantal conversies en complicaties tussen de groepen. Er werd geconcludeerd dat een verkorting van de wachttijd tot cholecystectomie bij patiënten met een milde biliaire pancreatitis zal leiden tot minder heropnames voor klachten van biliaire aard.

Conclusies en toekomst perspectief

Deel I – Interactie tussen probiotica en enterale voeding tijdens acute pancreatitis

Het eerste deel van dit proefschrift is toegespitst op de vraag: is er een verklaring voor de onverwachte resultaten van de PROPATRIA studie? Een

veelgestelde vraag is: *"hebben we teveel gevraagd van de dunne darm in deze ernstig zieke patiënten door het toedienen van probiotica en enterale voeding in het lumen van het proximale deel van de dunne darm? Heeft dit geleid tot een verhoogd zuurstofverbruik met daardoor locale hypoxie en uiteindelijk niet-occlusieve darmischemie?"* Als dit daadwerkelijk zo zou zijn, kan de darmischemie, zoals gezien bij negen patiënten in de probiotica-arm van de studie, dan fungeren als oorzaak en uitleg voor de verhoogde sterfte in deze experimentele groep van de PROPATRIA studie?[1] Dit concept is de focus geweest van verschillende studies in dit proefschrift, maar ook al deels onderzocht door Paul van Minnen, Harro Timmermans, Marc Besselink en Femke Lutgendorff. Besselink en collega's hebben laten zien dat probiotica meer mucosale schade kunnen veroorzaken in een subgroep patiënten met voorspeld ernstige pancreatitis en orgaanfalen, terwijl er geen mucosale schade werd gevonden bij patiënten zonder orgaanfalen.[25] Alhoewel deze analyse was verricht in een kleine subgroep patiënten uit de PROPATRIA studie (115 patiënten zonder orgaanfalen en 26 patiënten met orgaanfalen), wijzen de resultaten uit dat de aanwezigheid van orgaanfalen misschien wel de belangrijkste factor is in de zoektocht naar antwoorden na de PROPATRIA studie. Helaas hebben de studies in dit proefschrift ons geen nieuwe duidelijke aanwijzingen gegeven wat de verhoogde mortaliteit in de PROPATRIA studie heeft veroorzaakt. Eerder uitgevoerde experimentele studies door de Pancreatitis Werkgroep Nederland, met name de studies van Femke Lutgendorff, hebben laten zien dat probiotica, welke voorafgaand aan een episode van acute pancreatitis werden toegediend, een positief effect hadden op de mucosa van de dunne darm. Dit komt ook overeen met het theoretische concept waarbij probiotica geacht worden de functie en het transport van de dunne darm positief te beïnvloeden.[26,27] Tevens geven de beschikbare klinische data ons ook geen nieuwe aanwijzingen voor een mechanisme waarbij de probiotica, de enterale voeding, of de combinatie hiervan verantwoordelijk is voor de onverwachte resultaten van de PROPATRIA studie. Hierbij moet wel vermeld worden dat noch de resultaten van de *in vitro* studies, noch van de experimentele dierenstudies geëxtrapoleerd kunnen worden naar de humane situatie, en daarom al onze bevindingen alleen maar indirecte data zijn over de pathofysiologisce mechanismen die tijdens een episode van acute pancreatitis een rol spelen.

Zoals te lezen is in **Hoofdstuk 2**, worden er in TIM-1 potentieel schadelijke metabolieten voor de darmmucosa geproduceerd in de aanwezigheid van vezelrijke voeding en pancreasenzymen. Echter, de verzamelde monsters uit TIM-1 moeten verder onderzocht worden in Caco-2 celkweek experimenten om te onderzoeken wat daadwerkelijk de cytotoxiciteit is van deze samples op de darmmucosa. Als aanvulling zou elke probioticastam van Ecologic 641 onderzocht moeten worden in TIM-1 om te weten te komen welk metaboliet door welke stam wordt geproduceerd. Om de resultaten van TIM-1 beter

te begrijpen, is het noodzakelijk om duidelijk te krijgen of de intraluminale concentratie van pancreasenzymen tijdens een episode van (ernstige) pancreatitis verhoogd of verlaagd is, aangezien de beschikbare studies tegengestelde resultaten laten zien.[28,29] Echter, omdat in TIM-1 er geen grote verschillen in zuurproductie werden gevonden tussen de experimenten met varierende intraluminale condities, is het niet te verwachten dat aanvullende *in vitro* experimenten het mechanisme zullen verklaren dat verantwoordelijk is voor de verhoogde mortaliteit en darmischemie in de PROPATRIA studie.

Wanneer we kijken naar experimentele studies met ratten, heeft na de PROPATRIA studie tot op heden geen enkele studie een negatief effect van probiotica laten zien. Daarnaast laat de studie beschreven in **Hoofdstuk 3** ook geen negatieve effecten van probiotica en enterale voeding zien. Daarom geldt ook voor toekomstige dierexperimentele studies dat het niet te verwachten is dat deze inzicht zullen geven in de mogelijk negatieve effecten van probiotica.

Bij de groep patiënten in Praag met voorspeld ernstige pancreatitis zonder orgaanfalen op het moment dat de eerste dosis probiotica en enterale voeding werd toegediend, werden geen negatieve effecten van probiotica gevonden. Daarnaast werd, net als in de PROPATRIA studie, geen positief effect gevonden op het aantal infectieuze complicaties. Alhoewel deze monocenter studie suggereert dat een specifieke probioticamix niet schadelijk is voor patiënten met voorspeld ernstige pancreatitis zonder orgaanfalen, moet wel opgemerkt worden dat een relatief klein cohort patiënten wordt beschreven en het een retrospectieve analyse betreft. Tevens werd een andere probioticamix gebruikt dan bij de PROPATRIA studie. Vanwege deze tekortkomingen is een directe vergelijking met de PROPATRIA studie zeer lastig.

De volgende conclusies kunnen worden getrokken. Dit proefschrift heeft geen duidelijke nieuwe inzichten opgeleverd waarmee de resultaten van de PROPATRIA studie verklaard kunnen worden. We moeten accepteren dat de huidige kennis en technieken mogelijk suboptimaal zijn om specifieke mechanismen op het level van de dunne darm te onderzoeken. Daarom zal het erg moeilijk zijn om nieuwe studies te ontwikkelen waarmee het mechanisme van niet-occlusieve darmischemie en de daarbijbehorende locale en systemische effecten adequaat onderzocht kan worden. Hierdoor staan we voor het volgende dilemma: moeten we doorgaan met het ontwikkelen en uitvoeren van studies naar niet-occlusieve darmischemie of moeten we accepteren dat we momenteel niet in staat zijn om een antwoord te vinden op de vragen die ontstaan zijn na de PROPATRIA studie?

Wetenschappelijk gezien zou het herhalen van de PROPATRIA studie een logische vervolgstap zijn, waarbij dezelfde groep patiënten als tijdens de PROPATRIA studie geïncludeerd zou moeten worden. Alleen op deze manier kan bepaald worden of de gevonden resultaten reproduceerbaar zijn. Naast zeer sterke ethische bezwaren en terughoudendheid om weer zo een studie uit te voeren, ontbreekt er ook een zinnige studiehypothese. Bij de PROPATRIA

studie was vermindering van het aantal infectieuze complicaties bij patiënten met voorspeld ernstige pancreatitis de primaire uitkomstmaat. Echter, er werd geen effect gezien op het precentage infectieuze complicaties, waardoor er geen wetenschappelijke basis is om de studie te herhalen.

Bestaat er nog de mogelijkheid dat er geen relatie bestaat tussen de verhoogde mortaliteit en de toevoeging van probiotica bij patiënten met voorspeld ernstige pancreatitis? Waarschijnlijk niet. We hebben alleen een bevestiging gevonden dat (bepaalde) probioticastammen waarschijnlijk niet schadelijk zijn in een subgroep patiënten met acute pancreatitis zonder orgaanfalen, maar we zijn helaas niet in staat om het mechanisme na te bootsen waarin probiotica in extreme condities, zoals bij patiënten met orgaanfalen, mogelijk niet-occlusieve darmischemie kunnen veroorzaken. Het feit dat dit mechanisme niet gevonden is, betekent niet dat dit mechanisme ook niet bestaat. Het aantal te includeren patiënten in de PROPATRIA studie was berekend om een verschil in infectieuze complicaties te vinden en niet een verschil in mortaliteit. Echter, dit mag niet de reden zijn om de verhoogde mortaliteit in de PROPATRIA studie te beschouwen als een toevalsbevinding.

Omdat het niet te verwachten is dat nieuwe *in vitro* en *in vivo* studies ons dichterbij een uitleg zullen brengen voor de onverwachte resultaten van de PROPATRIA studie, en omdat er geen nieuwe gerandomiseerde studie in de nabije toekomst uitgevoerd zal worden, moeten we accepteren dat met de huidige kennis en technieken we geen verklaring kunnen vinden voor de verhoogde mortaliteit en incidentie van darmischemie bij enteraal gevoede patiënten met voorspeld ernstige pancreatitis die profylactisch behandeld zijn met probiotica in de PROPATRIA studie.

Deel II – Diagnose van infectie en interventie strategieën bij acute pancreatitis

In de laatste jaren zijn minimaal invasieve procedures de nieuwe standaard geworden in de gezondheidszorg. Lang gold open necrosectomie als de gouden standaard voor de behandeling van (verdenking op) geïnfecteerde necrose. Echter, de PANTER studie heeft laten zien dat, zelfs bij gecompliceerde ziektes zoals ernstige acute pancreatitis, minimaal invasieve interventiestrategieën superior zijn ten opzichte van conventionele strategieën.[2] Daarnaast wordt ook de behoefte aan invasieve diagnostische middelen bij het diagnosticeren van geïnfecteerde necrose betwist. Met in het achterhoofd de policy van de PANTER studie om chirurgische interventie zo lang mogelijk uit te stellen, beantwoordt het tweede deel van dit proefschrift de volgende vragen: hoe kan accuraat de diagnose geïnfecteerde necrose gesteld worden, wat is de rol van PCD vergeleken met open chirurgische necrosectomie bij verdenking op geïnfecteerde necrose, en hoe moeten persisterende pancreasfistels behandeld worden bij patiënten die een interventie hebben ondergaan na acute pancreatitis? Daarnaast hebben we gekeken naar de optimale timing van

cholecystectomie na een episode van milde biliaire pancreatitis. Deze vragen persisteerden na de PANTER en PROPATRIA studies. Met een gecombineerde database van beide studies werden patiënten geselecteerd om deze vragen te beantwoorden, samen met systematische reviews van de literatuur. De volgende conclusies kunnen getrokken worden uit het tweede deel van dit proefschrift:

1) Met de policy van het uitstellen van interventie bij patiënten met (verdenking op) geïnfecteerde necrotiserende pancreatitis is de toegevoegde waarde van FNA laag. FNA moet alleen gebruikt worden bij patiënten bij wie er op basis van klinische en radiologische tekenen nog onduidelijkheid bestaat over de aanwezigheid van geïnfecteerde necrose.
2) Bij patiënten met geïnfecteerde necrotiserende pancreatitis kan een aanzienlijk deel behandeld worden met alleen PCD, zonder dat er aanvullende chirurgische necrosectomie nodig is. Daarom moet PCD de eerste keus van interventie zijn bij verdenking op geïnfecteerde necrose.
3) Persisterende pancreasfistels, ontstaan na een interventie ten behoeve van (geïnfecteerde) necrotiserende pancreatitis, kunnen veilig behandeld worden met ETS als alternatief voor conservatieve behandeling.
4) Bij patiënten met een milde biliaire pancreatitis moet de tijd tussen ziekenhuisontslag na indexopname en cholecystectomie zo kort mogelijk gehouden worden om het aantal recidiverende klachten van biliaire aard te minimaliseren.

Zoals beschreven in **Hoofdstuk 5** en **6**, is met het zo lang mogelijk uitstellen van een interventie FNA een onderzoek geworden met gelimiteerde waarde. Echter, het is mogelijk dat een subgroep patiënten met vroege tekenen van geïnfecteerde necrotiserende pancreatitis wel degelijk baat heeft bij een vroege interventie. Hierbij moet dan gedacht worden aan PCD, aangezien dit een veel minder heftige immuunrespons veroorzaakt vergeleken met chirurgische necrosectomie. In deze subgroep patiënten zou PCD alleen geïnitieerd zijn wanneer er duidelijk bewijs is van geïnfecteerde necrose. Bij onduidelijke radiologische en klinische kenmerken van geïnfecteerde necrose kan FNA alsnog een belangrijke rol innemen in de diagnostiek naar geïnfecteerde necrose. In de nabije toekomst zal de POINTER studie (uitgestelde of vroege drainage bij patiënten met geïnfecteerde necrotiserende pancreatitis) uitwijzen wat de optimale timing is voor interventie bij patiënten met (verdenking op) geïnfecteerde necrotiserende pancreatitis en wat de rol is van FNA bij het vroeg diagnosticeren van infectie.

Een persisterende pancreasfistel is een beruchte complicatie van chirurgische of radiologische interventie bij patiënten met geïnfecteerde necrotiserende pancreatitis. In **Hoofdstuk 7** is beschreven dat ETS een veilige en goed uitvoerbare behandeling is als alternatief voor conservatieve behandeling. Toekomstige gerandomiseerde studies moeten uitwijzen of

ETS de gouden standaard moet worden voor peristerende pancreasfistels of alleen beschouwd mag worden als een veilig alternatief voor conservatieve behandeling. Naast de duur voor het droogvallen van de pancreasfistel moet ook onderzocht worden wat de kwaliteit van leven is voor patiënten en moet gekeken worden naar de kosten-effectiviteit.

In **Hoofdstuk 8** staat beschreven dat cholecystectomie na een episode van milde biliaire pancreatitis zo snel mogelijk na herstel van de pancreatitis uitgevoerd moet worden, aangezien een uitgestelde cholecystectomie na milde biliaire pancreatitis is geassocieerd met een hoger recidief van biliaire complicaties. Omdat de studies die in de systematische review zijn geïncludeerd van matige methodologische kwaliteit zijn, is de PONCHO studie opgestart om te onderzoeken of vroege cholecystectomie geprefereerd moet worden boven uitgestelde cholecystectomie. De resultaten van de PONCHO studie (studienummer ISRCTN72764151) worden in de loop van 2014 verwacht. De optimale timing van cholecystectomie na een episode van ernstige biliaire pancreatitis blijft vooralsnog onduidelijk, echter het schaarse bewijs dat beschikbaar is suggereert dat een vroege cholecystectomie na ernstige biliaire pancreatitis is geassocieerd met een verhoogd percentage infectieuze complicaties.[30,31] Nadat bewezen is wat de optimale timing is voor cholecystectomie na een episode van milde biliaire pancreatitis, moeten toekomstige studies zich richten op de optimale timing van cholecystectomie na ernstige biliaire pancreatitis.

Referenties

1. Besselink MG, van Santvoort HC, Buskens E, et al. Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. Lancet 2008; 371:651-659.
2. van Santvoort HC, Besselink MG, Bakker OJ, et al. A step-up approach or open necrosectomy for necrotizing pancreatitis. N Engl J Med 2010; 362:1491-1502.
3. van Minnen LP, Timmerman HM, Lutgendorff F, et al. Modification of intestinal flora with multispecies probiotics reduces bacterial translocation and improves clinical course in a rat model of acute pancreatitis. Surgery 2007; 141: 470-80.
4. Banks PA, Freeman ML; Practice Parameters Committee of the American College of Gastroenterology. Practice guidelines in acute pancreatitis. Am J Gastroenterol 2006; 101: 2379-2400.
5. van Santvoort HC, Bakker OJ, Bollen TL, et al. A conservative and minimally invasive approach to necrotizing pancreatitis improves outcome. Gastroenterology 2011; 141:1254-1263.
6. Rau B, Bothe A, Beger HG. Surgical treatment of necrotizing pancreatitis by necrosectomy and closed lavage: changing patient characteristics and outcome in a 19-year, single-center series. Surgery 2005; 138:28-39.
7. Rodriguez JR, Razo AO, Targarona J, et al. Debridement and closed packing for sterile or infected necrotizing pancreatitis: insights into indications and outcomes in 167 patients. Ann Surg 2008; 247:294-299.
8. Bakker OJ, van Santvoort HC, van Brunschot S, et al. Endoscopic transgastric vs surgical necrosectomy for infected necrotizing pancreatitis: a randomized trial. JAMA 2012; 307:1053-1061.
9. van Baal MC, van Santvoort HC, Bollen TL, et al. Systematic review of percutaneous catheter drainage as primary treatment for necrotizing pancreatitis. Br J Surg 2011; 98:18-27.
10. Rau B, Bothe A, Beger HG. Surgical treatment of necrotizing pancreatitis by necrosectomy and closed lavage: changing patient characteristics and outcome in a 19-year, single-center series. Surgery 2005; 138:28-39.
11. Büchler MW, Gloor B, Müller CA, et al. Acute necrotizing pancreatitis: treatment strategy according to the status of infection. Ann Surg 2000; 232:619-626.
12. Rodriguez JR, Razo AO, Targarona J, et al. Debridement and closed packing for sterile or infected necrotizing pancreatitis: insights into indications and outcomes in 167 patients. Ann Surg 2008; 247:294-299.
13. Freeny PC, Hauptmann E, Althaus SJ, et al. Percutaneous CT-guided catheter drainage of infected acute necrotizing pancreatitis: techniques and results. AJR Am J Roentgenol 1998; 170:969-975.
14. Ho HS, Frey CF. Gastrointestinal and pancreatic complications associated with severe pancreatitis. Arch Surg 1995; 130:817-822.
15. Tsiotos GG, Smith CD, Sarr MG. Incidence and management of pancreatic and enteric fistulas after surgical management of severe necrotizing pancreatitis. Arch Surg 1995; 130:48-52.
16. Fotoohi M, D'Agostino HB, Wollman B, et al. Persistent pancreatocutaneous fistula after percutaneous drainage of pancreatic fluid collections: role of cause and severity of pancreatitis. Radiology 1999; 213: 573-578.

17. Connor S, Ghaneh P, Raraty M, et al. Minimally invasive retroperitoneal pancreatic necrosectomy. Dig Surg 2003; 20:270-277.
18. Boerma D, Rauws EA, van Gulik TM, et al. Endoscopic stent placement for pancreaticocutaneous fistula after surgical drainage of the pancreas. Br J Surg 2000; 87:1506-1509.
19. Telford JJ, Farrell JJ, Saltzman JR, et al. Pancreatic stent placement for duct disruption. Gastrointest Endosc 2002; 56:18-24.
20. Varadarajulu S, Noone TC, Tutuian R, et al. Predictors of outcome in pancreatic duct disruption managed by endoscopic transpapillary stent placement. Gastrointest Endosc 2005; 61:568-575.
21. UK guidelines for the management of acute pancreatitis. Gut 2005; 54 Suppl 3:iii1-9.
22. Forsmark CE, Baillie J. AGA institute technical review on acute pancreatitis. Gastroenterology 2007; 132:2022-2044.
23. Uhl W, Warshaw A, Imrie C et al. IAP Guidelines for the surgical management of acute pancreatitis. Pancreatology 2002; 2:565-573.
24. Bakker OJ, van Santvoort HC, Hagenaars JC, et al. Timing of cholecystectomy after mild biliary pancreatitis. Br J Surg 2011; 98:1446-1454.
25. Besselink MG, van Santvoort HC, Renooij W et al. Intestinal barrier dysfunction in a randomized trial of a specific probiotic composition in acute pancreatitis. Ann Surg 2009; 250:712-719.
26. Lutgendorff F, Nijmeijer RM, Sandström PA et al. Probiotics prevent intestinal barrier dysfunction in acute pancreatitis in rats via induction of ileal mucosal glutathione biosynthesis. PLoS One 2009; 4:e4512
27. Lutgendorff F, Trulsson LM, van Minnen LP et al. Probiotics enhance pancreatic glutathione biosynthesis and reduce oxidative stress in experimental acute pancreatitis. Am J Physiol Gastrointest Liver Physiol 2008; 295:G1111-1121
28. O'Keefe SJ, Lee RB, Li J et al. Trypsin secretion and turnover in patients with acute pancreatitis. Am J Physiol Gastrointest Liver Physiol 2005; 289: G181-187.
29. Czakó L, Yamamoto M, Otsuki M. Exocrine pancreatic function in rats after acute pancreatitis. Pancreas 1997; 15:83-90.
30. Nealon WH, Bawduniak J, Walser EM. Appropriate timing of cholecystectomy in patients who present with moderate to severe gallstone-associated acute pancreatitis with peripancreatic fluid collections. Ann Surg 2004; 239: 741-751.
31. Heider RT, Brown A, Grimm IS et al. Endoscopic sphincterotomy permits interval laparoscopic cholecystectomy in patients with moderately severe gallstone pancreatitis. J Gastrointest Surg 2006; 10:1-5.

Chapter 11

Acknowledgements
List of Publications
Curriculum Vitae

Dankwoord

Tijdens mijn promotieonderzoek heb ik met vele mensen samengewerkt. Met de één wat intensiever dan met de ander, maar uiteindelijk heeft iedereen zijn of haar steentje bijgedragen aan dit proefschrift. Zonder de bijdrage van de mensen om mij heen was het nooit gelukt om tot dit eindresultaat te komen. Daarom wil ik hen allen bedanken die dit proefschrift mogelijk hebben gemaakt. Naar een aantal mensen wil ik graag wat extra woorden van dank uitspreken.

Prof. dr. H.G. Gooszen, geachte promotor, professor, ik weet nog de dag dat ik voor het eerst tegenover u zat op uw kamer om over wetenschappelijk onderzoek te praten. Al snel opperde u om aan de slag te gaan op het datacentrum van de Pancreatitis Werkgroep Nederland en ik viel met mijn neus in de boter. Kort nadat ik mij gemeld had op het datacentrum werden de resultaten bekend van PROPATRIA, waarna ik uiteindelijk werd aangesteld op het POSTPATRIA project. De verhuizing van Utrecht naar Nijmegen had wat voeten in de aarde, maar u heeft zich altijd vol ingezet om dit project tot een goed einde te brengen. U heeft mij het vertrouwen gegeven dat ik nodig had. Ik kreeg de volledige vrijheid bij het opzetten van mijn experimenten en ik hoop dat ik het vertrouwen heb waargemaakt met dit proefschrift. De laatste loodjes waren zwaar en soms had ik wat aanmoediging nodig, maar uiteindelijk hebben we het POSTPATRIA project tot een goed einde kunnen brengen. U was ook altijd geinteresseerd naar mijn leven naast het onderzoek, iets wat ik altijd erg gewaardeerd heb. En ja, professor, Tijn slaapt nog steeds onder het mooie lakentje. Volgens mij kan ik me geen betere promotor wensen. Ik wil u bedanken voor alles wat u de afgelopen jaren voor mij heeft gedaan en ik ben oprecht trots dat u mijn promotor bent.

Prof. dr. ir. G.T. Rijkers, geachte promotor, beste Ger, vanaf moment één was je mijn vraagbaak voor het experimentele deel van dit proefschrift. Ik zie ons nog samen naar een vergadering van de proefdiercommissie gaan om het pancreatitismodel toe te lichten. Ook de vele meetings bij TNO om te discussiëren over de TIM-resultaten staan me nog goed bij. Je hebt me enorm op weg geholpen in de wereld van de experimentele wetenschap en mij bijgestaan waar nodig gedurende mijn promotietraject. Hier ben ik je erg dankbaar voor. Je aanstelling aan de Roosevelt Academie als hoogleraar en daarmee ook je vertrek uit Nijmegen heeft ervoor gezorgd dat we elkaar wat minder vaak zagen, maar telefonisch en per mail hebben we altijd goed contact gehouden. Het hoogleraarschap is je van harte gegund en het is me een genoegen dat ik onder jou mag promoveren.

Dr. M.G. Besselink, gachte copromotor, beste Marc, wat heb ik een bewondering voor je. Jouw enthousiasme en doorzettingsvermogen waren

een grote inspiratiebron voor me. Ik vraag me nog steeds af hoe je het steeds voor elkaar kreeg, maar zonder uitzondering kreeg ik altijd binnen 24 uur alle toegestuurde documenten terug met zinvolle opmerkingen en aanpassingen. Ik kon met alles bij je terecht, op elk moment van de dag. Jij was degene die me onder jouw vleugels nam na mijn eerste schreden in het datacentrum. Je nam me op sleeptouw en enthousiasmeerde me voor veel studies die uiteindelijk allemaal in dit proefschrift terecht zijn gekomen. Vanaf dag één heb ik ongelooflijk veel van je opgestoken en wat ben ik blij dat jij mijn compromotor bent. Heel veel dank voor jouw begeleiding, je tomeloze enthousiasme en alles wat je verder voor me gedaan hebt.

Prof. Dr. L.M.A. Akkermans, beste Louis, tijdens één van uw bezoekjes aan het datacentrum in Utrecht raakten we aan de praat over experimenteel onderzoek en de vervolgplannen na PROPATRIA. of ik daar misschien in geïnteresseerd in zou zijn… Dat hoefde ik me geen twee keer te laten zeggen. Mijn aanstelling ging niet geheel zonder slag of stoot en ik moest uiteindelijk mijn studie Geneeskunde tijdelijk onderbreken. U hielp me met de administratie en de examencommissie te overtuigen om mij tussentijds mijn doctoraal toe te kennen, waarna ik full-time aan de slag kon bij de Pancreatitis Werkgroep Nederland. Daarna was u met name betrokken bij de experimentele studies. Naast uw enorme kennis op wetenschappelijk gebied was u ook een meester in het vertellen van mooie verhalen, grotendeels allemaal zelf meegemaakt. Zo herinner ik me nog het verhaal over het naar u vernoemde plantje…fantastisch! Ik vond het een eer dat u mij wilde toespreken tijdens mijn buluitreiking en ik hoop dat we in de toekomst nog veel contact zullen hebben. Dank voor alles!

Leden van de manuscriptcommissie, **prof. dr J.P.H. Drenth, prof. dr. M. Ritskes-Hoitinga en prof. dr. I.H.M. Borel Rinkes**, dank voor het plaatsnemen in de manuscriptcommissie en voor het beoordelen van mijn proefschrift.

Leden van de promotiecommissie, **prof. dr. J.L.L. Kimpen**, dank voor het plaatsnemen in de corona. **Prof. dr. H.M. van Goor**, beste Harry, dank voor de altijd oprechte interesse in mij en mijn onderzoek. Het hoogleraarschap is je van harte gegund. Ik vond het een genoegen om met je samen te werken en wie weet gaan we in de toekomst nog eens samen iets publiceren. **Prof. dr. J.J.G.M. van den Oord**, beste Ome Joost, wat een eer dat ik mijn proefschrift tegenover u mag verdedigen. **Dr. V.B. Nieuwenhuijs**, beste Vincent, onze eerste ontmoeting was in Australië waar ik een tijdje wetenschappelijk onderzoek onder jouw begeleiding. Terug in Nederland hebben we altijd contact gehouden en hoe mooi is het dat ik nu mijn proefschrift tegenover jou mag verdedigen. Dank voor alles wat je voor me gedaan hebt!

Onderzoekers van de Pancreatitis Werkgroep Nederland, ik heb maar anderhalf jaar op het datacentrum doorgebracht, maar wat een mooie tijd. Een hechte groep en altijd bezig om jezelf te verbeteren. Regelmatig tot in de late uurtjes of in de weekenden, het datacentrum had bijna letterlijk een personele bezetting van 24/7. **Hjalmar van Santvoort** en **Olaf Bakker**, beiden betrokken bij een aantal artikelen uit mijn proefschrift, ik heb veel van jullie geleerd. Jullie kritische blik leidde vaak tot een verbetering van een onderzoeksvoorstel of artikel, maar ook wel eens tot felle discussies. Niet alles ging zonder slag of stoot, maar uiteindelijk kwam er altijd een oplossing en kwam het de kwaliteit alleen maar ten goede. Dank voor jullie medewerking aan de inhoud van dit proefschrift! **Thomas Bollen**, als student heb ik nog lange tijd voor jou gewerkt om alle CT-scans van patiënten met acute pancreatitis te verzamelen. Inmiddels alle ziekenhuizen in Nederland gezien en in de loop van de tijd een mooie dataset verzameld. Dank ook voor jouw hulp aan dit proefschrift. **Stefan Bouwense**, we begonnen ongeveer gelijktijdig in Nijmegen op het datacentrum. Daarna in het CWZ nog een half jaar collega's geweest. Ons promotietraject had ongeveer eenzelfde verloop, dat wil zeggen, even een soort van korte break gehad toen we in opleiding kwamen. Uiteindelijk weer vol aan de bak gegaan en jij bent nu ook binnenkort aan de beurt om het alles verlossende "Hora est" te horen. Het ga je goed en ik hoop dat we ook in de toekomst contact blijven houden. Wie weet ooit nog een keer een hotelkamer van 4m^2 delen samen met Yama Issa in Stockholm? **Usama Achmed Ali**, de chronische pancreatitis-man. Bezeten van onderzoek en statistiek en een leuke collega. Al snel vertrok je naar Amerika om je met een nieuw project bezig te houden. Inmiddels weer terug en klaar om je eigen proefschrift af te ronden. 2015 moet jouw jaar worden, met opleiding en promotie. Alle succes toegewenst, het is je gegund! **Yama Issa**, opvolger van Usama, inmiddels volledig je eigen weg gevonden in onderzoeksland. Wat hebben we vaak over onderzoek en andere zaken gesproken toen je nog in Nijmegen op het datacentrum zat. Helaas werd uiteindelijk het AMC jouw uitvalsbasis, maar gelukkig hebben we altijd contact gehouden. Het ga je goed en ook voor jou ligt er een mooie promotie en opleidingsplek voor de chirurgie in het verschiet! **Rian Niimeijer**, inmiddels in opleiding tot MDL-arts en gepromoveerd. Samen verzorgden we het experimentele onderzoek van de werkgroep. Totaal verschillend van het klinisch onderzoek, maar zeker niet minder leuk. Ellenlang doorbrengen in het lab, zelf je proefopzet maken, je experimenten uitvoeren en vervolgens zelf je bepalingen doen, dat heeft ook wel iets. Dank voor de goede gesprekken over van alles en nog wat. Goed om te zien dat je je eigen weg hebt gekozen en het prima naar je zin hebt bij de MDL. **Nicolien Scheeper**, mega energiek, altijd enthousiast. APEC opgezet en momenteel langstdienende op het datacentrum. Het jagershuisje in Loenen was echt fantastisch! Mountainbiken, BBQ, wild spotten in de nacht, wat een mooi weekend was dat. Hopelijk komt er nog een keer zo een weekend! Heel veel succes met APEC en alle andere lopende

studies. Het kan niet anders dan dat ook jij een mooi proefschrift en een mooie carrière als MDL-arts tegemoet gaat. **David da Costa, Bob Holleman** en **Janneke van Grinsven**, de jongste onderzoekers op het datacentrum. Ik was al weg uit Nijmegen toen jullie begonnen. Ik hoop dat jullie net zo een mooie tijd bij de Pancreatitis Werkgroep Nederland hebben als dat ik heb gehad. Succes met al jullie projecten en dat het mag uitmonden in een mooi proefschrift.

Sandra van Brunschot, collega-onderzoeker, collega op de werkvloer, maar bovenal een hele goede vriendin. Wij zijn bijna tegelijkertijd in het datacentrum begonnen en van begin af aan hadden we een goede klik. Vele mooie momenten hebben we meegemaakt als onderzoeker, zoals bijvoorbeeld de EPC in Praag. Wat een week was dat en wat hebben we een lol gehad! Vooralsnog geen gezamenlijke publicatie, maar dat kan nooit lang meer duren. Dat we weer directe collega's zijn, spreekt toch wat makkelijker af. Rowena en ik kijken altijd uit naar de etentjes met jou en Ben en we hopen dat we nog veel van die avonden mogen meemaken. **Ben van den Elshout**, voor jou ook nog een paar woorden. Ik leerde je kennen als partner van Sandra, maar je bent uitgegroeid tot een echte vriend. Ongelofelijk gezellig en veel humor, kortom, een echte Brabander. Ik heb nog de foto van ons tweeën voor ogen na een supersnelle afdeling met mountainbiken: Ben en Mark op hun best! Echte vrienden helpen je waar nodig, en die hulp kregen we van jullie bij onze verhuizing! Jullie hulp de hele dag en natuurlijk de legandarische uitspraak: "het zou leuk als je nu weeën zou krijgen" zullen we nooit vergeten. Sandra en Ben, ik hoop dat we vrienden voor het leven blijven en dat we nog vele mooie momenten mogen meemaken!

Uiteraard wil ik ook graag de researchverpleegkundigen van het datacentrum bedanken voor hun hulp en gezelligheid tijdens mijn periode in Utrecht en Nijmegen. Altijd stond er een kop koffie klaar en was er tijd voor een gezellig praatje. **Vera Zeguers, Annie Bakker, Anneke Roeterdink, Hetty van der Eng** en **Helen van Wezel**, dank voor de welkome afleiding in de wereld van de wetenschap!

Mijn kamergenoten in Nijmegen, ook jullie bedankt voor de gezelligheid en wanneer nodig een luisterend oor. **Tjarda Tromp**, hard werkend, maar ook erg gezellig. Als we samen aan het werk waren, dan was het altijd eerst een paar uur knallen, maar daarna standaard tijd voor een kop koffie en een babbel. Dank voor de gezellige momenten! **Marjan de Vries**, je begon met een volledig nieuw project en hebt alles van de grond af aan opgebouwd. Ik vind het bewonderenswaardig hoe jij je als niet-medicus zo goed staande kon houden tussen al die eigenwijze medici. Je bent een harde werker, maar gezelligheid en praatjes waren zeker zo belangrijk voor je. Met jouw instelling ligt er een mooie carrière voor je in het verschiet, waar je ook later terecht komt!

Richard ten Broek, jouw promotie is nu bijna een feit en je bent inmiddels ook in opleiding tot chirurg. Dank voor dat je mijn vraagbaak wilde zijn wat betreft statistiek en tekenprogramma's.

Een groot deel van mijn onderzoeksperiode heb ik in het dierenlab doorgebracht. Graag wil ik iedereen bedanken die mij heeft geholpen bij mijn experimenten. **Ilona van den Brink** en **Francien van de Pol**, jullie hebben mij wegwijs gemaakt in het dierenlab en samen hebben we het pancreatitismodel nieuw leven ingeblazen. Het was een lange aanloop, met iedere keer weer een tegenslag, maar uiteindelijk hadden we een goed lopend model. Jullie waren altijd enthousiast en vrolijk, en schoten te hulp waar nodig. Zonder jullie was het nooit gelukt om mijn experimenten tot een goed einde te brengen. Ondanks dat jullie vaak op de achtergrond acteren, zijn jullie onmisbaar voor elke onderzoeker. Heel veel dank voor een mooie tijd in het dierenlab en voor al jullie hulp en inzet.

Daphne Reijnen, ook jij hebt veel geholpen met mijn experimenten, met name ook in de weekenden. Daarnaast hielp jij me, samen met Ilona en Francien, goed op weg in de wereld van de dierexperimenten. Heel erg veel dank voor jouw hulp en gezelligheid in het dierenlab. Ook zonder jou had ik mijn experimenten niet tot een goede einde kunnen brengen!

Ik wil ook graag de proefdierdeskundigen **Roel Snepers** en **Philip Mulkens** bedanken voor hun steun, aanwijzingen en tips tijdens mijn experimenten.

André Verheem, jij kwam uit Utrecht om ons een 3-daagse cursus te geven om zo het pancreatitsmodel onder de knie te krijgen. Heel veel dank voor de tijd en moeite die je in ons gestoken hebt om ons het model eigen te maken. Zonder jouw hulp geen experiment.

Michiel van Rens, als student kwam jij me helpen op het dierenlab. Je hebt het experiment van begin af aan meegemaakt en mij ongelooflijk veel werk uit handen genomen. Je deed alles geheel vrijblijvend en allemaal in je vrije tijd. En goed ook! Ik had met een gerust hart een week weg kunnen gaan, je kon het experiment met Ilona, Francien en Daphne makkelijk draaiende houden. Die auteursplek bij het artikel is dan ook meer dan verdiend. Met jouw humor en instelling ga je alles bereiken wat je maar wilt in de toekomst, daarvan ben ik overtuigd. Inmiddels afgestudeerd en ANIOS chirurgie, misschien in de toekomst ook directe collega's?

Graag wil ik ook de mensen bedanken die me hebben geholpen bij de analyses van de proefdierexperimenten. **Wilbert Peters, Hennie Roelofs** en **Christopher Geven**, veel dank voor jullie hulp bij de amylasebepalingen.

De experimenten bij TNO met het TIM-1 model heb ik alleen maar kunnen uitvoeren door de goede begeleiding van **Koen Venema, Marjorie Koenen** en **Mark Jelier**. Ik wil jullie allen bedanken voor jullie hulp aan mijn experimenten.

Prof. dr. I.D. Nagtegaal, beste Iris, dank dat je me wilde helpen met de histologische beoordeling van mijn prepareten. Mede door jou is mijn interesse in de pathologie gewekt en heb ik er ook voor gekozen om een keuzecoschap pathologie te doen. Achteraf gezien ook een goede keuze, aangezien ik veel geleerd heb en het zes weken erg naar mijn zin heb gehad.

Dr. J.J. Hermans, beste John, we hadden een mooi idee om met een nieuw soort CT-scan het pancreas van de rat af te beelden tijdens een aanval van acute pancreatitis. Helaas waren onze ideeën iets te vooruitstrevend en is het uiteindelijk gebleven bij een pilot-studie. Ik vond het in ieder geval leuk en leerzaam om met je samen te werken en wie weet gaat het in de toekomst wel lukken om onze ideeën ten uitvoer te brengen.

Dr. P. Kohout, dear Pavel, thank you for your support to chapter 4. You asked me to come to Prague to collect and analyze data of patients with acute pancreatitis and treated with probiotics. I remember your recommendation for a place to stay during my period in Prague: “You should make a reservation in hotel The Swan, it is close to the hospital and, more importantly, they serve good food and beer!”. I had a great time in Prague and I hope we will meet again soon. I wish you all the best!

Willem Renooij, voorheen voorzitter van de Mucosale-barrière werkgroep, de onderzoeksgroep in Utrecht. Helaas is het niet gelukt om het Caco-2 celkweek project in Nijmegen op poten te zetten. Toch wil ik je heel erg bedanken voor de tijd en moeite die je in me hebt gestoken om mij de basis van de basale wetenschap bij te brengen. Je pensioen is je van harte gegund en ik wens je nog vele mooie jaren toe in je buitenhuisje in Frankrijk.

Ik wil de medewerkers van Winclove Probiotics BV bedanken voor hun hulp en steun aan verschillende experimenten uit dit proefschrift. Dank voor het leveren van de probiotica, maar met name veel dank voor het meedenken en hulp bij het interpreteren van de resultaten van de TIM-1 studie. **Peter Pekelharing, Frans Rombouts, Sarah Meeuws, Luuk van Duijn** en **Isolde van der Vaart**, hartelijk dank!

Chirurgen en assistenten van het Tweesteden Ziekenhuis en St. Elisabeth Ziekenhuis, ook jullie staan in mijn dankwoord. Dank voor jullie interesse in mijn onderzoek, maar vooral ook in het leven naast het onderzoek!

Mijn hockeyteam, Den Bosch Heren 6, voor jullie ben ik inmiddels Van Baal, de rattenman. Ik hoop dat ik na het afronden van dit proefschrift wat meer tijd heb om samen met jullie een "Ouwe Jan" te gaan drinken in BLD. Dank voor jullie begrip dat ik er niet altijd bij kan zijn, maar hopelijk gaat dit snel veranderen!

Ronald en Karin Gijsberts, dank voor de welkome afleiding die jullie regelmatig boden op de zaterdagavond! Inmiddels wonen we wat verder van elkaar af, maar dat doet niets af aan onze vriendschap. Dat we nog samen nog vele avonden met een wijntje en biertje mogen meemaken!

Erik Manning, heel veel dank voor jouw creatieve input en de schildering van mijn cover! Je hebt er een waar kunstwerk van gemaakt!

Mijn schoonfamilie, **Michael, Ria, Inge, Désirée, Vincent, Josefien, Kim** en **Cynthia**, dank voor de afleiding die jullie regelmatig boden!

En dan, last but not least, mijn familie. **Ghislaine van Baal**, Ghiesje, mijn kleine zusje. Inmiddels al een volwassen dame, maar je blijft voor altijd mijn kleine zusje. Na je tenniscarrière heb je je eigen leventje opgebouwd in Nijmegen en dat doet me goed. Het eerste jaar logopedie was niet helemaal naar wens verlopen, maar ik weet zeker dat het je dit jaar allemaal mee zal zitten. Je werkt er immers hard genoeg voor. Hopelijk heb je dan nog wel wat tijd om af en toe nog het kaartspel te spelen. Dat is er afgelopen paar jaar door mij een beetje bij ingeschoten, maar ik beloof dat ik mijn leven zal beteren. Ik zal ook wat vaker langskomen in Nijmegen om gezellig een hapje te komen eten! **Juliette van Baal**, Juultje, in Amsterdam bezig aan je eigen proefschrift. Je zal merken dat het je meer bezighoudt en opslokt dan je vooraf zou verwachten. Met name de laatste loodjes zijn erg pittig. We zien en bellen elkaar eigenlijk veel te weinig, maar met onze drukke agenda's is het gewoon lastig afspreken. Je bent inmiddels weer helemaal into de gynaecologie en volgens mij past dat ook het beste bij je. Eindelijk heb je die promotieplek gevonden, iets waar je al lang naar op zoek was. Je kunt het, dat heb je in Engeland al laten zien. Heel veel succes met je eigen proefschrift en ik hoop dat je een mooie toekomst in de gynaecologie tegemoet gaat. Het is je gegund! Lieve zusjes, ik wil jullie allebei bedanken voor alles, gewoon, omdat jullie mijn lieve zusjes zijn!

Mijn paranimfen, **Geert** en **Jaap van Baal**, mijn twee broertjes. Hoe mooi is het dat jullie zometeen naast mij staan! De afgelopen twee jaar hebben jullie

elke keer weer gevraagd wanneer we bij elkaar konden komen om de promotie door te spreken, maar bovenal, wanneer we die fles korenwijn konden gaan leegdrinken. Nu is het eindelijk zo ver! De dag is inmiddels doorgesproken en die fles korenwijn heeft goed gesmaakt. Lieve broertjes, we spreken en zien elkaar te weinig, maar ik hoop dat dat gaat veranderen na 11 december. Geert, ik heb supermooie herinneringen aan jouw huwelijksdag, maar vooral ook hele mooie herinneringen aan de nacht tevoren en de ochtend zelf. Zaten we daar, met z'n drieën, het laatste ontbijtje met ons vrijgezelle broertje. Gewoon, broertjes onder elkaar, wat was dat een mooi moment! Voor mij in ieder geval heel speciaal en iets wat me altijd zal bijblijven. Lieve broertjes, heel veel dank dat jullie mijn paranimfen willen zijn, maar voor jullie geldt ook, heel veel dank, gewoon omdat jullie mijn broertjes zijn!

Lieve **pap** en **mam**, eindelijk is het zover. Het heeft even geduurd, maar het boekje is nu een feit. Van begin af aan hebben jullie mij gesteund, bij alles wat ik deed. Af en toe met wat scepsis, maar uiteindelijk is het allemaal goedgekomen. Jullie motto was altijd: "als je iets doet, doe het dan goed. Nooit half werk leveren en altijd je talenten benutten." Een mooi motto, wat ik bij alles wat ik doe in gedachten probeer te houden. Ik ben jullie ongelooflijk dankbaar voor de kansen en mogelijkheden die jullie ons geboden hebben, zodat wij ons hebben kunnen ontwikkelen tot de personen die jullie kinderen nu zijn. De sporten die we vroeger hebben mogen doen, de muziekinstrumenten, maar later ook jullie steun zodat ik acht maanden in Australië onderzoek kon gaan doen. Pap, die twee weken die je toen langs kwam zal ik nooit vergeten. Wat vond ik het mooi om een rat te opereren terwijl je toekeek. We hebben daar mooie momenten meegemaakt met veel hoogtepunten, maar die ene dag in het dierenlab staat bij mij bovenaan het lijstje! Mam, altijd zorgen om je kinderen, en je altijd volledig weggecijferen voor ons. Samen hebben we een aantal jaar het Lulof Open georganiseerd. Stressvolle weken, maar o zo mooi om dat samen met jou te doen. Ik kijk met heel veel plezier terug op deze periode en ik vind het oprecht jammer dat dat nu voorbij is. We waren een goed team, waarschijnlijk ook omdat we volgens pap hetzelfde karakter hebben. Inmiddels ben je aan je tweede leven begonnen en ik geniet er van je zo te zien opbloeien. Lieve pap en mam, mijn dank is eigenlijk niet in woorden te beschrijven. Ik ben trots op jullie als ouders en heb, zeker nu ik vader ben, heel veel respect voor hoe jullie het voor elkaar hebben gekregen om vijf kinderen op te voeden. Oneindig veel dank en dat we met de hele familie nog heel veel mooie momenten mogen meemaken!

Mijn kinderen, lieve **Tijn** en **kleine uk**, jullie zijn het mooiste geschenk in mijn leven, mooier dan ik ooit had kunnen bedenken. Tijn, met één lach

maak je mijn hele dag goed en smelt ik ter plekke. Helaas kun je niet bij de promotie aanwezig zijn, maar ik zal zeker een boekje voor je bewaren. Deze dag komt net even te vroeg, alhoewel je al wel met drie weken levenservaring je eerste wetenschappelijke congres hebt bijgewoond met bijbehorend aanwezigheidscertificaat. Lieve Tijn, je weet het zelf nog niet, maar je maakt mama en mij elke dag weer zielsgelukkig! Kleine uk, jij mag wel bij de promotie aanwezig zijn, veilig in de buik van mama, dat wel. Jij gaat ons leven helemaal compleet maken. We kijken heel erg uit naar je komst en kunnen bijna niet wachten tot het zover is. Lieve uk, nog even goed groeien en over een paar maanden mag je papa en mama de gelukkigste ouders ter wereld maken!

Lieve **Rowena**, wijffie, de laatste woorden van dit proefschrift zijn voor jou. Wat moet ik zeggen? Woorden schieten tekort. Ik heb je leren kennen, net voordat ik met mijn promotie-onderzoek begon. Je hebt alles van begin af aan meegemaakt en je hebt me altijd en overal gesteund waar mogelijk. Meer dan ik ooit had durven dromen! Zelfs tijdens het klussen vlak voor onze verhuizing naar Nijmegen moest ik nog regelmatig even checken hoe het met mijn ratjes ging, en nooit heb je geklaagd! De laatste anderhalf jaar zijn ongelofelijk hectisch geweest, maar gelukkig weten we ons elke keer weer overal doorheen te slaan. Dat bewijst maar weer wat voor een sterke vrouw je bent. Wijffie, lieve schat, ik ben supertrots op je en hou zielsveel van je. Ik wil je bedanken voor wie je bent en wat je allemaal hebt gedaan voor mij. We gaan samen een hele mooie toekomst tegemoet en ik hoop dat we nog heel erg lang van elkaar mogen genieten!

List of publications

Van Baal MC, van Rens MJ, Geven CB, van de Pol FM, van den Brink IW, Hannink G, Nagtegaal ID, Peters WH, Gooszen HG, Rijkers GT. Interaction between probiotics and enteral nutrition in an experimental acute pancreatitis model in rats. *Accepted Pancreatology*

Van Baal MC, Koenen M, Akkermans LM, Venema K, Gooszen HG, Rijkers GT. Metabolic consequences of interaction between enteral nutrition and multispecies probiotics in an in vitro model of the small intestine. *Submitted*

Van Baal MC, Bakker OJ, Bollen TL, Rijkers GT, van Goor H, Boermeester MA, Dejong CH, van Eijck CH, van der Harst E, Gooszen HG, van Santvoort HC, Besselink MG, for the Dutch Pancreatitis Study Group. The role of routine fine-needle aspiration in the diagnosis of infected necrotizing pancreatitis. *Surgery. 2014 Mar;155(3):442-8*

Van Baal MC, Kohout P, Besselink MG, van Santvoort HC, Zazula R, Gooszen HG. Probiotic prophylaxis in predicted severe pancreatitis: a monocenter retrospective cohort. *Pancreatology 2012 Sep-Oct;12(5):458-62*

Van Baal MC, Besselink MG, Bakker OJ, van Santvoort HC, Schaapherder AF, Nieuwenhuijs VB, Gooszen HG, van Ramshorst B, Boerma D, for the Dutch Pancreatitis Study Group. Timing of cholecystectomy after mild biliary pancreatitis: a systematic review. *Ann Surg 2012 May;255(5):860-6*

Van Baal MC, van Santvoort HC, Bollen TL, Bakker OJ, Besselink MG, Gooszen HG, for the Dutch Pancreatitis Study Group. Percutaneous catheter drainage as primary treatment for necrotizing pancreatitis: a systematic review. *Br J Surg 2011 Jan;98(1):18-27*

Bakker OJ, **van Baal MC**, van Santvoort HC, Besselink MG, Poley JW, Heisterkamp J, Bollen TL, Gooszen HG, van Eijck CH, for the Dutch Pancreatitis Study Group. Endoscopic treatment of pancreatic fistulas in patients with necrotizing pancreatitis. *Ann Surg 2011 May;253(5):961-7*

Van Loo E, **van Baal MC**, Gooszen HG, Ploeg R, Nieuwenhuijs VB. Long-term results and quality of life after surgery for chronic pancreatitis. *Br J Surg 2010 Jul;97(7):1079-1086*

Steenks M, **van Baal MC**, Nieuwenhuijs VB, de Bruijn MT, Schiesser M, Teo EH, Callahan T, Padbury RT, Barritt G. Intermittent ischemia maintains function following ischemia reperfusion in steatotic livers. *HPB (Oxford) 2010 May;12(4):250-261*

Van Baal MC, Besselink MG, van Santvoort HC, Nieuwenhuijs VB, Gooszen HG. The step-up approach to infected necrotizing pancreatitis. In: de Campos T, Rassian S (eds): Acute pancreatitis. Editora Atheneu, Sao Paulo, Brazil, 1st edition, chapter 24. *In press*

Curiculum Vitae

Mark Christiaan Paulus Marie van Baal was born on April 10th, 1984 in Hoofddorp, the Netherlands. In 2002 he graduated from the Gymnasium at OSG Erasmus, Almelo. He studied Medicine at the University of Utrecht from 2002 to 2012. In 2005, he interrupted his study for nine months and went to Adelaide, Australia, to participate in a research project on reperfusion injury of the liver in obese rodents at Flinders Medical Center, Adelaide, under supervision of prof. dr. G.J. Barritt and dr. V.B. Nieuwenhuijs. In 2007, during medical school, he joined the research group of prof. dr. H.G. Gooszen at the department of Surgery, University Medical Center Utrecht, to perform clinical research on acute pancreatitis. In 2010, after the PROPATRIA study, again he interrupted his medical school for two more years to perform experimental studies on the interaction between probiotics and enteral nutrition in acute pancreatitis under the supervision of prof. dr. H.G. Gooszen en prof. dr. ir. G.T. Rijkers in the Radboudumc, Nijmegen. For this research project he received a grant of the Dutch Digestive Foundation. In November 2011 he continued his medical school and graduated in June 2012. Thereafter, he worked as a resident in the Canisius Wilhelmina Hospital, Nijmegen for six months. In January 2013, he started residency in general surgery under the supervision of dr. M.S. Ibelings at the Tweesteden Hospital, Tilburg. The fourth and fifth year of his surgical training are scheduled at the University Medical Center Utrecht under the supervision of dr. M.R. Vriens. For the sixth and final year of his residency, he will return to the Tweesteden Hospital, Tilburg.