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


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.

Methods

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 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.2–4 Systemic antibiotic prophylaxis has long been studied as a measure to prevent secondary infection in acute pancreatitis.1 However, two double-blind, placebo-controlled trials2 and two meta-analyses3,4 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.4 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.5–7 Certain strains of probiotic bacteria may prevent infectious complications by reducing small-bowel bacterial overgrowth, restoring gastrointestinal barrier function, and modulating the immune system.8,9 A reduction of infectious complications has been reported in several clinical studies with probiotics in patients undergoing elective abdominal operations10,11 and in patients with acute pancreatitis.12 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 nation-wide 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.

Lancet 2008; 371: 651–59

Published Online
February 14, 2008
DOI:10.1016/S0140-6736(08)66207-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 (B van Ramshorst MD), Prof R J Ploeg MD), University Medical Center Groningen, Groningen, Netherlands; 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 van Ramshorst MD); Department of Surgery, Canisius Wilhelmina Hospital, Nijmegen, Netherlands (E Rosman MD); Department of Gastroenterology, Meander Medical Center, Amersfoort, Netherlands (A M Brink MD); Department of Surgery, Leiden University Medical Center, Leiden, Netherlands (A F M Schaapberder MD); Department of Surgery and NUTRIM, University Hospital Maastricht, Maastricht, Netherlands (C H C Dejong MD);
Patients with acute pancreatitis and an Acute Physiology and Chronic Health Evaluation (APACHE II) score of 8 or more.\textsuperscript{7} Imrie/modified Glasgow score of 3 or more,\textsuperscript{8} or C-reactive protein over 150 mg/L,\textsuperscript{9} 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. Adherence to practice guidelines. The institutional review board of each participating medical 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,\textsuperscript{8} 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.\textsuperscript{8} In brief, the study product (Ecologic 641, Winclove Bio Industries, Amsterdam, Netherlands) consisted of six different strains of freeze-dried, viable bacteria: \textit{Lactobacillus acidophilus}, \textit{Lactobacillus casei}, \textit{Lactobacillus salivarius}, \textit{Lactococcus lactis}, \textit{Bifidobacterium bifidum}, and \textit{Bifidobacterium infantis}, in a total daily dose of \(10^{10}\) 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.\textsuperscript{20,21} Probiotic species that were ever reported to have been associated with an infectious complication, irrespective of underlying disease, were excluded.\textsuperscript{21} 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.\textsuperscript{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 PaO\textsubscript{2} below 60 mm Hg despite F\textsubscript{1}O\textsubscript{2} 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 noradrenaline and dopamine) support (cardiocirculatory insufficiency), adapted from the Atlanta classification.\textsuperscript{21} 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 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.

---

**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.*
Table 1: Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Probiotics (N=152)</th>
<th>Placebo (N=144)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>60.4 (16.5)</td>
<td>59.0 (15.5)</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>91 (60%)</td>
<td>83 (58%)</td>
</tr>
<tr>
<td>Body-mass index (kg/m²)</td>
<td>27.1 (6.1)</td>
<td>27.8 (5.9)</td>
</tr>
</tbody>
</table>

Cause of pancreatitis

Biliary

Alcohol

Unknown

Medication

Hypertriglyceridaemia

Other

American Society of Anesthesiologists class

I (healthy status)

II (mild systemic disease)

III (severe systemic disease)

Severity of pancreatitis

APACHE II score

Imrie score (first 48 h)

C-reactive protein concentration (mg/L) (highest first 48 h)

SOFa score (on admission)

MODS (on admission)

Organ failure before randomisation

Multiorgan failure before randomisation

Endoscopic sphincterotomy

Time from first symptoms to admission (days)

Time from admission to first dose (days)

Time from admission to enteral nutrition (days)

Contrast-enhanced CT

Necrotising pancreatitis

≤30% pancreatic parenchymal necrosis

>30% pancreatic parenchymal necrosis

No contrast-enhanced CT done

CT severity index

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).

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). Most of the deaths were caused by multiorgan failure: 20 (83%) of those in the probiotics group and 23 in the placebo group had multiorgan failure without signs of bowel ischaemia. In all cases, the randomisation code was broken (both patients had received probiotics). This information was revealed only to members of the monitoring and steering committees.

An overwhelming majority of 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 placebo group.
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. 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 group of trials of antibiotic prophylaxis in predicted severe acute pancreatitis. The adverse effects of probiotics noted here were unexpected. Several studies have associated probiotics with a reduction in infectious complications. 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; 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 group.
group. 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. 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. 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. This second study used a probiotic preparation previously found to be effective in preventing infectious complications in patients undergoing abdominal operations.

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. 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. The combination of strains was found capable of inhibiting the in-vitro growth of a wide variety of pathogens cultured from pancreatic necrosis. Again, the combination of strains had better growth-inhibiting capacities than did the individual strains. 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. 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

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

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

SSN=sequential study number, patient number 1 was the first patient in the trial.
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, and several cases of non-occlusive mesenteric ischaemia have been reported in acute pancreatitis. 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%. A clinical study in patients with severe pancreatitis showed a significant increase in a biological marker for entercyte death and small-bowel ischaemia. 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. This effect is probably local, since ischaemia usually occurs at the site of administration of enteral feeding. 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. 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. 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, whereas mortality increased when glutamine was administered after the induction of a low flow state. 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, CHlVE, LMAA, HGG, and several other members of the study group participated in the design of the study. MGHB, HCCvS, 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
Adjudication committee: M A Boermeester, H van Goor, H G Gooszen, M G H Besselink, H C van Santvoort.
Monitoring committee: University Medical Center Utrecht—H M Boel Rinkes, Y van der Graaf, B Oldenburg, W Renooij; University Hospital Maastricht—E Stobberingham.

Investigators
St Antonius Hospital, Nieuwegein (45 patients)—B L Weusten, R Timmer; Gelderse Vallei Hospital, Ede (33 patients)—P M Kruit; University Medical Center Utrecht (28 patients)—K J van Erpecum, G A Cirkel, V Zeguaas, A Roeterdink, H G Rijnhart, M S van Leeuwen, B U Ridwan, U Ahmed Ali; Radboud University Nijmegen Medical Centre, Nijmegen (21 patients)—A Nootbooom, J B Jansen, G T Bourgaerts, 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 Hokker, M R Kouki Spanjer, H T Buitenhuis, S U van Vliet, S Ramcharan; Rijnstate Hospital, Arnhem (16 patients)—E J Spillenaar Bilgen, P van Erdenb; University Hospital Maastricht (16 patients)—P Ruten; St Elisabeth Hospital, Tilburg
(13 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 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 Winclowe 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). Winclowe Bio Industries, Amsterdam supplied both the probiotics and placebo.

References
1 Anon. UK guidelines for the management of acute pancreatitis. Gut 2005; 54 (suppl 3); iii-iix.