

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



Marc G H Besselink, Hjalmar C van Santvoort, Erik Buskens, Marja A Boermeester, Harry van Goor, Harro M Timmerman, Vincent B Nieuwenhuijs, Thomas L Bollen, Bert van Ramshorst, Ben J M Witteman, Camiel Rosman, Rutger J Ploeg, Menno A Brink, Alexander F M Schaapherder, Cornelis H C Dejong, Peter J Wahab, Cees J H M van Laarhoven, Erwin van der Harst, Casper H J van Eijck, Miguel A Cuesta, Louis M A Akkermans, Hein G Gooszen, for the Dutch Acute Pancreatitis Study Group

Summary

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

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

Findings One person in each group was excluded from analyses because of incorrect diagnoses of pancreatitis; thus, 152 individuals in the probiotics group and 144 in the placebo group were analysed. Groups were much the same at baseline in terms of patients' characteristics and disease severity. Infectious complications occurred in 46 (30%) patients in the probiotics group and 41 (28%) of those in the placebo group (relative risk 1.06, 95% CI 0.75–1.51). 24 (16%) patients in the probiotics group died, compared with nine (6%) in the placebo group (relative risk 2.53, 95% CI 1.22–5.25). Nine patients in the probiotics group developed bowel ischaemia (eight with fatal outcome), compared with none in the placebo group ($p=0.004$).

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

Introduction

The incidence of acute pancreatitis in Europe and the USA is increasing by about 5% per year, mainly owing to an increase in biliary pancreatitis.^{1–3} About a fifth of patients will develop necrotising pancreatitis, which is associated with a 10–30% mortality rate, mostly attributed to infectious complications and infection of (peri)pancreatic necrotic tissue in particular.¹ These infections are thought to be the sequelae of a cascade of events starting with small-bowel bacterial overgrowth, mucosal barrier failure, and a proinflammatory response leading to bacterial translocation of intestinal bacteria.^{4–6} Systemic antibiotic prophylaxis has long been studied as a measure to prevent secondary infection in acute pancreatitis.¹ However, two double-blind, placebo-controlled trials^{7,8} and two meta-analyses^{9,10} have failed to show a beneficial effect, and many clinicians have abandoned this strategy. In the two antibiotic trials, the incidence of extrapancreatic infections (eg, bacteraemia, pneumonia) and pancreatic infection remained high.^{7,8} Consequently, there is a clear need for other strategies to prevent infectious complications in patients with acute pancreatitis.

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

Methods

Patients

The design and rationale of the study have been described in detail elsewhere.¹⁶ Adult patients admitted with a first

Lancet 2008; 371: 651–59

Published Online
February 14, 2008
DOI:10.1016/S0140-6736(08)60207-X

See [Editorial](#) page 624

See [Comment](#) page 634

Department of Surgery (M G H Besselink MD, H C van Santvoort MD, H M Timmerman PhD, Prof L M A Akkermans PhD, Prof H G Gooszen MD) and Julius Center for Health Sciences and Primary Care (E Buskens MD), University Medical Center Utrecht, Utrecht, Netherlands; Department of Epidemiology (E Buskens) and Department of Surgery (V B Nieuwenhuijs MD, Prof R J Ploeg MD), University Medical Center Groningen, Groningen, Netherlands; Department of Surgery, Academic Medical Center, Amsterdam, Netherlands (M A Boermeester MD); Department of Surgery, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands (H van Goor MD); Department of Radiology (T L Bollen MD) and Department of Surgery (B van Ramshorst MD), St Antonius Hospital, Nieuwegein, Netherlands; Department of Gastroenterology, Gelderse Vallei Hospital, Ede, Netherlands (B J M Witteman MD); Department of Surgery, Canisius Wilhelmina Hospital, Nijmegen, Netherlands (C Rosman MD); Department of Gastroenterology, Meander Medical Center, Amersfoort, Netherlands (M A Brink MD); Department of Surgery, Leiden University Medical Center, Leiden, Netherlands (A F M Schaapherder MD); Department of Surgery and NUTRIM, University Hospital Maastricht, Maastricht, Netherlands (C H C Dejong MD);

Department of Gastroenterology, Rijnstate Hospital, Arnhem, Netherlands (P J Wahab MD); Department of Surgery, St Elisabeth Hospital, Tilburg, Netherlands (C J H M van Laarhoven MD); Department of Surgery, Medical Center Rijnmond Zuid, Rotterdam, Netherlands (E van der Harst MD); Department of Surgery, Erasmus Medical Center, Rotterdam, Netherlands (C H J van Eijck MD); and Department of Surgery, Vrije Universiteit Medical Center, Amsterdam, Netherlands (Prof M A Cuesta MD)

Correspondence to: Prof Hein G Gooszen, University Medical Center Utrecht, PO Box 85500, HP G04.228, 3508 GA Utrecht, Netherlands h.gooszen@umcutrecht.nl

episode of acute pancreatitis were enrolled in eight university medical centres and seven major teaching hospitals in the Netherlands. Acute pancreatitis was defined as abdominal pain in combination with serum amylase or lipase concentrations that were raised to at least three times the institutional upper limit of normal. Patients were not enrolled in the study if any of the following criteria were present: pancreatitis after endoscopic retrograde cholangiopancreatography; suspected malignancy of the pancreas or biliary tree; non-pancreatic infection or sepsis caused by a second disease; diagnosis of pancreatitis first made at operation; or a medical history of immune deficiency.

Patients with acute pancreatitis and an Acute Physiology and Chronic Health Evaluation (APACHE II) score of 8 or more,¹⁷ Imrie/modified Glasgow score of 3 or more,¹⁸ or C-reactive protein over 150 mg/L,¹⁹ predicting a severe course of disease, were eligible for randomisation.

All patients or their legal representatives gave written informed consent. This study was investigator-initiated and investigator-driven and done in accordance with the principles of the Declaration of Helsinki and good clinical practice guidelines. The institutional review board of each participating hospital approved the protocol.

Procedures

Randomisation was done with a computer-generated permuted-block sequence and balanced by participating centre and by presumed cause (biliary vs non-biliary) in blocks of four. Patients were randomly assigned to receive either a multispecies probiotic preparation or a placebo twice daily at the first possible occasion, but no later than 72 h after onset of symptoms of pancreatitis.

The study was double-blinded. Both the probiotic and placebo preparations were packaged in identical, numbered sachets that were stored in identical, numbered containers. The study product and placebo were both white powders, identical in weight, smell, and taste. All doctors, nurses, research staff, and patients involved remained unaware of the actual product administered during the entire study period. An independent monitoring committee was informed in cases of serious adverse events that were possibly associated with the study product. At the time of a prespecified interim analysis,¹⁶ the monitoring committee advised about whether to continue the trial.

The rationale for the design of the multispecies probiotic preparation has been described in detail elsewhere.²⁰ In brief, the study product (Ecologic 641, Winclove Bio Industries, Amsterdam, Netherlands) consisted of six different strains of freeze-dried, viable bacteria: *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus salivarius*, *Lactococcus lactis*, *Bifidobacterium bifidum*, and *Bifidobacterium lactis* (previously classified as *Bifidobacterium infantis*), in a total daily dose of 10¹⁰ bacteria, plus cornstarch and maltodextrins. The individual probiotic cultures are sold by major probiotic producers as ingredients for probiotic supplements or dairy food and carry the European

Union qualified presumption of safety (QPS). Individual strains were selected on the basis of their capacity to inhibit growth of pathogens most often cultured from infected necrotising pancreatitis in vitro.^{20,21} Probiotic species that were ever reported to have been associated with an infectious complication, irrespective of underlying disease, were excluded.²⁰ Placebo sachets contained only cornstarch and maltodextrins. Both the probiotic and placebo preparations were checked according to national regulations for any contamination with known pathogens and for the presence of endotoxins. Three different batches of probiotics and placebo were produced, tested, and used during the study.

After randomisation, each patient had a nasojejunal feeding tube inserted. The study product or placebo was administered twice daily and added to the continuously running fibre-enriched tube feeding (Nutrison Multi Fibre, Nutricia, Zoetermeer, Netherlands). The study product or placebo was dissolved in sterilised distilled water and administered for a maximum of 28 days. If placement of the nasojejunal tube was delayed for more than 12 h, the first dose of the study product or placebo was taken orally. Nasojejunal tubes were placed either by upper gastrointestinal endoscopy or under fluoroscopic guidance. When nasojejunal tubes became blocked or were pulled out, a new tube was re-inserted at the first possible opportunity, generally within 24 h. The amount of tube feeding was gradually increased over the first days with an energy target of 125 kJ/kg (up to 90 kg) on day 4 after start of enteral nutrition. When patients started oral intake, the nasojejunal tube was removed and the study product or placebo was dissolved in tap water and ingested orally for the remainder of the 28 days. Administration of the study product or placebo was stopped when a patient was diagnosed with infected pancreatic necrosis. Patients discharged before 28 days were only allowed to stop treatment if CT showed the absence of pancreatic necrosis or fluid collection. During the study, patients were not allowed to use any commercially available product containing probiotics. During administration of the study product or placebo, nursing staff recorded the number of sachets administered and registered any potential side-effect (eg, abdominal complaints).

Antibiotic prophylaxis was not given routinely in patients with necrotising pancreatitis. The use of antibiotics was recorded, irrespective of indication. When endoscopic retrograde cholangiopancreatography was indicated in cases of biliary pancreatitis, antibiotic prophylaxis was allowed. A standard baseline (intravenous) contrast-enhanced CT scan was done 7 days after admission to detect pancreatic necrosis. One experienced radiologist (TLB), unaware of treatment allocation, re-read all CT scans to assess the CT severity index.²² In cases of a clear clinical diagnosis of infected (peri)pancreatic necrosis (persistent fever and clinical deterioration in the third or fourth week of disease in the presence of documented necrosis or air bubbles in the collections with necrosis on CT, while other sources of infection were absent),

fine-needle aspiration of (peri)pancreatic collections was not mandatory to confirm the clinical suspicion. A positive culture was mandatory for the endpoint of infected necrosis. During surgical intervention or percutaneous drainage for (suspected or documented) infected necrosis, tissue or fluid samples were sent for routine microbiological assessment. Body temperature was measured at least twice daily and, in cases of fever, blood cultures were drawn. Further diagnostic and therapeutic measures were left to the treating clinicians' discretion.

The primary endpoint was the composite of any of the following infectious complications: infected pancreatic necrosis, bacteraemia, pneumonia, urosepsis, or infected ascites, during admission and 90-day follow-up (panel). All infections were weighted equally; multiple infections in the same patient were deemed to be one endpoint. Secondary endpoints (during admission and 90-day follow-up) were mortality, sequential organ failure assessment (SOFA) scores, (multi)organ failure during admission, onset of (multi)organ failure after randomisation, need for surgical intervention because of (documented or suspected) infected necrosis or intra-abdominal catastrophe, hospital stay, intensive-care stay, use of antibiotics, and abdominal complaints (nausea and abdominal fullness with visual analogue scales [VAS; cutoff 3.0 on a ten-point scale], and presence of diarrhoea as assessed by the patient [at days 5, 10, 14, 21, 28, and 35]). Per patient, the percentage intake of the study product or placebo was calculated and categorised as less than 80%, 80–89%, 90–95%, and over 95%. Microbiological data of the initial positive culture for each of the infectious complications of the primary endpoint were collected.

Organ failure was defined as PaO₂ below 60 mm Hg despite F_iO₂ of 30% or the need for mechanical ventilation (pulmonary insufficiency), serum creatinine over 177 mmol/L after rehydration or need for haemofiltration or haemodialysis (renal failure), and systolic blood pressure below 90 mm Hg despite adequate fluid resuscitation or need for vasopressor (mainly noradrenalin and dopamine) support (cardiovascular insufficiency), adapted from the Atlanta classification.²³ Multiorgan failure was defined as failure of at least two organ systems on the same day. Organ failure before randomisation was defined as any organ failure that started before the day of randomisation. Because the administration of the study product or placebo could start at any time during the day of randomisation, start of organ failure on that day was left out of this definition. Onset of organ failure after randomisation was defined as initial (for the first time) onset of organ failure after the day of randomisation.

Data collection was done by local physicians, who completed case record forms. During the study an independent data monitor checked at least 10% of the individual patients' data against the primary source data, on site in the participating centres. After completion of the follow-up of the last patient but before any analysis or unblinding, two authors (MGHB and HCvS) checked all

Panel: Definitions included in the primary endpoint

Infected pancreatic necrosis—positive culture of peripancreatic fluid or pancreatic necrosis obtained by either fine-needle aspiration, during the first percutaneous drainage, or during the first surgical intervention

Bacteraemia—positive blood culture. For non-pathogens (eg, coagulase-negative staphylococci) at least two samples had to be positive

Pneumonia—coughing, dyspnoea, chest film showing infiltrative abnormalities, lowered arterial blood gas with positive sputum culture. If in intensive care, a positive endotracheal culture is mandatory

Urosepsis*—dysuria with bacteraemia on the same day, without a urinary catheter in situ

Infected ascites†—bacteria detected in aspirate of intraperitoneal fluid or abdominal fluid sampled during surgical exploration

*Before any analysis, the adjudication committee restricted the definition of urinary tract infection to urosepsis. †Before any analysis, the adjudication committee added this group of infections to the infectious complications endpoint.

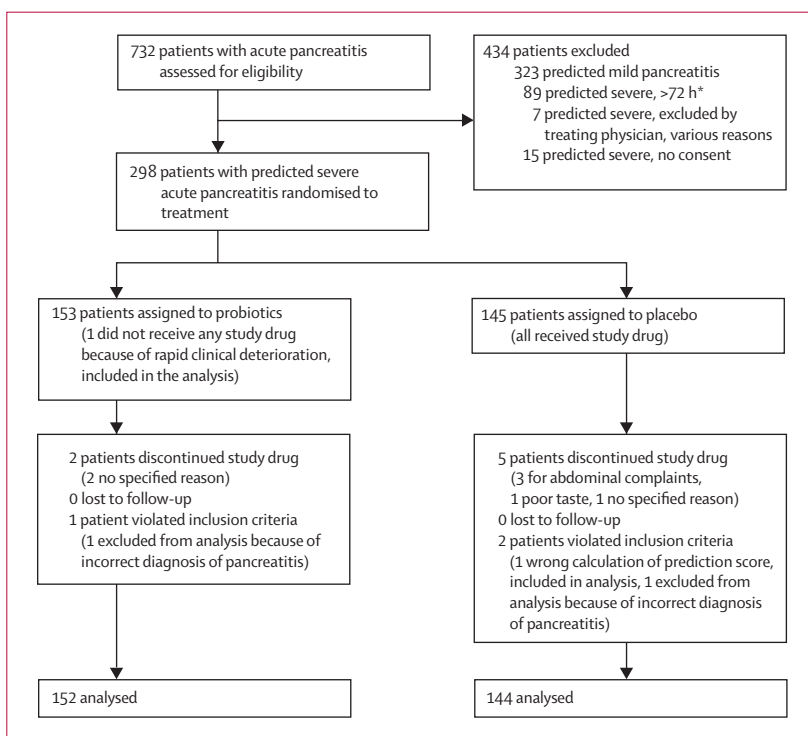


Figure 1: Trial profile

*Not randomised because of clinical symptoms of pancreatitis for more than 72 h at time of diagnosis of predicted severe acute pancreatitis. Patients were either initially missed for randomisation, were transferred from other hospitals more than 72 h after onset of symptoms, or already had complaints for more than 72 h on admission.

primary and secondary endpoints on site with primary source data. Before any analysis and without knowledge of treatment allocation, the blinded adjudication committee judged all exclusions, endpoints that were not fully specified in the protocol in individual patients, and serious adverse events. Only after agreement was reached on all endpoints were analyses done with blinding of the products administered preserved. After the results of the blinded analyses were presented to the monitoring committee, the randomisation code was broken on Oct 26, 2007.

	Probiotics (N=152)	Placebo (N=144)
Age (years)	60.4 (16.5)	59.0 (15.5)
Sex (male)	91 (60%)	83 (58%)
Body-mass index (kg/m ²)	27.1 (6.1)	27.8 (5.9)
Cause of pancreatitis		
Biliary	92 (61%)	75 (52%)
Alcohol	27 (18%)	28 (19%)
Unknown	21 (14%)	28 (19%)
Medication	4 (3%)	6 (4%)
Hypertriglyceridaemia	4 (3%)	3 (2%)
Other	4 (3%)	4 (3%)
American Society of Anaesthesiologists class		
I (healthy status)	62 (41%)	62 (43%)
II (mild systemic disease)	76 (50%)	64 (44%)
III (severe systemic disease)	14 (9%)	18 (13%)
Severity of pancreatitis		
APACHE II score*	8.6 (4.4)	8.4 (4.5)
Imrie score (first 48 h)	3.3 (1.7)	3.4 (1.6)
C-reactive protein concentration (mg/L) (highest first 48 h)	268 (127)	270 (122)
SOFA score (on admission)	2.1 (2.0)	1.9 (1.6)
MODS (on admission)	1.6 (1.6)	1.5 (1.5)
Organ failure before randomisation*	9 (6%)	5 (3%)
Multiorgan failure before randomisation	5 (3%)	1 (0.7%)
Endoscopic sphincterotomy	48 (32%)	35 (24%)
Time from first symptoms to admission (days)	0 (0-3)	0 (0-3)
Time from admission to first dose (days)	2 (0-4)	2 (0-3)
Time from admission to enteral nutrition (days)	2 (0-7)	2 (0-7)
Contrast-enhanced CT		
Necrotising pancreatitis†	46 (30%)	34 (24%)
≤30% pancreatic parenchymal necrosis	16 (11%)	14 (10%)
>30% pancreatic parenchymal necrosis	30 (20%)	20 (14%)
No contrast-enhanced CT done	6 (4%)	12 (8%)
CT severity index‡	4 (0-10)	4 (0-10)

Data are n (%), mean (SD), or median (range). APACHE II=Acute Physiology and Chronic Health Evaluation score, determined on admission. MODS=multiple organ dysfunction score (range 0-24, higher scores indicating more severe disease). SOFA=sequential organ failure assessment (range 0-24, higher scores indicating more severe disease). *Patients with multiorgan failure are included in the group patients with organ failure. †Done on day 7-10 after admission. ‡CT severity index ranges from 0 to 10, higher scores indicating more extensive pancreatic parenchymal necrosis and peripancreatic fluid collections.

Table 1: Baseline characteristics

Statistical analysis

We calculated that 200 patients with predicted severe acute pancreatitis would be required to detect a 20% reduction in the absolute risk of the occurrence of infectious complications (from 50% to 30% of patients during admission and 90-day follow-up) for the study to attain an 80% statistical power, at a two-sided α of 0.05. This sample size calculation took into account the fact that up to 40% of patients with predicted severe pancreatitis are ultimately diagnosed with mild pancreatitis (ie, no local or systemic complications) and thus do not progress to severe or necrotising pancreatitis. After the first 100 patients were randomised and had completed follow-up, the number of infectious complications was calculated in the total group

of randomised patients without unblinding the data. The rate of infectious complications was lower than expected (28%), so the monitoring committee advised increasing the total sample size from 200 to 296 patients to maintain statistical power. After 184 patients had been randomised and had completed follow-up, a blinded interim analysis was done for the primary endpoint and mortality. Although a non-significant difference in mortality was observed ($p=0.10$), the monitoring committee concluded that this had been caused by skewed randomisation because more patients in the group with higher mortality required admission to intensive care within 72 h after admission ($p=0.15$), whereas the overall mortality was well within the expected range (11%). According to the predefined stopping rule¹⁶ the monitoring committee recommended that the study should be completed.

All data analyses were done in accordance with a pre-established analysis plan. The incidence of the primary endpoint was compared between the groups and the results are presented as relative risk with exact 95% CI. The Kolmogorov-Smirnov test was used to assess whether continuous data were normally distributed ($p>0.05$). For continuous variables, differences between groups were tested with Student's *t* test for normally distributed data or Mann-Whitney *U* test for non-normally distributed data. Fisher's exact test was used for proportions in all cases. In cases of significant difference in the incidence of either the primary endpoint or mortality between groups, Kaplan-Meier curves with log-rank tests were generated.

All analyses were done on the basis of the intention-to-treat principle. Prespecified subgroup analyses were done for cause of pancreatitis and for presence of pancreatic parenchymal necrosis. We used logistic regression models to do a formal test for interaction to assess whether treatment effects differed significantly between these subgroups. A two-sided *p* value of less than 0.05 was deemed to be statistically significant. All statistical analyses were done with SPSS (version 12.0.1).

Role of the funding source

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

Results

732 consecutive patients with a first episode of acute pancreatitis were registered prospectively between March, 2004, and March, 2007 (figure 1). 298 patients were predicted to have a severe disease course (135 patients with APACHE II score ≥ 8 , 204 with Imrie score ≥ 3 , 252 with C-reactive protein >150 mg/L), and were randomly assigned treatment with probiotics or with placebo (figure 1). Two patients—one in each group—were excluded from the final analysis because of an incorrect

diagnosis of acute pancreatitis; one was ultimately diagnosed with acute cholecystitis and the other with post-pancreatic surgery anastomotic leakage. One patient who did not receive any study product and one who, in retrospect, had predicted mild pancreatitis were included in the final analysis (figure 1). Study groups were comparable for all baseline characteristics (table 1).

All but five patients started treatment within 72 h of onset of symptoms. Median intake of probiotics or placebo per patient was 100% (25% lower limit 91%). No difference in the categorised percentage intake between the groups was found (data not shown; $p=0.78$). No infections were confirmed to be caused by any of the probiotic strains administered. During the study, two serious adverse events were reported; both patients died. The monitoring committee convened on both occasions: in one patient, a ruptured caecum with ischaemia was found during emergency laparotomy and the second patient had small-bowel ischaemia diagnosed at emergency laparotomy. In both cases, the randomisation code was broken (both patients had received probiotics). This information was revealed only to members of the monitoring and steering committees. A review of published work did not reveal any evidence of a relation between bowel ischaemia and the use of probiotics. The monitoring committee subsequently advised that the study continue. The institutional review board was informed on both occasions.

There was no significant difference in the occurrence of the composite primary endpoint between the two groups, nor were there any significant differences between the groups in its individual components (table 2). The relative risk for the primary endpoint was 1.06 (95% CI 0.75–1.51). Table 3 shows the pathogens cultured from the 87 patients with an infectious complication; no significant differences between the groups were observed.

There were significantly more deaths in the probiotics group than there were in the placebo group ($p=0.01$; table 2 and figure 2); the relative risk for mortality was 2.53 (95% CI 1.22–5.25). Most of the deaths were caused by multiorgan failure: 20 (83%) of those in the probiotics group and seven (78%) of those in the placebo group. Other causes of death were respiratory failure after aspiration (one) and cerebral infarction/bleeding (three) in the probiotics group, and ruptured aneurysm (one) and cerebral infarction (one) in the placebo group.

Bowel ischaemia was detected during operation or autopsy in nine patients in the probiotics group; eight of these patients died as a result. No cases of bowel ischaemia were seen in the placebo group ($p=0.004$ for difference between groups; table 4). The nine cases of bowel ischaemia were all diagnosed within the first 14 days of admission in seven different hospitals; four university and three teaching hospitals. In all nine patients, contrast-enhanced CT (either the baseline CT or an earlier CT) showed unequivocal evidence of acute pancreatitis. All these patients had early onset of organ failure (median 2 days after admission, range 1–6 days). At the time of diagnosis of bowel

	Probiotics (N=152)	Placebo (N=144)	p value
Primary endpoint			
Any infectious complication*	46 (30%)	41 (28%)	0.80
Infected necrosis	21 (14%)	14 (10%)	0.29
Bacteraemia	33 (22%)	22 (15%)	0.18
Pneumonia	24 (16%)	16 (11%)	0.31
Urosepsis	1 (0.7%)	2 (1%)	0.61
Infected ascites	4 (3%)	0 (0%)	0.12
Secondary endpoint			
Use of antibiotics, any indication	75 (49%)	76 (53%)	0.56
Percutaneous drainage	14 (9%)	8 (6%)	0.23
Surgical intervention, any indication	28 (18%)	14 (10%)	0.05
Necrosectomy	24 (16%)	14 (10%)	0.16
Intensive care admission	47 (31%)	34 (24%)	0.19
Intensive care stay (days)	6.6 (17.1)	3.0 (9.3)	0.08
Hospital stay (days)	28.9 (41.5)	23.5 (25.9)	0.98
Organ failure during admission, any onset†‡	41 (27%)	23 (16%)	0.02
Multiorgan failure during admission, any onset‡	33 (22%)	15 (10%)	0.01
Organ failure, onset after randomisation†§	21 (14%)	16 (11%)	0.60
Multiorgan failure, onset after randomisation§	18 (12%)	11 (8%)	0.25
Nausea	20 (13%)	23 (16%)	0.51
Abdominal fullness	36 (24%)	43 (30%)	0.24
Diarrhoea	25 (16%)	28 (19%)	0.55
Bowel ischaemia	9 (6%)	0 (0%)	0.004
Mortality	24 (16%)	9 (6%)	0.01

Data are mean (SD) or n (%). *Patients with one or more infectious complication. †Patients with multiorgan failure are included in the organ failure group. ‡Patients with organ failure present at any time during admission, irrespective of the date of onset of organ failure, are included. §Patients in whom organ failure developed (for the first time) after the day of randomisation are included. Patients in whom organ failure (in any organ) started before the day of randomisation or on the day of randomisation are not included.

Table 2: Endpoints

ischaemia, six patients had vasopressor support (14 patients in the placebo group and 23 in the probiotics group had vasopressor support in the first 14 days). Patients had received a median of six doses of probiotics (range 4–22 doses) before diagnosis of bowel ischaemia. The small bowel was involved in eight of the nine patients (including the survivor). During autopsy (six patients), five patients with small-bowel ischaemia had no sign of occlusive disease in the mesenteric vessels.

Apart from the patients with bowel ischaemia, 11 patients died in the 2 weeks after admission: eight in the probiotics group and three in the placebo group. These patients died of multiorgan failure without signs of bowel ischaemia.

No significant differences were noted between the groups for the serial SOFA scores (data not shown). Although more patients in the probiotics group than in the placebo group developed organ failure during the study there was no difference between the groups with regard to organ failure that started after the day of randomisation ($p=0.6$). During the study, 102 (34%) patients developed the most severe form of acute pancreatitis (organ failure or pancreatic parenchymal necrosis); 56 (37%) in the probiotics group and 46 (32%) in the

	Probiotics (N=152)	Placebo (N=144)
Gram-positive bacteria		
<i>Staphylococcus</i> spp	20	20
<i>Staphylococcus aureus</i>	10	11
Coagulase-negative staphylococci	9	5
<i>Enterococcus</i> spp	10	3
<i>Streptococcus</i> spp	3	3
Other gram-positive microorganisms†	3	3
Gram-negative bacteria		
Enterobacteriaceae	28	20
<i>Escherichia coli</i>	17	7
<i>Klebsiella</i> spp	8	8
Other gram-negative microorganisms‡	4	8
Fungi		
<i>Candida</i> spp	5	1
<i>Chrysosporium</i> sp	1	0
Unknown	0	1

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

Table 3: Pathogens isolated from 87 patients with an infectious complication*

placebo group. 18 patients did not undergo a CT: the treating physician deemed CT unnecessary in 17 patients, or the patient refused because of good clinical condition; one patient in the placebo group died on day 4 before CT could be done. The latest point at which a baseline CT was done was 10 days after admission.

Predefined subgroup analyses were done for the presence of pancreatic parenchymal necrosis (any extent) and cause (biliary vs non-biliary) for both the primary endpoint and mortality. The tests for interaction were not significant—ie, we could not confirm an interaction between probiotic administration and pancreatic necrosis or underlying cause for either the primary endpoint or for mortality. In the subgroup of patients with pancreatic parenchymal necrosis, one or more infectious complication consistent with the primary endpoint occurred in 32 (70%) of 46 patients in the probiotics group versus 18 (53%) of 34 patients in the placebo group (p=0.16). In patients with pancreatic parenchymal necrosis, 19 (41%) of 46 patients in the probiotics group died, compared with five (15%) of 34 in the placebo group (p=0.01).

Discussion

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

The rate of infectious complications in our study is in line with a large German multicentre study (31%) on antibiotic prophylaxis in predicted severe acute pancreatitis.⁸ Although antibiotic prophylaxis was strongly discouraged in our study, antibiotics were used in about half the patients, although only a third of all patients had a documented infection. Antibiotics were sometimes started pre-emptively, on the basis of clinical suspicion of infection before bacterial culture results becoming available. Obviously, this clinical indication for antibiotic treatment leads to false-positive diagnoses of infectious complications. The overall rate of antibiotic use in our study was no different from that in the placebo groups of trials of antibiotic prophylaxis in acute pancreatitis.^{7,8}

The adverse effects of probiotics noted here were unexpected. Several studies have associated probiotics with a reduction in infectious complications.^{13,14} Most of these studies have been done in patients undergoing elective abdominal operations. However, one randomised study in 90 critically ill patients showed a non-significant increase in septic complications in the probiotics group;²⁴ another randomised study in 61 children admitted to a paediatric intensive-care unit was discontinued prematurely because of a non-significant increase in infections in the probiotics

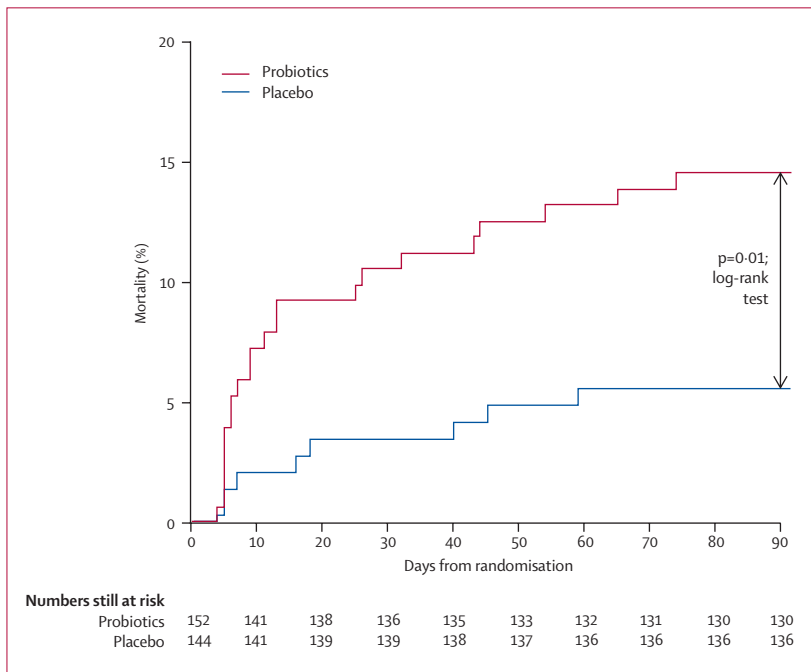


Figure 2: Kaplan-Meier time-to-event analysis for mortality in the first 90 days after randomisation
 A follow-up of longer than 90 days was obtained in 266 (90%) patients. Three deaths occurred after 90 days: two in the probiotics group (day 112 and 125) and one in the placebo group (day 140).

SSN	Sex	Age (years)	Day of diagnosis	Days of treatment before diagnosis	Vasopressor support at day of diagnosis	Day of onset of organ failure	Day of death	Findings
Patient 10	Female	40	5	3	0	1	5	Emergency laparotomy day 5: perforated caecum with adjacent ischaemia. At autopsy: mucosal ischaemia 80 cm of small bowel
Patient 93	Male	61	12	11	0	1	13	Emergency laparotomy day 12: resection of 50 cm ischaemic proximal jejunum. At autopsy: necrosis and inflammatory changes of the small bowel wall
Patient 121	Male	62	4	2	1	2	4	At autopsy: only the proximal jejunum vital, rest of the small bowel ischaemic
Patient 124	Female	88	6	4	1	6	6	At autopsy: inflammatory changes of the duodenum wall and necrotising oesophagitis
Patient 160	Female	62	4	2	1	1	4	Emergency laparotomy day 4: ischaemia of most of the small bowel
Patient 202	Male	60	12	10	1	2	26	Emergency laparotomy day 12: necrosis of 40 cm jejunum. At autopsy: necrotising jejunitis
Patient 235	Male	57	9	9	1	2	125	Emergency laparotomy day 9: resection of 90 cm of ischaemic ileum. Patient died 4 months later from cerebral infarction
Patient 243	Male	22	4	3	0	2	Survived	Emergency laparotomy day 4: ischaemic proximal jejunum
Patient 297	Male	57	3	3	1	2	6	Emergency laparotomy day 3: ischaemia and inflammation of the entire small and large bowel

SSN=sequential study number, patient number 1 was the first patient in the trial.

Table 4: Clinical characteristics of nine patients with bowel ischaemia in the probiotics group

group.^{25,26} To date, the main criticism of most randomised controlled trials of probiotic prophylaxis is methodological shortcomings—eg, analyses not done by intention to treat and sample sizes too small to provide convincing evidence on relevant clinical endpoints.

Two small placebo-controlled randomised controlled trials of probiotic prophylaxis have been done in patients with acute pancreatitis. The first study randomised 45 patients with both predicted mild and predicted severe pancreatitis of solely non-biliary causes.¹⁵ The infection rate was lower in the probiotics group than in the placebo group; no effect on mortality was noted. However, this study was criticised because patients with biliary pancreatitis were excluded, the sample size was small, and analyses were not by intention to treat.^{27,28} In the second trial, done by the same research group in 62 patients with predicted severe pancreatitis, the difference in the rate of infectious complications seen in the first trial could not be reproduced.²⁹ This second study used a probiotic preparation previously found to be effective in preventing infectious complications in patients undergoing abdominal operations.^{13,14}

Because the findings of our trial are in marked contrast with the previous reports, we scrutinised our results and methodology for explanations other than a deleterious effect of probiotics. Randomisation was successful, since there were no significant differences in baseline characteristics between groups. In the probiotics group there was a (non-significantly) higher proportion of patients with organ failure before randomisation as well as a greater proportion of patients with more than 30% pancreatic parenchymal necrosis than in the placebo group. When we assessed this imbalance by use of logistic regression, the (adjusted) mortality remained significantly higher in the probiotics group than in the placebo group (data not

shown). There was no indication that treatment effects differed in the subgroup analyses. We also considered whether the composition of the product or the doses used explained the effects noted. The daily dose was similar to doses used in previous studies and, although the combination of probiotic strains administered was different from the preparations used so far, the individual strains have an unblemished reputation as probiotics, both in (smaller) clinical studies and in daily practice in the food industry. The six probiotic strains used in this study were selected from 69 different probiotic bacteria on the basis of their capacity to inhibit growth of gut-derived pathogens and to modulate immune responses.²⁰ The combination of strains was shown to result in a better antimicrobial spectrum, induction of interleukin 10, and silencing of pro-inflammatory cytokines than the individual components.²⁰ The combination of strains was found capable of inhibiting the in-vitro growth of a wide variety of pathogens cultured from pancreatic necrosis.²¹ Again, the combination of strains had better growth-inhibiting capacities than did the individual strains.²¹ Additionally, when the preparation was administered before induction of severe acute pancreatitis in rats, a significant reduction of both infectious complications and late mortality was noted.³⁰ The same preparation was also used in three small clinical studies under elective circumstances in healthy volunteers, patients with ileostomy, patients about to undergo pancreaticoduodenectomy, and patients with primary sclerosing cholangitis, and no adverse events were noted (unpublished data, trial registry ISRCTN45167712, ISRCTN71637623, and NCT00161148). However, these patients were less ill than the patients in the present study.

Previous randomised trials with probiotics have been of much smaller sample size and with fewer critically ill patients than in the present study. Consequently, the power

of these studies was too small to detect differences in mortality or uncommon adverse events such as bowel ischaemia. In our study, probiotics caused a significant increase in mortality, most likely a result of deleterious effects on the (small) bowel wall. After administration of probiotics, no significant increase in new onset organ failure was seen. Possibly, probiotics especially exert their adverse effects in patients in whom organ failure has already occurred. Because the exact mechanism causing the bowel ischaemia seen here is, at present, unknown, we cannot exclude or confirm that another product—eg, a combination of strains or one strain alone—would have resulted in similar results. However, in view of the fatal nature of these complications, the administration of any type of probiotic in this category of patients must strongly be advised against until the mechanism of the complications has been unravelled.

The occurrence of non-occlusive mesenteric ischaemia is well known in critically ill patients,³¹ and several cases of non-occlusive mesenteric ischaemia have been reported in acute pancreatitis.³² Such complications could explain why only two of the nine cases of mesenteric ischaemia seen in this study were reported as a serious adverse event. Evidence exists to suggest that intestinal bloodflow at the mucosal level is generally reduced in acute pancreatitis. An experimental study in rats found a reduction in bloodflow to the intestinal mucosa of up to 85%.³³ A clinical study in patients with severe pancreatitis showed a significant increase in a biological marker for enterocyte death and small-bowel ischaemia.³⁴ In a severely ill patient going through a phase of severe systemic inflammation or organ failure, an already critically reduced bloodflow and oxygen supply in the small-bowel mucosa might be further compromised by the administration of enteral feeding, known for its increased demand for local oxygen.^{35,36} This effect is probably local, since ischaemia usually occurs at the site of administration of enteral feeding.^{35,36} However, until now, this occurrence has not been recognised as an argument to refrain from enteral nutrition in critically ill patients because the beneficial effects outweigh the small risk of developing ischaemia.

We can only speculate as to the mechanism of bowel ischaemia in the probiotics group. The administration of 10 billion probiotic bacteria per day on top of enteral nutrition might have even further increased local oxygen demand, with a combined deleterious effect on an already critically reduced bloodflow. A second possible explanation could be that the presence of probiotics caused local inflammation at the mucosal level. Experimental studies have shown that gut epithelial cells under metabolic stress react to commensal bacteria with an inflammatory response.³⁷ One could postulate that increasing the bacterial load in the small bowel could lead to aggravation of local inflammation, again with a further reduction of capillary bloodflow and ultimately ischaemia. Notably, three of the six autopsy reports of patients with bowel ischaemia mentioned inflammatory changes of the small-bowel wall.

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

Our findings show that probiotics should not be administered routinely in patients with predicted severe acute pancreatitis, and that the particular composition used here should be banned for the present indication. Whether other (combinations of) strains might have resulted in different results is debatable, but, until the underlying mechanism is actually revealed, administration of probiotics in patients with predicted severe acute pancreatitis must be regarded as unsafe. Most importantly, probiotics can no longer be considered to be harmless adjuncts to enteral nutrition, especially in critically ill patients or patients at risk for non-occlusive mesenteric ischaemia.

Contributors

MGHB, EB, MAB, HvG, HMT, VBN, BvR, BJMW, RJP, AFMS, CHCD, CHJvE, LMAA, HGG, and several other members of the study group participated in the design of the study. MGHB, HCvS, and TLB collected the data. MGHB and EB did the statistical analysis. MGHB drafted the first and subsequent versions of the report with input from all authors. HGG supervised the current study. All authors read and approved the final report.

Committee members

Steering committee: H G Gooszen, M G H Besselink, H C van Santvoort, H M Timmerman, L M A Akkermans, V B Nieuwenhuijs, E Buskens, H van Goor.

Adjudication committee: M A Boermeester, H van Goor, H G Gooszen, M G H Besselink, H C van Santvoort.

Writing committee: M G H Besselink, H C van Santvoort, E Buskens, M A Brink, H v Goor, H M Timmerman, V B Nieuwenhuijs, T L Bollen, L M A Akkermans, H G Gooszen.

Monitoring committee: University Medical Center Utrecht—

I H M Borel Rinkes, Y van der Graaf, B Oldenburg, W Renooij; University Hospital Maastricht—E Stobberingh.

Investigators

St Antonius Hospital, Nieuwegein (45 patients)—B L Weusten, R Timmer; Gelderse Vallei Hospital, Ede (33 patients)—P M Kruij; University Medical Center Utrecht (28 patients)—K J van Erpecum, G A Cirkel, V Zegers, A Roeterdink, H G Rijnhart, M P Schwartz, M S van Leeuwen, B U Ridwan, U Ahmed Ali; Radboud University Nijmegen Medical Centre, Nijmegen (21 patients)—A Nootboom, J B Jansen, G T Bongaerts, H C Buscher; Canisius Wilhelmina Hospital, Nijmegen (20 patients)—A C Tan, L Ootes, B Houben; Meander Medical Center, Amersfoort (19 patients)—M Mundt, R Frankhuisen, E C Consten; Leiden University Medical Center, Leiden (19 patients)—A Haasnoot; University Medical Center Groningen (19 patients)—H S Hofker, M R Kruijt Spanjer, H T Buitenhuis, S U van Vliet, S Ramcharan; Rijnstate Hospital, Arnhem (16 patients)—E J Spillenaar Bilgen, P van Embden; University Hospital Maastricht (16 patients)—J P Rutten; St Elisabeth Hospital, Tilburg

(15 patients)—T A Drixler; Academic Medical Center, Amsterdam (14 patients)—O van Ruler, D J Gouma, M J Bruno; Medical Center Rijnmond Zuid, Rotterdam (13 patients)—J F Lange, N A Wijffels, L A van Walraven, F J Kubben; Erasmus Medical Center, Rotterdam (13 patients)—J B C van der Wal; G van't Hof, E J Kuipers; Vrije Universiteit Medical Center, Amsterdam (seven patients)—C J Mulder.

Conflict of interest statement

HMT is an employee of Winlove Bio Industries, Amsterdam, and the University Medical Center Utrecht. All of the other authors declare that they have no conflict of interest.

Acknowledgments

We thank the medical and nursing staff in the participating centres for their assistance in enrolment and care of patients in this study. Senter, an agency of the Dutch Ministry of Economic Affairs, funded this study (grant number TSGE3109). Winlove Bio Industries, Amsterdam supplied both the probiotics and placebo.

References

- Anon. UK guidelines for the management of acute pancreatitis. *Gut* 2005; **54** (suppl 3): iii1–iii9.
- Frey CF, Zhou H, Harvey DJ, White RH. The incidence and case-fatality rates of acute biliary, alcoholic, and idiopathic pancreatitis in California, 1994–2001. *Pancreas* 2006; **33**: 336–44.
- Yadav D, Lowenfels AB. Trends in the epidemiology of the first attack of acute pancreatitis: a systematic review. *Pancreas* 2006; **33**: 323–30.
- 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**: 252–62.
- 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**: 403–04.
- Van Leeuwen PA, Boermeester MA, Houdijk AP, et al. Clinical significance of translocation. *Gut* 1994; **35**: S28–34.
- Dellinger EP, Tellado JM, Soto NE, et al. Early antibiotic treatment for severe acute necrotizing pancreatitis: a randomized, double-blind, placebo-controlled study. *Ann Surg* 2007; **245**: 674–83.
- Isenmann R, Runzi M, Kron M, et al. Prophylactic antibiotic treatment in patients with predicted severe acute pancreatitis: a placebo-controlled, double-blind trial. *Gastroenterology* 2004; **126**: 997–1004.
- 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. *Pancreatol* 2007; **7**: 531–38.
- Mazaki T, Ishii Y, Takayama T. Meta-analysis of prophylactic antibiotic use in acute necrotizing pancreatitis. *Br J Surg* 2006; **93**: 674–84.
- Bengmark S. Ecological control of the gastrointestinal tract. The role of probiotic flora. *Gut* 1998; **42**: 2–7.
- Guarner F, Malagelada JR. Gut flora in health and disease. *Lancet* 2003; **361**: 512–19.
- Rayes N, Seehofer D, Theruvath T, et al. Supply of pre- and probiotics reduces bacterial infection rates after liver transplantation—a randomized, double-blind trial. *Am J Transplant* 2005; **5**: 125–30.
- Rayes N, Seehofer D, Theruvath T, et al. Effect of enteral nutrition and synbiotics on bacterial infection rates after pylorus-preserving pancreatoduodenectomy: a randomized, double-blind trial. *Ann Surg* 2007; **246**: 36–41.
- Olah A, Belagyi T, Issekutz A, Gamal ME, Bengmark S. Randomized clinical trial of specific lactobacillus and fibre supplement to early enteral nutrition in patients with acute pancreatitis. *Br J Surg* 2002; **89**: 1103–07.
- 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. *BMC Surg* 2004; **4**: 12.
- Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. *Crit Care Med* 1985; **13**: 818–29.
- Blamey SL, Imrie CW, O'Neill J, Gilmour WH, Carter DC. Prognostic factors in acute pancreatitis. *Gut* 1984; **25**: 1340–46.
- Werner J, Hartwig W, Uhl W, Muller C, Buchler MW. Useful markers for predicting severity and monitoring progression of acute pancreatitis. *Pancreatol* 2003; **3**: 115–27.
- Timmerman HM, Niers LE, Ridwan BU, et al. Design of a multispecies probiotic mixture to prevent infectious complications in critically ill patients. *Clin Nutr* 2007; **26**: 450–59.
- Ridwan BU, Koning CJ, Besselink MG, et al. Antimicrobial activity of a multispecies probiotic (Ecologic 641) against pathogens isolated from infected pancreatic necrosis. *Lett Appl Microbiol* 2008; **46**: 61–67.
- Balthazar EJ, Robinson DL, Megibow AJ, Ranson JH. Acute pancreatitis: value of CT in establishing prognosis. *Radiology* 1990; **174**: 331–36.
- 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**: 586–90.
- Jain PK, McNaught CE, Anderson AD, Macfie J, Mitchell CJ. Influence of synbiotic containing *Lactobacillus acidophilus* La5, *Bifidobacterium lactis* Bb 12, *Streptococcus thermophilus*, *Lactobacillus bulgaricus* and oligofructose on gut barrier function and sepsis in critically ill patients: a randomised controlled trial. *Clin Nutr* 2004; **23**: 467–75.
- Singhi S. Probiotics in the critically ill: handle with care! *Pediatr Crit Care Med* 2007; **8**: 499–501.
- Honeycutt TC, El KM, Wardrop RM III, et al. Probiotic administration and the incidence of nosocomial infection in pediatric intensive care: a randomized placebo-controlled trial. *Pediatr Crit Care Med* 2007; **8**: 452–58.
- Weale R, Edwards A. Letter 1: 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). *Br J Surg* 2003; **90**: 122–23.
- Rahman SH, Catton JA, McMahon MJ. Letter 2: 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). *Br J Surg* 2003; **90**: 123.
- Olah A, Belagyi T, Poto L, Romics L Jr, Bengmark S. Synbiotic control of inflammation and infection in severe acute pancreatitis: a prospective, randomized, double blind study. *Hepatogastroenterology* 2007; **54**: 590–94.
- 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.
- Kolkman JJ, Mensink PB. Non-occlusive mesenteric ischaemia: a common disorder in gastroenterology and intensive care. *Best Pract Res Clin Gastroenterol* 2003; **17**: 457–73.
- Hirota M, Inoue K, Kimura Y, et al. Non-occlusive mesenteric ischemia and its associated intestinal gangrene in acute pancreatitis. *Pancreatol* 2003; **3**: 316–22.
- Andersson R, Wang X, Ihse I. The influence of abdominal sepsis on acute pancreatitis in rats: a study on mortality, permeability, arterial pressure, and intestinal blood flow. *Pancreas* 1995; **11**: 365–73.
- Rahman SH, Ammori BJ, Holmfeld J, Larvin M, McMahon MJ. Intestinal hypoperfusion contributes to gut barrier failure in severe acute pancreatitis. *J Gastrointest Surg* 2003; **7**: 26–35.
- McClave SA, Chang WK. Feeding the hypotensive patient: does enteral feeding precipitate or protect against ischemic bowel? *Nutr Clin Pract* 2003; **18**: 279–84.
- Melis M, Fichera A, Ferguson MK. Bowel necrosis associated with early jejunal tube feeding: a complication of postoperative enteral nutrition. *Arch Surg* 2006; **141**: 701–04.
- Nazli A, Yang PC, Jury J, et al. Epithelia under metabolic stress perceive commensal bacteria as a threat. *Am J Pathol* 2004; **164**: 947–57.
- Heyland DK, Novak F, Drover JW, Jain M, Su X, Suchner U. Should immunonutrition become routine in critically ill patients? A systematic review of the evidence. *JAMA* 2001; **286**: 944–53.
- Sukhotnik I, Khateeb K, Mogilner JG, et al. Dietary glutamine supplementation prevents mucosal injury and modulates intestinal epithelial restitution following ischemia-reperfusion injury in the rat. *Dig Dis Sci* 2007; **52**: 1497–504.
- Omata J, Fukatsu K, Ueno C, Maeshima Y, Saitoh D, Mochizuki H. Intraluminal glutamine administration during ischemia worsens survival after gut ischemia-reperfusion. *J Surg Res* 2007; **132**: 260–64.