Intestinal Crises in the Newborn

Loss of Intestinal Absorptive Capacity after Necrotizing Enterocolitis

Marie-Chantal Struijs

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Loss of intestinal absorptive capacity after necrotizing enterocolitis

Intestinale crises in de pasgeborene

Verlies van absorptiecapaciteit van de darm na necrotiserende enterocolitis

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PART I

INTRODUCTION

Socrates

Chapter 1

General introduction

Intestinal crises in the newborn consist of a spectrum of gastrointestinal disorders, either congenital or acquired in the first month after birth. In the acquired group necrotizing enterocolitis (NEC) is generally recognized as the most important cause of intestinal crisis with significant mortality and long lasting morbidity. Other acquired disorders are volvulus and milk curd syndrome. Examples of congenital gastrointestinal disorders are gastroschisis, intestinal atresia, omphalocele, and meconium peritonitis (1-3). NEC is the main subject of this study.

CASE

Girl, born at gestational age of 24 5/7 weeks with a birth weight of 685 grams; Apgar scores were 5, 7, and 9 after 1, 5 and 10 minutes. She was admitted to the neonatal intensive care unit and ventilated due to respiratory distress. Shortly after birth she has a few episodes of hypotension, requiring resuscitation with iv fluids and dopamine. After 3 days she could be extubated and enteral feeding was introduced and could be advanced. On the fourth day, she had multiple incidents of apnea and bradycardia, which required re-intubation. Echocardiography demonstrated a large patent ductus arteriosus, and an abdominal film, indicated because she was vomiting and had a distended abdomen, demonstrated intestinal pneumatosis. Necrotizing enterocolitis is diagnosed (Bell stage II) and enteral feeding is stopped. Symptomatic therapy consisted of insertion of a nasogastric tube, and the initiation of antibiotics. Due to a deteriorating clinical condition, a laparotomy was performed on the seventh day after birth.

Pathogenesis

NEC is the most common gastrointestinal disorder affecting mainly premature neonates (90% of the cases). Among infants with a low birth weight (500-1500 grams) the mean prevalence of NEC is 7% (4, 5). The pathogenesis remains poorly understood, a recent review even was entitled: Necrotizing enterocolitis - 150 years of fruitless search for the cause (6). Intestinal immaturity (e.g. circulatory regulation, barrier function, innate immunity, motility, and digestion), genetic predisposition, feeding with formula milk, abnormal bacterial colonization, and hypoxic-ischaemic injury have all been suggested, alone or in combination, to play a role in the pathogenesis of NEC. An estimated 20-40% of babies with NEC require surgical intervention, and the associated case fatality rate still approaches 50% (4, 5).

Circulatory disturbances have been linked to the pathogenesis of NEC since the mid 1900s (6-12). In the New York Babies Hospital, 64 cases of necrotizing enterocolitis were

observed during the period from 1954 to 1974. Santulli postulated the hypothesis of mesenteric hypoperfusion as follows: 'Indirect injury to the mucosa may result from selective circulatory ischaemia. This is the most acceptable theory of pathogenesis. It is supported by our clinical and pathological data.' (8).

In 1969, Lloyd et al. proposed the diving reflex theory (also known as the 'master switch of life') as a causative factor for the pathogenesis of NEC. In this study he observed that 80% of infants with gastrointestinal perforations experienced a significant episode of perinatal asphyxia or shock. The diving reflex theory is based on the assumption that extrinsic neurogenic redistribution of cardiac output occurs to preserve blood flow to the brain, heart, and kidneys at the expense of blood flow to the splanchnic organs (10, 13). However, the diving reflex theory was abandoned for the following reasons: first of all, NEC rarely occurs in the first week postnatally. Therefore perinatal asphyxia could not be responsible for the histological changes observed in NEC. Secondly, repeated case-control studies demonstrated that infants with NEC rarely suffered from perinatal asphyxia (14-16). The third argument that pleads against the diving reflex theory is that adrenergic stimulation (the basis for the diving reflex) does not cause flow reduction or tissue hypoxia (16-18).

Currently, the exact role of the circulation in the pathogenesis of NEC remains to be determined. We can conclude that hypoxia-ischaemia is probably not the sole explanation for the pathogenesis of NEC (18, 19). However, whether it plays a role in combination with other factors in the initiation of NEC or whether it is a secondary effect, has yet to be determined. It is very likely that prior to tissue destruction, ischaemia occurs given the histology findings in the intestines of patients with NEC, which generally demonstrate ischemic features (17). Currently, intestinal barrier dysfunction is suggested to play an important role in the pathogenesis of NEC, especially NO-mediated (nitrix oxide) intestinal barrier failure. Release of inflammatory mediators leads to overproduction of NO, which reacts with superoxide to produce peroxynitrite ($ONOO^-$) in the intestinal epithelium. This induces enterocyte apoptosis (programmed cell death) and causes inhibition of tissue repair mechanisms (enterocyte proliferation and migration). Subsequently, a vicious cycle follows with further tissue destruction and eventually intestinal perforation (20, 21).

Another line of research has focused on the susceptibility of the premature infant to NEC. It has been previously shown that immature intestinal cells have an exaggerated inflammatory response to pathogenic stimuli (22). This might be due to an increased or an abnormal toll-like receptor (TLR) signaling. At least in healthy newborn rats, TLR4 gene expression is shown to decrease after birth, however when the rats were stressed by a hypoxic condition or when they were formula fed, their TLR4 expression was increased. TLR4 causes activation of NF-κB which initiates synthesis of pro-inflammatory cytokines (23-25). This could be attenuated by heat shock treatment since this inhibited

NF-κB activation through increased heat shock protein 70 expression in a rodent model of NEC (26).

Pre-operative evaluation

Diagnosing NEC is a challenging problem. At present, there is no definitive diagnostic instrument, either laboratory and/or radiological, available to accurately diagnose NEC and to follow its course, unless NEC is in its end stage. Diagnosis is mainly based on clinical findings, supported by laboratory measurements (such as a decrease in platelet count and increase in lactate levels), and imaging techniques such as abdominal films, ultrasound and/or explorative laparoscopy/laparotomy (5, 27). Recent studies have mainly focused on biomarkers to evaluate infants at risk for NEC or to evaluate the severity of NEC (28). One of the most promising biomarkers is intestinal fatty acid binding protein (I-FABP) since this can be determined in urine. Urinary I-FABP has been shown to predict disease severity in 14 infants with NEC (29). Although these are promising results, we are still waiting for larger validation studies.

For decades the radiological evaluation of plain abdominal X-rays is considered as a cornerstone diagnostic procedure to diagnose NEC. The most used staging system in NEC is the modified Bell staging criteria. These consist of 3 stages and are based on a combination of systemic, abdominal and radiographic signs (Table 1) (30).

Supportive non-operative care

Once NEC is suspected, the medical management is aimed at bowel rest, which includes: discontinuation of enteral feeds, nasogastric tube insertion for decompression, parenteral nutrition, and broad-spectrum antibiotics. This approach is continued until the patient improves clinically. In case the patient clinically deteriorates surgical treatment should be considered. The only absolute indication for a surgical intervention is intraperitoneal free air as seen on abdominal films (which is indicative for a bowel perforation) (31). Relative indications are clinical deterioration despite optimal medical management (also expressed in worsening of biochemical parameters), increasing pneumatosis with portal venous gas, or an abdominal mass with persistent intestinal obstruction or sepsis (4, 31-34).

Table 1 Modified Bell staging criteria

Stage	Classification of NEC	Systemic signs	Abdominal signs	Radiographic signs
I A	Susptected NEC	Temperature instability, apnea, bradycardia, lethargy	Gastric retention, abdominal distention, emesis, hemepositive stool	Normal of intestinal dilation, mild ileus
I B	Suspected NEC	Same as I A	Same as I A but with grossly bloody stool	Same as I A
II A	Definite NEC, mildly ill	Same as I A	Same as I B plus absent bowel sounds with or without abdominal tenderness	Intestinal dilation, ileus, pneumatosis intestinalis
II B	Definite NEC, moderately ill	Same as I A plus metabolic acidosis and trombocytopenia	Same as I B plus absent bowel sounds, definite abdominal tenderness with or without abdominal cellulitis or right lower quadrant mass	Same as II A plus ascites
III A	Advanced NEC, severely ill, intact bowel	Same as II B plus hypotension, severe apnea, combined respiratory and metabolic acidosis, DIC, and neutropenia	Same as II B plus signs of peritonitis, marked tenderness, and abdominal distention	Same as II B
III B	Advanced NEC, severely ill, perforated bowel	Same as III A	Same as III A	Same as II B plus pneumoperitoneum

DIC: disseminated intravascular coagulation

Adapted from: Neu J. Necrotizing enterocolitis: the search for a unifying pathogenic theory leading to prevention. Pediatr Clin North Am 1996;43:409-432.

CASE CONTINUED

At laparotomy, 7 cm of necrotic jejunum was found 15 cm after the ligament of Treitz. The necrotic tissue was resected and a double-barrel jejunostomy was created. This procedure had to occur very fast, since the girl was hypoxic multiple times due to her pulmonary hypertension based on a patent ductus arteriosus.

Intra-operative considerations

The two most used techniques for surgical intervention are peritoneal drainage and laparotomy. Two multicenter studies comparing these treatments showed that there were no significant differences in outcomes, but the infants treated with peritoneal drainage often required subsequent laparotomy (35, 36). A summarizing systematic

review showed that mortality was increased by more than 50% with peritoneal drainage (37). In our institution, the use of peritoneal drainage has been abandoned and primary laparotomy is still the surgical technique of choice.

At laparotomy, the different options during surgery include: resection with a primary anastomosis, resection with ostomy formation, no resection but a proximal diverting jejunostomy (in extensive NEC), or 'clip and drop' (technique in which non-viable bowel is resected, the remaining parts of the bowel are clipped, and a relaparotomy follows within 48-72 hours) (32, 38). The extent of the disease, the patient's weight, and the patient's clinical status influence the decision of which surgical intervention is chosen. There is still considerable debate whether primary anastomosis or ostomy is the most preferable surgical option. An ostomy is generally considered as the most safe approach since healing of the anastomosis might be suboptimal in the presence of peritonitis, inflamed bowel ends, and reduced intestinal blood supply (32, 39). In addition, a primary anastomosis is associated with high rates of breakdown, sepsis, and stricture formation (40). An ostomy has the disadvantage of significant morbidity such as prolapse, excoriation of the surrounding skin, and stenosis. Also, achieving adequate enteral feeds can be difficult and an ostomy with a high output carries the risk of electrolyte imbalances and dehydration (32, 41).

CASE CONTINUED

Postoperatively, enteral feeding was not well tolerated and due to an open abdominal wound, stools from the ostomy leaked into the wound. After 2 months the ostomy was reverted, however, three weeks postoperatively there were signs of abdominal distension, feeding intolerance and infection again. An X-ray demonstrated pneumatosis. Another laparotomy was performed showing a perforated Meckel's diverticulum and this was resected with primary anastomosis. Two weeks postoperatively, she had a distended abdomen and blood in the stool. No definitive cause was found and after a short episode of withholding enteral feeding and antibiotics, she quickly recovered. Due to the longterm need for parenteral nutrition, she had TPN associated cholestatic jaundice, which fortunately completely resolved with only medical management.

Currently, she is at home and has recovered well. Her weight is 2SD below her growth curve, and she has hypotonia due to prematurity.

Post-operative care

Unfortunately, complications occur in 15 to 68 percent of cases after ostomy formation. Ostomy related complications are stricture formation, parastomal hernia, prolapse,

wound infection, wound fistula, wound dehiscence, and small bowel obstruction (42-45). Especially premature infants are at high risk for ostomy related complications and more specifically patients with NEC as they have a lower gestational age and birth weight (43).

Following ostomy formation, surgeons tend to postpone ostomy closure for at least 8 weeks or until the infant weighs two kilograms because of the risk of postoperative abdominal adhesions and the supposed morbidity associated with anesthesia and ventilation anticipated in case of earlier closure (46-48). The timing of ostomy closure is very variable, mainly based on the surgeons' preference or local protocols and currently without any evidence based guidelines. Early closure might not only avoid ostomy related complications but it could also be favorable since having an ostomy can be associated with diarrhea, severe fluid and electrolyte losses, and growth retardation (41). Moreover, ostomy closure during the same hospital admission is also favorable for parents and caregivers since caring for an ostomy might prolong the hospital admission and is sometimes not easy for parents at home.

Once the neonates have survived an episode of NEC, intestinal adaptation is of major importance. Factors that are of influence are the length and the function of the remaining intestine, where the absolute length of the remaining intestine is less important than the percentage of remaining intestinal length (49-51). Results regarding the importance of the ileocecal valve are mixed: some studies show an improved outcome with a retained ileocecal valve, whereas others fail to show any differences (31). Few studies have focused on the role of the colon in adaptation. They have shown that adaptation times were shorter with an intact colon (52, 53).

Short bowel syndrome and subsequent intestinal failure is a major problem following surgery for NEC. Approximately 25% of survivors after surgery for NEC are diagnosed subsequently with short bowel syndrome and intestinal failure (54, 55). During surgery for NEC, it is therefore essential to measure the length of the remaining bowel and the length of the resected bowel. These data can aid in determining the prognosis after surgery. Once the length of the remaining bowel is too short compared to age-related standards or when the function of the remaining bowel is inadequate to achieve full enteral feeding, infants are dependent on parenteral nutrition for a certain period. There is still a need to optimize the current composition of parenteral nutrition (56-58). Currently, different trials are investigating the lipid emulsions in parenteral nutrition such as SMOF lipid and Omegaven (59, 60). Especially omega-3 fatty acids are focus of study since they are safe and effective in the treatment of parenteral nutrition associated liver disease, but at high costs (61). In addition, the administration of parenteral nutrition is associated with different complications such as catheter-related central line problems, such as sepsis and vascular thrombosis, and (often progressive) parenteral nutrition-associated liver disease (62, 63). Once life-threatening complications start to occur despite optimal medical and surgical treatment, intestinal transplantation should be

considered. It is important to refer early and be listed on the waiting list for transplantation early in order to achieve long-term survival after transplantation. Currently, around 115 intestinal transplantations are performed each year worldwide (64).

Aims and outline of this thesis

The various above described aspects related to NEC inspired the research presented in this thesis. Although the circulation is not considered to be the primary determinant in the pathogenesis of NEC, it might still play an important role based on the coagulation necrosis that is observed in the intestines during surgery. Therefore, we evaluated the microcirculation in the intestines of infants with NEC using a new non-invasive biomarker: sidestream darkfield imaging (SDF), pre- and intra-operatively. Our research in the postoperative period focused on the optimal way of recovery, mainly addressing the optimal timing of ostomy closure since this is currently based on institutional preferences and literature addressing this topic is currently lacking. In addition, a new amino acid solution in infants after surgery for congenital gastrointestinal disorders was evaluated. Eventually, when all treatment options for intestinal failure have been tried, intestinal transplantation is the only option. Therefore, we evaluated the referral criteria for intestinal transplantation.

This thesis consists of three parts.

Part I will focus on the preoperative and intraoperative evaluation, with special attention for the role of the circulation in the pathogenesis of NEC.

Chapter 2 evaluated the microcirculation of infants with NEC and other gastrointestinal pathology using sidestream darkfield imaging. In **chapter 3** normal values of intestinal length were established for premature infants up to children of 5 years of age.

Part II focuses on postoperative considerations, mainly addressing the optimal timing of ostomy closure.

In **chapter 4**, a randomized controlled trial was performed to evaluate a new amino acid solution for infants after major gastrointestinal surgery. In **chapter 5**, a systematic review of available literature was performed to determine the optimal timing of ostomy closure in infants with NEC. In **chapter 6** we retrospectively evaluated whether early ostomy closure was equal in respect to adhesion formation at the time of ostomy closure. Also, the costs of early versus late ostomy closure in infants with NEC were compared. In **chapter 7** we demonstrated that the colon does not play a role in intestinal adaptation in infants with short bowel syndrome. The referral criteria for intestinal transplantation were evaluated in **chapter 8**.

Part III summarizes the results of these studies, puts them in perspective and speculates on further areas of current and future research topics (**chapters 9 and 10**).

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PART II

PRE- AND INTRA-OPERATIVE EVALUATION

We must believe that we
are gifted for something, and
that this thing, at whatever cost,
must be attained

Marie Curie

Chapter 2

Microcirculatory evaluation of the surgical newborn: a new biomarker?

Marie-Chantal Struijs
Erik AB Buijs
John Vlot
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Richard Keijzer
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Submitted

ABSTRACT

Background: Vascular accidents are generally considered to play a role in the pathogenesis of necrotizing enterocolitis (NEC) and major gastrointestinal disorders such as intestinal atresia/gastroschisis. Using a new non-invasive technique, sidestream darkfield imaging (SDF), the microcirculation in neonates with NEC and the mesenteric circulation during laparotomy in neonates with NEC and intestinal atresia/gastroschisis was evaluated.

Methods: This prospective study was subdivided in 2 parts: pre-operative and intra-operative measurements. Pre-operative measurements were performed in the armpit of neonates with NEC. Intra-operative measurements were performed on the mesenteric border of the intestines at standardized places (necrotic and non-affected intestinal tissue). SDF images were analyzed in an automated vascular analysis program (vessel density and blood flow); statistical analysis was performed in SPSS 17. A *P* value <0.01 was considered statistically significant.

Results: 32 patients were included; 15 NEC patients (6 also measured pre-operatively), and 17 patients with gastroschisis (n=8), atresia (n=4), and other (n=5). In the NEC group, pre-operative vessel density and blood flow did not decrease in the days before surgery. During surgery, no significant differences were found in the vessel density and blood flow of affected (necrotic) and non-affected tissue. Vessel density was lower, although not significant, in the non-affected intestinal tissue of the NEC group (4.4 mm/mm^2) compared with the gastrointestinal disorders group (7.8 mm/mm^2). In gastroschisis no significant differences were found. Atresia patients had a decreased vessel density and blood flow in the atretic part compared with the non-affected tissue.

Conclusions: SDF cannot be used to predict which neonates will need surgery for NEC. We did find a difference in the mesenteric perfusion of non-affected intestinal tissue during surgery as a potential new biomarker to determine how much intestinal tissue needs to be resected at laparotomy.

INTRODUCTION

Necrotizing enterocolitis (NEC) is the most common and sometimes devastating neonatal gastrointestinal disorder. It mainly affects premature infants (90% of the cases) and the mean prevalence in this population is 3-7%. Surgery is warranted in 20-40% of neonates and the associated case fatality rate with surgical intervention is 50% (1, 2). The pathogenesis remains poorly understood (3). A combination of intestinal immaturity (e.g. circulatory regulation, barrier function, innate immunity, motility, and digestion), genetic predisposition, feeding with formula milk, abnormal bacterial colonization, and hypoxic-ischaemic injury have been suggested to play a role in the pathogenesis of NEC (1, 2).

Previous research has already attempted to identify the exact role of the hypoxic-ischemic insult and whether it plays a primary or secondary role in the pathogenesis of NEC (4-7). Hypoxia-ischaemia as sole cause in the pathogenesis is probably not true (8, 9). But whether hypoxia-ischemia plays a role in combination with other factors in the initiation of NEC or whether it represents a secondary effect, remains unclear. It is however very likely that before tissue destruction is visible, ischaemia occurs (6).

Recent studies have mainly focused on biomarkers to evaluate infants at risk for NEC or to evaluate the severity of NEC (10). One of the most promising biomarkers is intestinal fatty acid binding protein (I-FABP) since this can be determined in urine (11). It would be ideal to have a biomarker to examine the intestinal ischemia hypothesis in the pathogenesis of NEC. Recently, sidestream darkfield imaging (SDF) has been used to evaluate the microcirculation in critically ill newborns with sepsis and extracorporeal membrane oxygenation (12, 13). SDF is a noninvasive method which assesses the microcirculation using concentrically green light emitting diodes (LEDs) surrounding a central light guide to provide sidestream darkfield illumination. The light is scattered in the tissue and is reflected by hemoglobin (14-17).

In this study, we determined the microcirculatory profile in neonates with (suspected) NEC using SDF and compared this with healthy age-matched control neonates. We extended our observations intra-operatively, comparing neonates with NEC undergoing surgical resection with other neonates undergoing laparotomy for intestinal atresia or gastroschisis: congenital gastrointestinal disorders with a proven vascular insufficiency origin.

METHODS

Study design

A single centre, prospective study was conducted at the neonatal and pediatric intensive care units of a tertiary university children's hospital. It was subdivided in two parts: pre-operative and intra-operative measurements; and three groups of participants were identified (Figure 1). Group 1 (NEC) consisted of all neonates with (suspected) NEC, either receiving medical and/or surgical treatment. Suspected NEC was defined according to the modified Bell's criteria as abdominal distension, abnormal stool consistency, blood in stool, systemic symptoms (temperature instability, apnea, bradycardia) and/or intestinal pneumatosis on abdominal films (18). Neonates also admitted to the neonatal intensive care unit but without suspected NEC were included in group 2 (controls). Group 3 (surgical controls) consisted of all neonates undergoing laparotomy either for a congenital gastrointestinal disorder such as intestinal atresia/ gastroschisis requiring surgery. Neonates were excluded when they had a severe cardiac anomaly (requiring corrective surgery within 60 days), severe chromosomal abnormalities, severe respiratory anomalies such as diaphragmatic hernia, and/or severe anomalies of the central nervous system.

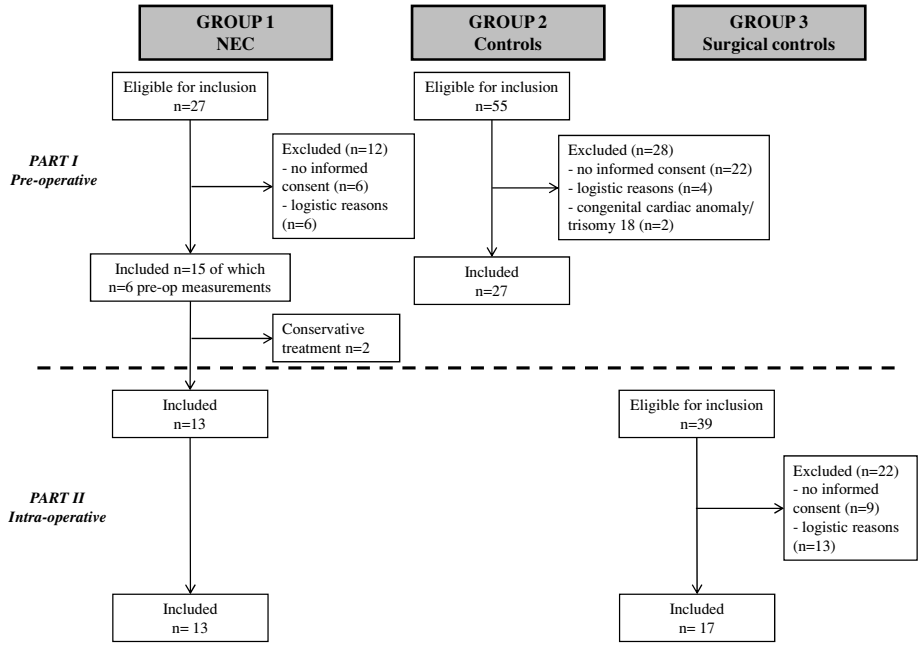

Figure 1 Flow chart

Pre-operative measurements were performed in group 1 and 2 for 7 consecutive days if possible. Neonates from group 1 (the ones that required surgery) and group 3 were measured intra-operatively.

The study protocol was approved by the institutional review board. Informed consent was obtained from the parents before start of the study and in case the participant required surgery for NEC, informed consent was obtained before the intra-operative measurements.

Study procedures

During the pre-operative part measurements were performed daily (up to maximum of 7 days) using the SDF device (Microscan BV, Microvision Medical, Amsterdam, The Netherlands).

The SDF was applied to different regions during the study period. In premature infants below 1500 grams the skin in the armpit was used as advocated by Genzel et al. (19). Before the measurements, saline was used to lubricate the skin to obtain optimal images. In all other neonates the buccal membrane was the site of choice for the measurements as published before by us (20). The recording was done during 2 to 5 minutes.

Intra-operatively measurements were performed by the operating surgeon on the mesenteric border. If possible, the following predetermined sites were measured for NEC patients: greater curvature of the stomach, macroscopic prenecrosis area (non-affected area) and necrotic area. For all other patients, the greater curvature of the stomach was always measured and then different sites were chosen according to the underlying disease process. For example in patients with intestinal atresia the distended bowel and atretic part were measured. Data were recorded on a Sony DSR-20P digital video recorder (Digital HD Videocassette recorder GV-HD700E, Sony Corporation, Tokyo, Japan).

Data collection

For all patients demographic data such as gender, gestational age, birth weight, Apgar scores, and maternal pre-eclampsia/HELLP were collected. For each study day, clinical characteristics were collected, such as blood pressure, heart rate, physical examination (abdominal distension etc), laboratory values, medical therapy, and nutritional data. All data are derived from our computerized patient data management system (PDMS).

For the SDF measurements, analysis was performed in AVA (Automated Vascular Analysis 3.0, Dept. of Medical Technological Development, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands). If possible, 3 fragments of each 10 seconds were selected for analysis. All fragments were randomized and all analyses in AVA were performed by the first author. Small vessels were defined as 10 µm and non-small vessels as 10-50 µm. For all fragments total vessel density (TVD), perfused

vessel density (PVD), microvascular flow index (MFI), and proportion of perfused vessels (PPV) were calculated (21). The results from all these measurements were averaged per day and used for statistical analysis.

Statistical analysis

Data were analyzed using SPSS (version 17; SPSS, Chicago, IL). In view of multiple tests performed a *P* value of < 0.01 was considered statistically significant instead of the conventional *P* value of < 0.05. Demographic data were described using descriptive statistics. Linear mixed model analysis was used for the analysis in part I, comparison of neonates with NEC (group 1) versus neonates without NEC (group 2). Whether mean blood pressure, saturation, temperature of the patient, pO_2, haemoglobin count, haematocrit count, and administration of indocid was of influence on the SDF measurements was also evaluated with mixed model analysis. Individual NEC patients were evaluated with one-way ANOVA. The last SDF values obtained before surgery were compared with the intra-operative values using Pearson and Spearman correlation analysis. Part II was analyzed using the paired (analysis within the group) and unpaired t-test (comparison of group 1 versus group 3).

RESULTS

Study Sample

Between November 2009 and April 2011, in total 121 patients were screened for eligibility of which 59 patients were included, subdivided in group 1 NEC (15 patients), group 2 controls (27 patients), and group 3 surgical controls (17 patients; gastroschisis (n=8), atresia (n=4), and other (n=5)) (Figure 1).

There was a non significant male predominance in the surgical controls group (group 3), (Table 1). Also, the gestational age at birth and birth weight was significantly (respectively *p*=<.01 and *p*=<.01) higher in this group. In the NEC group (group 1), medical therapy was not sufficient in 13 out of 15 patients (87%), therefore surgery was required and intra-operative measurements were performed in these 13 patients. Seven patients died in the NEC group (group 1) due to severe, extensive NEC and concomitant cardiorespiratory failure (47%), this was significantly more than in the other two groups (*p*=<.01). In the surgical controls group (group 3), 2 patients died (12%). One patient died due to multi-organ failure related to blow out of the stomach as a consequence of milk curd, and the other patient died due to cardiorespiratory failure during a septic episode.

Table 1 Demographic characteristics

	Group 1 NEC	Group 2 Controls	Group 3 Surgical controls[#]	*P*
N	15	27	17	
Male gender	5 (33%)	10 (37%)	11 (65%)	.17[*]
Gestational age at birth (weeks)	28.9 (3.7)	27.1 (1.7)	35.3 (3.9)	<.01[†]
Birth weight (grams)	1164 (404)	908 (163)	2395 (802)	<.01[†]
SGA	2 (13%)	9 (33%)	5 (30%)	.45[*]
Apgar 1 min	5.9 (2.3)	5.78 (2.3)	7.7 (2.1)	<.01[†]
Apgar 5 min	7.6 (1.8)	7.6 (1.6)	8.7 (1.7)	.03[†]
Maternal PE/HELLP	4 (29%)	2 (8%)	1 (8%)	.19[*]
Patent ductus arteriosus	5 (33%)	13 (48%)	1 (6%)	.01[*]
Age at start study (days)	10 [4,50]	12 [3,40]	0 [0,27]	<.01[†]
Gestational age at start study (weeks)	31 (3.7)	29.3 (1.6)	36 (3.6)	<.01[†]
Surgery	13 (87%)	0 (0%)	17 (100%)	NA
Death	7 (47%)	0 (0%)	2 (12%)	<.01[*]

Data are represented as n (%), mean (SD) or median [range], NA not applicable. [*]Fisher's exact, [†]Kruskal-Wallis

SGA indicates small for gestational age; PE, pre-eclampsia; HELLP, hemolysis, elevated liver enzymes and low platelets

gastroschisis n=8 (47%), atresia n=4 (24%), other n=5 (29%)

Pre-operative part

Analysis in AVA was sometimes challenging due to presence of hairs or due to the skin color (Figure 2). Images of infants with pigmented skin were very difficult to analyze. No statistically significant differences were found in SDF measurements between the NEC group and the control group regarding pre-operative measurements divided per day. Also the MFI values were comparable for both groups in small and non-small vessels (respectively p=.94 and p=.20; Table 2).

No statistically significant influences from the mean blood pressure, saturation, temperature of the patient, pO_2, haemoglobin count, haematocrit count, and administration of indocid were found on SDF values.

Analysis of individual NEC cases to determine whether vessel density or blood flow is decreased when comparing the first measurement with the last measurement, did not yield uniform results. In total 4 cases could be evaluated of which 2 (50%) underwent surgery. In most cases the vessel density at the last measurement was decreased.

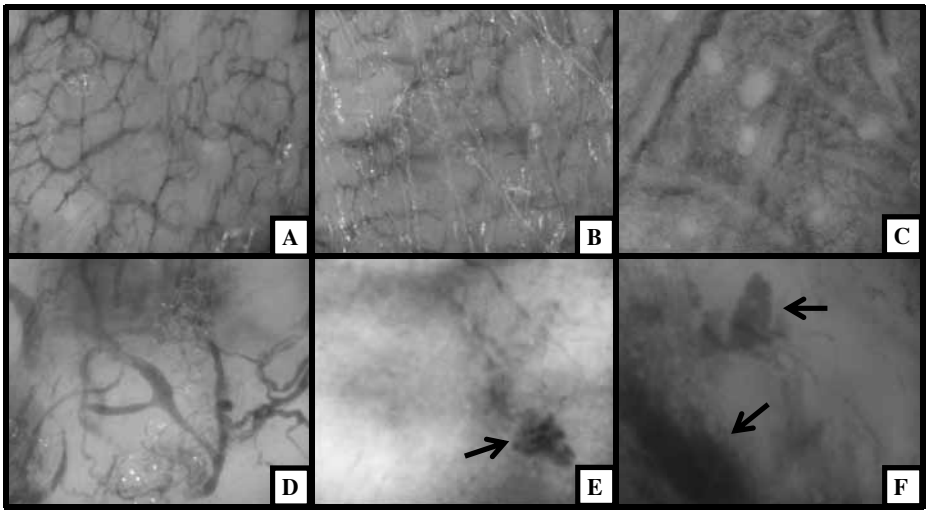

Figure 2 SDF images of pre- and intra-operative measurements
Images obtained with SDF. A. Pre-operative measurement in the armpit of NEC patient. B. Pre-operative measurement of infant with skin and hairs in the image. C. Pre-operative measurement of an infant with a dark coloured skin. D. Non-affected intestinal tissue of an infant with NEC. E. Necrotic intestinal tissue of an infant with NEC. F. Intestinal tissue of an infant with atresia. The arrows show vascular accidents in the mesenteric circulation.

Table 2 Pre-operative SDF measurements

	Group 1 NEC	Group 2 Controls	*P*
N	6	27	
TVD			
Small vessels	8.4 (4.8-13.5)	8.5 (2.4-14.8)	.89
Non-small vessels	11.7 (6.6-17.1)	11.3 (6-20.8)	.83
PVD			
Small vessels	15.4 (10.4-20.2)	15.1 (3.5-21.2)	.79
Non-small vessels	4.5 (2-7.3)	4.1 (1.6-9.4)	.57
MFI			
Small vessels	2.9 (2.25-3)	2.9 (2.25-3)	.94
Non-small vessels	3.0 (2.9-3.0)	3.0 (2.3-3.0)	.20
PPV			
Small vessels	39 (21.3-60.1)	39.6 (8.9-69.1)	.93
Non-small vessels	60 (40-71.7)	56.6 (4.4-82)	.42

Data are represented as mean (range)
TVD, total vessel density (mm/mm^2); PVD, perfused vessel density (mm/mm^2); MFI, microvascular flow index; PPV, proportion perfused vessels (%)

Table 3 Intra-operative SDF measurements

	Group 1 NEC			Group 3 Surgical controls		
	Non-affected intestinal tissue	Necrotic intestinal tissue	*P*	Non-affected intestinal tissue	Affected intestinal tissue	*P*
N	7	7		3	3	
TVD						
Small vessels	4.4 (1.3)[a]	5.7 (2.2)[a]	.23	7.8 (2.5)	3.7 (2.8)	.08
Non-small vessels	10.1 (2.5)[a]	8.1 (3.1)[a]	.43	9.4 (2.6)	10.1 (3.5)	.50
PVD						
Small vessels	-	-		5.2 (8.9)	0 (0)	.42
Non-small vessels	-	-		1.2 (2.1)	0 (0)	.42
MFI						
Small vessels	.21 (0.4)	0 (0)	.20	1.2 (1.6)[a]	0.25 (.4)[a]	.22
Non-small vessels	.21 (0.4)	0 (0)	.20	1.2 (1.6)[a]	.67 (0.7)[a]	.50
PPV						
Small vessels	-	-		14.6 (25.3)	0 (0)	.42
Non-small vessels	-	-		18.7 (32.5)	0 (0)	.42

Data are represented as mean (SD); [a] n=5; p-values from paired t-test
TVD, total vessel density (mm/mm^2); PVD, perfused vessel density (mm/mm^2); MFI, microvascular flow index; PPV, proportion perfused vessels (%)

Intra-operative part

Although intra-operative measurements were performed in 13 patients, only 7 patients in the NEC group and 3 patients in the surgical controls group could be fully analyzed. For the other patients formal analysis in AVA could not be performed due to poor image quality. Figure 2 (bottom part) shows three images of the SDF measurements. Patients with gastroschisis were very difficult to measure since the stomach and intestines had significant intestinal peel. Atresia patients showed vascular accidents on the intra-operative SDF measurements (Figure 2, image F).

No statistical significant differences were found when non-affected intestinal tissue was compared with necrotic intestinal tissue in the NEC group. Same results were found for the surgical controls group (Table 3, paired t-test). When comparing the NEC group with the surgical controls group, also no statistically significant differences were found in the SDF measurements (unpaired t-test).

DISCUSSION

In this prospective study no significant differences were found in vessel density and blood flow in a group of patients with suspected NEC compared with a group of patients without NEC. Intra-operative measurements in the NEC and surgical controls group showed no blood flow in necrotic intestinal tissue. However, non-affected intestinal tissue also had a decreased blood flow. In patients with NEC, vessel density was equal in necrotic intestinal tissue compared with non-affected intestinal tissue. Although not statistically significantly different, vessel density of the small vessels was higher in the surgical controls group.

At present, there is no definitive diagnostic instrument, either laboratory and/or radiological, available to accurately diagnose NEC and to follow its course, unless NEC is in its end stage. Therefore new techniques, such as SDF are proposed to gain more knowledge about the pathogenesis of NEC and to earlier diagnose infants with suspected NEC. It would be ideal to have a set of biomarkers that could predict which infants develop NEC or to evaluate which infants with NEC will eventually require surgery.

Vessel density, blood flow, and the proportion of perfused vessels did not differ between infants with suspected NEC and controls. When analyzing individual cases of infants with NEC, no parameter could be determined that could be predictive of which infants will eventually require surgery. It should be noted however that the number of individual cases that could be analyzed was relatively low. Further studies are needed to evaluate SDF as biomarker to predict which infants will need a laparotomy.

Vessel density and blood flow on the mesenteric border of the intestines (operative measurements) were not significantly lower in necrotic intestinal tissue compared with non-affected intestinal tissue. We did find a difference, although not significant, in the vessel density of non-affected intestinal tissue between NEC patients and surgical controls (4.4 vs 7.8 mm/mm^2). This difference might explain the fact that the intestinal tissue of NEC patients is more prone to develop intestinal necrosis due to less dense vessel structure. However, the blood flow in non-affected intestinal tissue was low; we expected this blood flow to be adequate. This could mean that the intestinal tissue surrounding the necrotic area is also affected, but still not necrotic yet and therefore no resection would be necessary yet (since it was not resected). Top et al. have found different results using OPS imaging (orthogonal polarization spectral imaging, precursor of SDF). They found a decreased functional capillary density of 0.4 cm/cm^2 on the mesenterium of necrotic bowel compared to a functional capillary density of 2.7 cm/cm^2 on mesenterium of vital bowel (22). In contrary, we found a similar vessel density between necrotic and non-affected bowel. Unfortunately, no other results in infants with NEC are available, but neonates with proven infection have been studied. OPS imaging demonstrated that functional small vessel density decreased 1 day before

changes in laboratory parameters occurred (23). Another finding in our study was that vessel density and blood flow was not correlated with blood pressure, temperature, and heart rate. These findings have also been found by Kroth et al. They also found that the microvascular parameters were not dependent on gestational age or postnatal age (19).

Another technique that could be used to evaluate the microcirculation in the abdomen is near infrared spectroscopy (NIRS). Fortune et al. evaluated the cerebro-splanchnic oxygenation ratio (CSOR) in 39 neonates of which a group of 10 patients had abdominal problems (5 patients with NEC). This group had a significantly lower median CSOR of 0.66 versus 0.96 in the control group (24). These data were confirmed by a study of Cortez et al., who showed that NEC patients had persistently low splanchnic oxygen saturation with loss of variability; this was preceded or followed by very high splanchnic oxygen saturations (25). The susceptibility of the intestinal tissue to episodes of apnea and bradycardia was studied by Petros et al. The recovery of the abdominal NIRS measurements after episodes of apnoea and bradycardia lasted twice as long as recovery of the peripheral saturation. These episodes, if frequent, could lead to chronic ischemia in infants who are susceptible to periods of low oxygenation such as preterm infants (26).

A new technique that recently has been introduced is visible light spectroscopy (T-stat); it measures microvascular haemoglobin oxygen saturation ($SgvO_2$). It uses shallow-penetrating visible light and the difference with NIRS is that it measures small, subsurface tissue volumes, whereas NIRS measures larger, deeper volumes of tissue (27-29). Few data are available using this new technique in intestinal mucosa. The $SgvO_2$ of intestinal mucosa was decreased in adult patients when hypoxia and ischemia was induced (29). Another study evaluated 3 adult patients with chronic mesenteric ischaemia. Mucosal saturations in these patients in ischaemic areas in the duodenum and proximal jejunum varied between 16% to 30%. In control patients these values were between 60% to 73% (30). The only available study in neonates was done during open heart surgery with hypothermic cardiopulmonary bypass. A visible light spectroscopy probe was placed in the esophagus as well as NIRS probes. In patients requiring antegrade cerebral perfusion, the cerebral oxygenation was maintained but the esophagus was not adequately perfused (31). Unfortunately, no results are available in patients with NEC, this would be an interesting subject for further studies.

During the study we were faced with a number of difficulties and limitations. The intra-operative measurements were difficult due to the condition of the patient. Measurements had to be done relatively fast. Also, the measurements are technically very challenging. Due to these reasons, unfortunately a limited number of intra-operative measurements could be used for analysis in AVA. Currently, analysis in AVA does not offer the possibility to note whether the continuous flow is slow, medium or fast. Unfortunately, the infants in the surgical controls group were significantly older than the NEC group. This is unavoidable due to the low number of infants below 32 weeks who

undergo surgery for another reason than NEC. A surprising finding in this study was the difficulties in analyzing images of infants with pigmented skin; this has never been described in previous literature.

SDF cannot be used to predict which neonates will need surgery for NEC. We did find a difference in the mesenteric perfusion of non-affected intestinal tissue during surgery as a potential new biomarker to determine how much intestinal tissue needs to be resected at laparotomy.

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Chapter 3

Establishing norms for intestinal length in children

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J Pediatr Surg 2009;44:933-938

ABSTRACT

Background: Existing data on pediatric intestinal length (IL) are limited because most studies report post-mortem values. Using prospective data, appropriate norms for IL were developed.

Methods: IL measurements, using a silk suture on the antimesenteric border, were prospectively made on patients between 24 weeks gestational age (GA) and 5 years of age undergoing laparotomy. Patients with gastrointestinal malformations or those above or below 2 standard deviations for growth parameters were excluded. A curve fitting process was applied to determine the best model for IL (small bowel and colon separately) from amongst, post-conception age, weight, and height at surgery.

Results: 108 patients participated in this study. Highly predictive ($R^2 > 0.8$) models for IL were determined for all predictor variables (post-conception age, weight and height) examined suggesting that all of these variables are excellent predictors determinants of IL. Although all models had statistically similar properties, the model using height had the best performance across the full range of the variable.

Conclusion: Although neither age, weight, nor height were definitely superior for the prediction of IL, we propose that until external validations of our models occur, height at surgery be used for the prediction of expected small intestinal and colon length in infants.

INTRODUCTION

Accurate quantification of the degree of intestinal loss is critical when assessing a child with Short Bowel Syndrome (SBS). Although not the only factor, intestinal length is a critical predictor of outcome in SBS (1, 2). One of the challenges in quantifying intestinal length in infants relates to developmental changes, with the added caveat that gestational age may be an important confounder in terms of expected intestinal length for any given body size. We have previously adopted the position that intestinal length following loss of intestine is best expressed as a percentage of predicted for gestational age rather than the absolute length in centimeters (3). However, existing data on gestational age appropriate intestinal lengths are limited by the fact that most studies report post-mortem measurements (4-12). We sought to develop norms for bowel length on the basis of prospectively collected data over preterm and early childhood. Although we previously have stated that predicted length should be stated based on gestational age appropriate norms, we also sought to examine whether expected length could be determined from body weight or height at surgery, or a combination of age, weight or height.

METHODS

Subjects

Subjects for this study were a convenience sample of patients (aged 24 weeks corrected gestational age up to 5 years of age) undergoing laparotomy between January 2003 and December 2005. Children with gastrointestinal anomalies such as diaphragmatic hernia, malrotation, gastrointestinal atresia, abdominal wall defects, Hirschsprungs disease and necrotizing enterocolitis (NEC) with long segment or circumferential necrosis were excluded from this study. Subjects were also excluded if they were above or below 2 standard deviations on age appropriate growth curves (13, 14). Approval for this study was obtained from our institutional research ethics board.

Bowel length measurement

All measurements were performed by a staff pediatric surgeon or a fellow in pediatric surgery. Upon entering the abdominal cavity, the small and large intestine were measured in situ along the antimesenteric border using a 3-0 silk suture. Small intestinal length was defined as the distance between the ligament of Treitz and the ileocecal valve. Accordingly, colon length was measured from the ileocecal valve to the proximal rectum at the peritoneal reflection. These lengths together with basic demographic characteristics of the patient were entered onto a datasheet.

Statistical analysis

All statistical analyses were done using SPSS (version 14; SPSS, Chicago, IL) with an alpha set at 0.05 with 0.1 being considered a trend. Analyses were done separately for small bowel and colon length.

In order to develop predictive models for bowel length, our intention was initially to perform univariate linear regression models with a number of predictors. Predictors were chosen a priori and included: post-conception age at surgery in weeks, weight at surgery in grams, and height/body length at the time of surgery in centimeters (cm). We then had planned to perform a multiple variable linear regression on all variables with a p-value < 0.2 on the univariate analyses. However, upon plotting our data, it became apparent that the distribution of intestinal length was non-linear. Furthermore, we noted that our predictors were highly correlated (Pearson r > 0.9), and as such, in order to avoid issues related to collinearity, these predictors could not be entered simultaneously into a multiple variable model.

Therefore, we selected an alternate approach to select the best model for bowel length from amongst our potential predictor variables. This method also takes into account the possibility that the relationship between bowel length and the predictor variable could be non-linear. Using the curve fit function in the SPSS regression module; we sought to identify the curve with the best overall fit between bowel length and each predictor variable. The specific curves examined for each predictor variable were: linear, logarithmic, inverse, power, S, and growth. It was decided, that the curve with the highest R-square (R^2) amongst each predictors that was examined, would be the model chosen for that predictor.

RESULTS

Study sample

One hundred and eight patients were included in the present study. The age range of the subjects at surgery was 25.5 – 280 weeks (5 years) post-conception. The postoperative diagnoses are included in Table 1. The majority of cases (n=29, 26.9%) were undergoing operation for necrotizing enterocolitis (NEC). Tumor was another major category of postoperative diagnosis (n=17, 15.7%), which mainly consisted of Wilm's tumor (n=5) and neuroblastoma (n=3). Obstruction (n=15, 13.9%) included intussusception (n=6), adhesive small bowel obstruction (n=3) and volvulus not related to malrotation (n=2). Weight range at operation was 540 – 19500 grams and the height range was 31 – 117 cm. Our predictor variables were highly correlated with Pearson correlations between age and weight of 0.944 ($p<0.001$), age and height of 0.930 ($p<0.001$), and weight and height of 0.977 ($p<0.001$).

Table 1 Postoperative diagnosis

	n	%
NEC	29	26.9
Tumor	17	15.7
Obstruction	15	13.9
Hepatobiliary	13	12.0
Stricture	9	8.3
Gastrointestinal perforation	9	8.3
Meconium ileus	6	5.6
Gastroesophageal reflux	3	2.8
Other	7	6.5

Small bowel length

Small bowel length increased from a mean of 70.0 centimeters (cm) [standard error – (se): 6.3] in those aged 24-26 weeks post-conception to 423.9 cm (se: 5.9) in those aged 49-60 months. Small bowel length increased from a mean of 83.1 cm (se: 9.2) in those weighing 500-999 grams to 407.0 cm (se: 13.2) in those weighing16000-19999 grams. Small bowel length increased from a mean of 97.4 cm (se: 6.0) in those measuring 30-39 cm to 396.4 cm (se: 15.3) in those measuring 100-120 cm in height. Table 2 shows the ranges of small bowel length for age, weight, and height.

The results of the curve fitting process are depicted in Table 3, which lists the R^2 for each of the various curves examined for each predictor variable. The model with the highest R^2 for post-conception age was the inverse curve ($R^2 = 0.845$), for weight the power curve ($R^2 = 0.846$), and height the S-curve ($R^2 = 0.852$). These curves and the corresponding equations are depicted in Figure 1.

Colon length

Colon length increased from a mean of 22.7 cm (se: 2.0) in those aged 24-26 weeks GA to 122.4 cm (se: 5.7) in those aged 49-60 months. Colon length increased from a mean of 23.6 cm (se: 1.4) in those weighing 500-999 grams to 122.2 cm (se: 5.5) in those weighing 16000-19999 grams. Colon length increased from a mean of 27.0 cm (se: 1.4) in those measuring 30-39 cm to 121.3 cm (se: 8.4) in those measuring 100-120 cm. Table 4 shows the ranges of colon length for age, weight, and height.

The results of the curve fitting process are depicted in Table 3 which lists the R^2 for each of the various curves examined for each predictor variable. The model with the highest R^2 for post-conception age was the logarithmic curve ($R^2 = 0.903$), for weight the linear function ($R^2 = 0.880$), and height the power curve ($R^2 = 0.880$). These curves and the corresponding equations are depicted in Figure 2.

Table 2 Small bowel length

	Mean (cm)	Standard error
Post-conception Age		
24 – 26 weeks	70.0	6.3
27 – 29 weeks	100.0	6.5
30 – 32 weeks	117.3	6.9
33 – 35 weeks	120.8	8.8
36 – 38 weeks	142.6	12.0
39 – 40 weeks	157.4	11.2
0 – 6 months	239.2	18.3
7 – 12 months	283.9	20.9
13 – 18 months	271.8	25.1
19 – 24 months	345.5	18.2
25 – 36 months	339.6	16.9
37 – 48 months	366.7	37.0
49 – 60 months	423.9	5.9
Weight at Surgery		
500 – 999 grams	83.1	9.2
1000 – 1499 grams	109.9	6.6
1500 – 1999 grams	120.1	4.6
2000 – 2999 grams	143.6	8.0
3000 – 4999 grams	236.5	23.8
5000 – 7999 grams	260.3	14.1
8000 – 9999 grams	300.1	22.0
10000 – 12999 grams	319.6	16.4
13000 – 15999 grams	355.0	19.2
16000 – 19999 grams	407.0	13.2
Height at Surgery		
30 – 39 cm	97.4	6.0
40 – 49 cm	129.0	5.6
50 – 59 cm	205.9	21.6
60 – 74 cm	272.0	11.1
75 – 89 cm	308.5	16.5
90 – 99 cm	382.5	15.2
100 – 120 cm	396.4	15.3

Table 3 R-square values for curve fitting procedure

Model	Age	Weight	Height
Small Bowel Length			
Linear	0.726	0.802	0.837
Logarithmic	0.829	0.829	0.848
Inverse	0.845	0.612	0.816
Power	0.758	0.846	0.840
S	0.843	0.722	0.852
Growth	0.609	0.731	0.790
Colon Length			
Linear	0.824	0.880	0.878
Logarithmic	0.903	0.831	0.859
Inverse	0.856	0.548	0.799
Power	0.844	0.875	0.880
S	0.883	0.661	0.859
Growth	0.708	0.832	0.859

DISCUSSION

The length of the small intestine is important in infants with SBS because it is associated with prognosis (1). We have previously highlighted the importance of expressing residual intestinal length as a percentage of the predicted length (3). Spencer et al. reported that a small bowel length of <10% of the expected intestinal length is associated with a relative risk of 5.74 for death (2). Although absolute bowel length was predictive of this adverse outcome, the magnitude of risk was lower. One of the limitations of using absolute remaining intestinal length relates to developmental changes, particularly in a population where prematurity is common.

Unfortunately, the definition of SBS is conflicting with the anatomic definition based on degree of intestinal loss and the functional definition based on degree and duration of parenteral support following loss of intestine. Our intestinal rehabilitation program, GIFT (Group for Improvement of Intestinal Function and Treatment), employs a combined definition that emphasizes the functional aspects (need for parenteral nutrition greater than 42 days after bowel injury), or a residual small bowel length less than 25% expected for gestational age (3, 15, 16). Therefore, knowledge of the proportion of the remaining intestine relative to expected length may assist in more accurately defining SBS, guiding nutritional management and providing prognostic information.

Quantification of normal intestinal length in neonates and infants has received little attention in the literature. To date, all studies have measured the intestinal length using a variety of approaches, thus generating results that are highly variable. Measurements

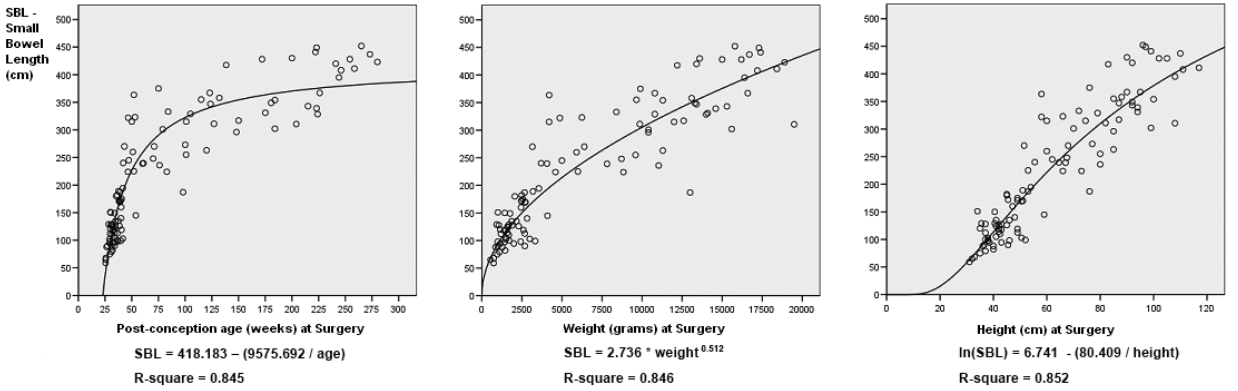

Figure 1 Graphs, based on optimal model from curve fit process, for prediction of small bowel length from each of post-conception age, weight, and height at operation.

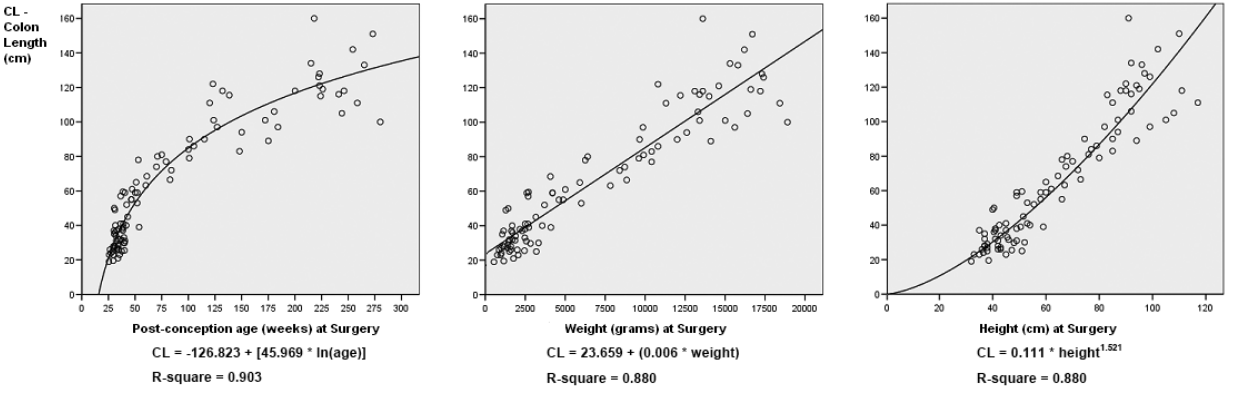

Figure 2 Graphs, based on optimal model from curve fit process, for prediction of colon length from each of post-conception age, weight, and height at operation.

Table 4 Colon Length

	Mean (cm)	Standard error
Post-conception Age		
24 – 26 weeks	22.7	2.0
27 – 29 weeks	24.4	1.2
30 – 32 weeks	37.7	2.2
33 – 35 weeks	27.8	1.7
36 – 38 weeks	40.1	4.3
39 – 40 weeks	32.7	2.1
0 – 6 months	56.8	2.7
7 – 12 months	57.1	2.2
13 – 18 months	84.8	2.3
19 – 24 months	107.8	4.5
25 – 36 months	95.0	3.4
37 – 48 months	122.5	5.9
49 – 60 months	122.4	5.7
Weight at Surgery		
500 – 999 grams	23.6	1.4
1000 – 1499 grams	32.5	2.9
1500 – 1999 grams	31.1	1.7
2000 – 2999 grams	38.5	3.0
3000 – 4999 grams	48.0	4.0
5000 – 7999 grams	66.7	4.2
8000 – 9999 grams	79.9	4.0
10000 – 12999 grams	97.3	5.9
13000 – 15999 grams	112.4	4.0
16000 – 19999 grams	122.2	5.5
Height at Surgery		
30 – 39 cm	27.0	1.4
40 – 49 cm	35.8	2.0
50 – 59 cm	44.8	3.2
60 – 74 cm	69.9	2.7
75 – 89 cm	95.0	4.0
90 – 99 cm	117.4	4.0
100 – 120 cm	121.3	8.4

of small intestinal length in a full term neonate vary between 176 and 305 cm. However, all of these values represent post-mortem measurements (4-12). In adults, the small intestinal length ranges from 2.4 to 3.7 meters when measured in vivo with a range of 4.7 to 9.7 meters post-mortem (17). These discrepancies are likely due to alterations occurring in the peri-mortem period with early shortening of the intestine as a result from smooth muscle contraction. However, as autolysis proceeds, there is relaxation and lengthening (18). The measurement technique is also important when interpreting intestinal length, as tension may increase length in the small intestine by 4% and in the large intestine by 14% (19).

Due to the limitations of existing post-mortem data which have been used to date for the quantification of intestinal length, we sought to examine intestinal length in live infants. To our knowledge, this is the first study to do so. We believe that this approach will provide more accurate measurements than those obtained from autopsy studies. As well, while many of the previous studies have tended to focus on premature infants, and express lengths over a small number of gestational age ranges, we sought to develop norms over a larger age range with more defined age categories. As such, measurements were done in infants from birth to 5 years of age. We also examined weight and height at surgery as separate predictors of intestinal length.

The initial goal of this project was to develop a predictive model for intestinal length, and we had intended to do so in a multiple variable manner. However, following preliminary analyses it became apparent that because of the substantial correlations between our predictor variables, a multiple variable approach would not be possible due to concerns of collinearity. As well, it was clear that in most cases the relationship between predictors was non-linear which prompted us to determine the best univariate relationship between the predictor and bowel length. Interestingly, given the high correlation between the predictor variables, predictive models that account for greater than 80% of the variance in bowel length could be developed for each of age, weight, and height. Therefore, it would seem that bowel length can be estimated, using any of these predictors, with the degree of variance being explained by each predictor being equivalently high. As such, we have developed reference tables and equations on the basis of our data for each predictor for both small and large bowel length. It is important to recognize that although the variance explained by each equation is high, none of these equations have been validated in an independent data set. Therefore, we believe that the ultimate approach to selecting the best predictor equation amongst our 3 equations for small and large bowel length would be to examine these equations in an independent dataset. The equation with the best performance in such an analysis could then be used prospectively to define the expected bowel length for children. It is our goal to perform the necessary validation studies.

Until such a validation is performed, our data suggests that height at surgery (in centimeters) be used to estimate small bowel length (SBL) using the following equation ln(SBL) = 6.741 – 80.409/height . Similarly colon length (CL) may be predicted using the equation CL = 0.111 * height$^{1.521}$. We chose height for a number of reasons. First, it appears that height is most predictive when one examines the variability from observed to expected values across the entire spectrum of height. This is in contrast to age and weight that seem to have minimal variability at the bottom range of the spectrum and significantly more variability in bowel length as an infant ages or gains weight. Second, height is easy to quantify and is relatively stable with time, and is not subject to drastic changes in the immediate peri-operative period as weight is. Finally, gestational age may be more difficult to accurately quantify than height.

This study has a number of strengths. First, it is the first paper to establish norms in live infants which eliminates the inaccuracy of measurement introduced by autopsy studies. Second, data were collected prospectively using a standard and simple method for bowel length measurement that should allow for application by other centers. Third, we included a wider range of ages that allows our data to be applied to a larger group of children. Despite the strengths, our study has some limitations. First, we did not take gender and ethnicity into consideration. Second, inter-observer variation could have impacted data quality. We believe that we controlled for this by protocolizing measurement technique. As well, such variation will occur in the "real" world when the norms established in this study are applied. Our final limitation could be the impact that prematurity may have in terms of the trajectory of growth, so that postnatal bowel growth for any given gestational age may not be the same as growth in the prenatal period.

In conclusion, we have presented the first data on intestinal lengths measured in live infants. We believe that these data will provide more accuracy than the previous published post-mortem data, although this assertion will need further validation. Overall, while expected intestinal length can be predicted based on post-conception age, weight, and height; we propose that height be used until such a time as external validation of the equations presented in this paper is done. We believe that our data will be very useful in the management of children with short bowel syndrome, by allowing one to accurately define the degree of residual intestine relative to that expected in an infant of similar size.

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PART III

POST-OPERATIVE CARE & CONSIDERATIONS

Medicine is so broad a field,
so closely interwoven with general interests,
dealing as it does with
all ages, sexes and classes, and yet
of so personal a character
in its individual appreciations,
that it must be regarded as one of those
great departments of work
in which the cooperation of men and women
is needed to fulfill
all its requirements

Elizabeth Blackwell

Chapter 4

Efficacy and safety of GLN-AA versus Standard-AA in infants: a first-in-man randomized double-blind trial

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Harma te Beest
Dick Tibboel

Submitted

ABSTRACT

Background & Aims: Efforts are directed at reaching the optimal composition of pediatric amino acids (AA) infusions. The goal was to demonstrate the safety and efficacy of a newly developed parenteral AA solution containing alanyl-glutamine (GLN-AA) compared to Standard-AA.

Methods: This is a randomized (2:1), double-blind, multicentre clinical pilot trial. Infants after surgical interventions were allocated to receive GLN-AA or Standard-AA over a minimum of 5 days to maximum of 10 days. AA profiles in blood samples obtained at baseline, day 7, and end of treatment were compared to normal ranges. Data regarding safety, and efficacy were also collected.

Results: Infants were comparable for gestational age at birth (36 vs 38 weeks), birth weight (2460 vs 2955 grams), and day of life during start intervention (1 vs 2 days). Plasma AA profiles in infants treated with GLN-AA (n=13) were closer the normal ranges than those in infants treated with Standard-AA (n=6). There were no clinical or statistical differences in adverse events, safety and efficacy parameters between both groups.

Conclusion: This first-in-man study shows that GLN-AA is safe in infants after surgical interventions, and is well tolerated. Compared to reference values, GLN-AA better reflects the amino acid requirements of the infant.

INTRODUCTION

The newborn infant is in a critical stage of development, and adequate nutrition is necessary to promote growth and optimal neurological development (1). Healthy newborn infants have a higher metabolic rate and energy requirement per unit body weight than older infants and children. Post-surgical infants may even need more energy, including higher amounts of amino acids (2, 3). There are standard AA solutions which are not optimally designed for term and preterm infants. In standard AA solutions, certain AAs are too low, too high or missing when fed to preterm or term infants.

For several possible reasons, infants with congenital gastrointestinal malformations undergoing surgical correction often are dependent on parenteral nutrition for a longer time. One, the intestinal tract has been shortened so that full enteral feeding is not tolerated. Second, a primary anastomosis has been made which cannot be "stressed" by enteral feeding. Although Ekingen et al. has shown that early post-operative feeding even in newborn surgical patients with an intestinal anastomosis is well tolerated (4). Moreover local practice determines the start of enteral feeding following a primary anastomosis. Third, intestinal motility may be impaired due to the nature of the congenital malformation (for example gastroschisis, extensive atresia), developing necrotizing enterocolitis, or the surgery itself. Although it is generally accepted that enteral nutrition needs to be introduced as soon as possible, these infants are dependent on parenteral nutrition for a certain period (3).

Recovery after surgery and normal growth will be suboptimal when the composition of amino acids (AA) infusions is not ideal (5-7). The numerous studies on AA supplements have brought conflicting results, and further trials have been advocated (8-10). A newly developed AA solution (GLN-AA, Fresenius Kabi, Bad Homburg, Germany) was designed exclusively for children including premature neonates to better suit the needs of children requiring AA. Fresenius decided under feasibility criteria to adjust the following components. It contains alanyl-glutamine as a precursor for glutamine in order to provide adequate amounts of glutamine, glycyl-tyrosine as a precursor for tyrosine, and acetyl-cysteine as a source of cysteine. Additionally arginine and taurine are increased compared to the reference solution (Standard-AA). GLN-AA was designed to better suit the AA requirements of parenterally fed infants and children.

In this report we describe a first-in-man study to demonstrate the safety and efficacy of GLN-AA compared to Standard-AA, as reflected by plasma AA concentrations in infants requiring parenteral nutrition after surgical interventions due to major congenital gastrointestinal malformations.

MATERIAL AND METHODS

Study design

This pilot phase II study was an international, multi-centre, randomized controlled, double-blind trial to compare the amino acid and safety profiles in infants treated with GLN-AA or Standard-AA for 5 to 10 days. Four pediatric surgical services in tertiary referral hospitals participated (Erasmus MC – Sophia, Rotterdam, The Netherlands; Klinik für Kinder- und Jugendmedizin, Mannheim, Germany; VU University Medical Center, Amsterdam, The Netherlands; Cliniques Universitaires Saint-Luc, Brussels, Belgium).

The inclusion criteria were the following: gestational age ≥ 34 weeks and age ≤ 23 months; birth weight for newborns ≥ 1840 grams or within +/- 2 SD for standard weight for infants other than newborns (these are based on the reference values applicable in Caucasian newborns derived from Nelson Textbook of Pediatrics, 11); surgical intervention of the gastrointestinal tract due to congenital malformations, an estimated need for parenteral nutrition of at least 5 days, and parental informed consent. The following exclusion criteria were applied: enteral nutrition ≥25% of total energy intake per day, considerable impairment of renal function, inborn congenital malformation other than of the bowel, severe congenital heart disease, major chromosomal abnormalities, inborn metabolic disorders and severe liver dysfunction. The study protocol was approved by all institutional review boards (Erasmus MC Rotterdam, the Netherlands; VU University Medical Center Amsterdam, The Netherlands; Klinik für Kinder- und Jugendmedizin Mannheim, Germany; Cliniques Universitaires Saint-Luc Brussels, Belgium).

Randomization and blinding

Patients were randomized to treatment using the method of randomly permuted blocks. The ratio of patients allocated to GLN-AA versus Standard-AA was 2:1. The next eligible patient for randomization received the lowest available randomization number at the study site. All investigators, physicians and nurses involved in the care of included patients were blinded to the allocation of treatment.

Treatment

GLN-AA (Fresenius Kabi, Bad Homburg, Germany) was the investigational solution; this 10% pediatric AA solution was administered isonitrogenously compared to the control solution (Vaminolact®, Fresenius Kabi, Bad Homburg, Germany; in this paper named Standard-AA), a 6.5% pediatric AA solution containing 18 AA, and taurine. Standard-AA is predominantly used for the supply of AA as part of pediatric parenteral nutrition. GLN-AA is a new solution, and compared with Standard-AA contains the 2 di-peptides glycyl-tyrosine as precursor for tyrosine, and alanyl-glutamine as precursor glutamine, and it contains acetyl-cysteine. Furthermore, the concentrations of taurine and arginine

are higher than those in Standard-AA, whereas phenylalanine is lower compensated by a higher amount of tyrosine.. GLN-AA does not contain glutamic acid and aspartic acid. The parenteral nutrition regimens were based on the requirements of the ESPGHAN Guidelines as published in 2005 (12).

Treatment was started the day after surgery, and lasted for a minimum of 5 days and a maximum of 10 days. Dosage of both study products was increased stepwise over the first 3 days, from 1.0±0.5 g/kg/day at day 1 to 2.0±0.5 g/kg/day at day 2 and 2.5 ± 0.25 g/kg/day at day 3. The latter dosage was then maintained until the end of study. Infusion lasted for a minimum of 20 hours per day. Treatment was stopped prematurely when enteral nutrition exceeded 25% of total energy intake. If parenteral nutrition with AA needed to be continued after 10 days, patients in both groups were given Standard-AA as long as clinically indicated.

Study endpoints

The primary study endpoint was the profile of all AA in plasma after last treatment, and comparison between both study groups. Secondary endpoints were safety and efficacy (growth outcome) parameters. Safety parameters are liver enzymes, blood gas values (pH, BE), blood glucose levels, incidence of sepsis, and adverse events including local tolerance of infusion. For all these values there was a predetermined list with cut-off values above which the study had to be stopped. Moreover a Data Safety Monitoring Board was appointed to regularly check these parameters. An adverse event was defined as any untoward medical occurrence in a patient which did not necessarily have a causal relationship with the treatment An adverse event could therefore be any unfavourable and unintended sign (i.e. an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. As pharmacokinetic variables plasma concentrations of the dipeptides (Ala-Gln and Gly-Tyr), and N-Acetyl-Cysteine (NAC) were defined. Efficacy parameters included body weight, head circumference, and pre-albumin levels.

Data collection and monitoring

All patient data were recorded in case report forms. Clinical parameters such as weight, blood glucose, study solution, concomitant medication, concomitant nutrition and (serious) adverse events were documented each study day. Local tolerance of the infusion site was scaled when the study solution was infused peripherally. Blood samples were drawn during steady state infusions conditions (except at baseline) at three time points (baseline, end of study day 7 and at day 10). These samples were drawn together with blood samples taken for routine care from with an intravenous puncture. Last follow-up visit was 28 days (± 7 days) after end of last infusion.

Analytical method

Free AA (all standard AA except cysteine, and the non-standard (non-canonic) AA taurine, citrulline and ornithine) and dipeptide (Ala-Gln, Gly-Tyr) concentrations in plasma samples were quantified by a triple-quadruple mass spectrometer after separation of matrix components by high-performance liquid chromatography on a cyano column (LC-MS/MS system consisted of a 1100 series binary pump (Agilent) and a Sciex API4000 (Applied Biosystems) mass spectrometer with turbo ion spray). Proteins were precipitated by sulphosalicylic acid. Thiols (including cysteine and N-Acetyl-L-Cysteine) of the plasma samples were reduced with dithiothreitol (methods for amino acid analysis according to Medizinisches Labor, Bremen, Germany).

Statistical analysis

For the sample size generation, power of 80% and level 1 error of α=0.025 was used. According to previous considerations, the limit of non-inferiority (border of accepted accordance) could be set to 25%, and the expected difference to 0%. To determine non-inferiority (using a one-sided t-test of equivalence in means), an effect size of 1.5 is necessary so that 12:6 patients would be sufficient.

Different populations were distinguished in the analyses (see Figure 1 flow chart). The 'safety population' was defined as all patients treated with study solution, regardless of how much and how they received study treatment. Patient characteristics analysis and the safety analysis were performed on this population. The 'full analysis population' was defined as all subjects in the safety population for whom one AA profile was available after having reached full-dose for at least one day. The efficacy analysis was performed on this population. The 'amino acid pattern analysis population' was defined as all patients for whom AA profiles were available after completion of at least 5 treatment days. This population was used only for the analysis of AA levels. Excluded infants for the 'full analysis' and 'amino acid pattern analysis' population did not show intolerance to study medication. They were excluded because enteral nutrition intake exceeded > 25% of total intake.

A *P*-value < 0.05 was considered statistically significant. Data were analyzed in SPSS (version 15) and SAS (Version 9.1.3) as appropriate. All statistical tests were two-sided with significance level of α=0.05. Data were analyzed with two-sided T-test, Fisher's exact test or Mann-Whitney U test as appropriate. An Analysis of Covariance (ANCOVA) model was applied to each efficacy parameter on the last available measurement.

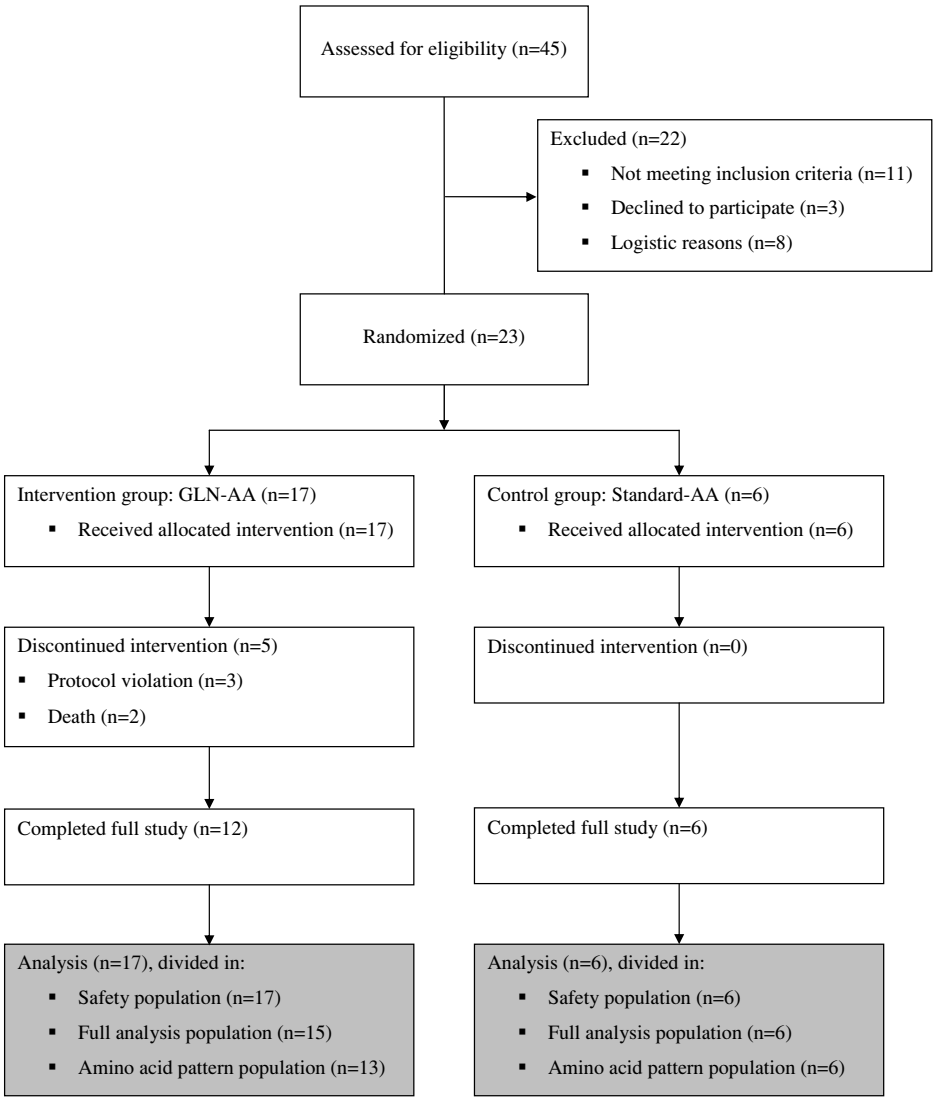

Figure 1 CONSORT diagram

RESULTS

Study sample

Between January 1, 2009 and December 18, 2009, 45 patients were screened for eligibility; 23 patients were enrolled from the 4 centers (10 in Erasmus MC – Sophia, 6 in Klinik für Kinder- und Jugendmedizin, 4 in VU University Medical Center, Amsterdam, and 4 in Cliniques Universitaires). 17 patients were assigned to the GLN-AA treatment group and 6 to the Standard-AA group (Figure 1).

Both treatment groups (safety population) were similar with regard to most demographic characteristics (Table 1). Gastroschisis was the primary diagnosis for surgery in both groups, 47% (8/17) of the patients in the GLN-AA group versus 50% (3/6) of the patients in the Standard-AA group.

Primary endpoint

This analysis concerned 19 patients (amino acid pattern analysis population), 13 in the GLN-AA group and 6 in the Standard-AA group. The last study day measurements of AA plasma levels of the GLN-AA group were closer to the midpoints of the respective normal ranges than the respective pre-treatment values (the normal ranges were defined by the analytical laboratory Medizinisches Labor, Bremen, Germany according to

Table 1 Main characteristics of the study population (n=23)

	Intervention group GLN-AA	**Control group Standard-AA**
N	17	6
Male gender	4 (24)	3 (50)
GA at birth, wk	36 [35-38]	38 [37-38]
Birth weight, g	2460 [2090-3100]	2960 [2480-3100]
Birth length, cm	45 [44-47][a]	49 [47-49][b]
Head circumference at birth, cm	32 [30-34][c]	34 [33-35][b]
Diagnosis		
Gastroschisis	8 (47)	3 (50)
Intestinal atresia	4 (23)	1 (17)
Omphalocele	2 (12)	2 (33)
Anal atresia	1 (6)	
Volvulus + meconium peritonitis	1 (6)	
Multiple congenital anomalies	1 (6)	
Age at random assignment, days	1 [1-3]	2 [1-3]
Younger than 3 days	12 (71)	4 (67)
Total infusion time, days	7.5 ± 3.1	8.9 ± 2.1
Daily AA intake, g/kg body weight	2.1 ±0.5	2.1 ±0.2

Data presented as n (%), median [IQR], or mean ± SD

[a]n=11, [b]n=4, [c]n=10, [d]n=15, [e]n=5

scientific data). The opposite is true for Standard-AA, last study day measurements were farther away from the midpoints of the respective normal ranges than the respective pre-treatment values. At the last study day measurement, 5 AA were outside normal ranges in each group, namely cysteine, glycine, serine, threonine and valine (Figure 2).

Secondary endpoints

Safety

This analysis concerned the safety population (n=23). Incidences of adverse events did not show clinically significant differences between the groups (*P*=1.000): 15 (88.2%) of the patients in the GLN-AA group and 6 (100%) of the patients in the Standard-AA group showed adverse events after the start of the first study infusion until the end of the follow-up period. These adverse events were judged not to be related to study solution or to study procedures. Six serious adverse events occurred in 3 patients (17.6%) in the GLN-AA group. Of these six, two serious adverse events, both leading to the death of the patient, were considered to be related to the underlying disorders present at birth.

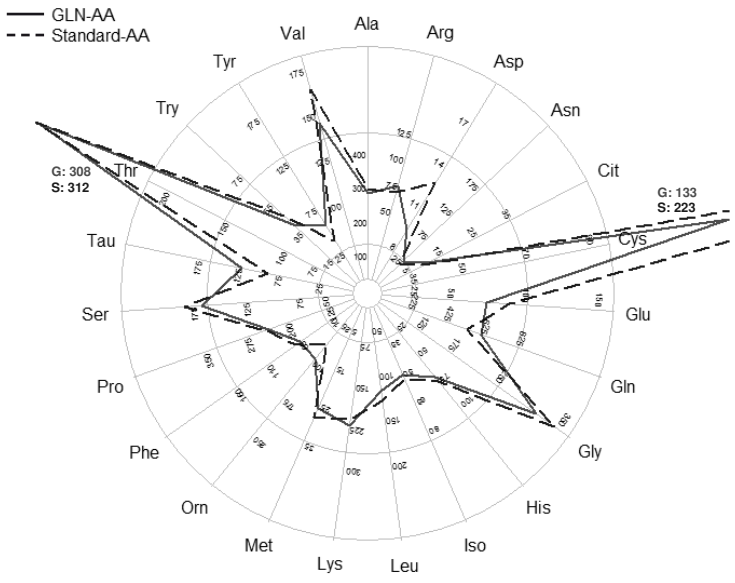

Figure 2 Plasma aminogram at last measurement

The reference ranges (µmol/L) are on the basis of concentrations in newborn infants; the inner circle is the lower limit of normal and the middle circle is the upper limit of normal. Ala: alanine, Arg: arginine, Asp: aspartic acid, Asn: asparagine, Cit: citrulline, Cys: cysteine, Glu: glutamic acid, Gln: glutamine, Gly: glycine, His: histidine, Iso: isoleucine, Leu: leucine, Lys: lysine, Met: methionine, Orn: ornithine, Phe: phenylalanine, Pro: proline, Ser: serine, Tau: taurine, Thr: threonine, Try, tryptophane, Tyr: tyrosine, Val: valine.

One of the patients, who should not have been included in the study from the start, had multiple congenital anomalies and developed anastomotic leakage, mediastinitis and sepsis after esophageal atresia correction, circulatory failure ensued. The other patient presented with therapy refractory persistent pulmonary hypertension of the newborn and was transferred for extracorporeal membrane oxygenation (ECMO). Emergency laparotomy revealed complete necrosis of the bowel, upon which continuation of treatment was considered futile.

AA infusion was peripherally infused for 28 days in total (5 patients) in the GLN-AA group and for 9 days in total (2 patients) in the Standard-AA group. As assessed on the local tolerance scale (grade 0-3) in only 2 (11.8%) patients, who received GLN-AA, symptoms i.e. of grade 1 ("erythema and/or some degree of swelling, no induration, no palpable venous cord") were reported.

Efficacy

This analysis was performed for a total of 21 patients (full analysis population). The body weight was expressed in z-scores as this better represents relative changes than the absolute weight. Comparing baseline and follow-up measurements, no significant dif-

Table 2 Safety parameters – Safety population (n=23)

	Normal range	**Intervention group GLN-AA N=17**		**Control group Standard-AA N=6**	
		Baseline	*Last measurement*	*Baseline*	*Last measurement*
Hematocrit, %	44-72	44 ± 7[a]	37 ± 6[a]	44 ± 7[b]	36 ± 4[b]
Platelets, 10^9/L	84-478	251 ± 68[c]	469 ± 151[c]	245 ± 91[b]	355 ± 138[b]
Glucose, mg/dL	54-162	97 ± 31	88 ± 28	101 ± 15	80 ± 10
CRP, mg/L	<10	26 ± 18[d]	21 ± 44[d]	48 ± 37	19 ± 37
γGT, U/L	13-147	79 ± 51[e]	144 ± 114[e]	95 ± 87	191 ± 134
ALT, U/L	<50	48 ± 61[a]	18 ± 16[a]	18 ± 10	12 ± 7
Bicarbonate, mmol/L	21-28	21 ± 4	23 ± 2	21 ± 4	25 ± 2
Creatinine, µmol/L	27-88	59 ± 27	28 ± 11	57 ± 14	29 ± 10
Ala-Gln, µmol/L[X]	-	<2.0 ± 0.0[f]	22.7 ± 26.1[f]	<2.0 ± 0.0	<2.0 ± 0.0
Gly-Tyr, µmol/L[Y]	-	<0.5 ± 0.0[f]	1.6 ± 1.2[f]	<0.5 ± 0.0	<0.5 ± 0.0
NAC, µmol/L[Z]	-	5.3 ± 0.9[f]	71.8 ± 22.7[f]	5.2 ± 0.5	<5.0 ± 0.0

Data are represented as mean ± SD

Ala-Gln = Alanyl-Glutamine/ Gly-Tyr = Glycyl-Tyrosine / NAC = N-Acetyl-Cysteine

[a]n=14, [b]n=5, [c]n=13, [d]n=11, [e]n=12, [f]n=16

[X] 2.0 µmol/L was the lower limit of quantification

[Y] 0.5 µmol/L was the lower limit of quantification

[Z] 5.0 µmol/L was the lower limit of quantification

ferences between study groups were observed for body weight, head circumference and pre-albumin. The amount of enteral nutrition at the last study day differed from 0 to 210 ml, most infants got breast milk as enteral nutrition.

Clinical laboratory evaluations

Blood samples of 23 patients (safety population) were analyzed (Table 2). Overall, no significant differences were found between both groups. Plasma levels of dipeptides (alanyl-glutamine and glycyl-tyrosine) and N-Acetyl-Cysteine (NAC) revealed major differences between the two treatment groups, reflecting the new composition of GLN-AA. All pre-treatment plasma levels of the dipeptide alanyl-glutamine were below the lower level of quantification (<2.0 µmol/L). All last study day measurement values of alanyl-glutamine in the Standard-AA patients had not changed, whereas in the GLN-AA group the mean last study measurement result had increased from below the quantification level of <2.0 µmol/L to 22.69 µmol/L. Similar results were shown for glycyl-tyrosine and NAC: the mean value increased in the GLN-AA group, but no changes were observed in the Standard-AA group.

DISCUSSION

In infants after surgical interventions, we conclude that GLN-AA is safe and well tolerated as part of parenteral nutrition. No clinical relevant differences with regard to frequency and distribution were observed in adverse events between both treatment groups. Moreover, in infants treated with GLN-AA the amino acid profile more closely resembled normal ranges than the amino acid profile in infants treated with Standard-AA, reflecting the new composition of GLN-AA.

AA plasma levels on the last study day in the GLN-AA group were closer to the mid-points of the normal ranges than those in the Standard-AA group. This means that the new composition of GLN-AA better reflects these infants' AA requirements and the goal of this new solution was achieved. All other AA levels, which were not changed compared to the Standard-AA, were comparable between both solutions. However, compared to normal ranges asparagine was lower and cysteine, glycine, serine, threonine, and valine were higher than the range of reference values in the new solution. These AA were also outside the range of reference values in the old Standard-AA solution. But in GLN-AA these AA better approach the normal values, however some improvement might still be necessary. The clinical relevance cannot be determined given the small data set.

Research has focused on obtaining the best pediatric AA mixtures (13-15). Obtaining an optimal AA solution is especially important for critically ill patients. They undergo increased proteolysis, there is increased hepatic synthesis of immune/inflammatory

proteins, and there is decreased muscle protein synthesis. They also have glucose and lipid intolerance due to an altered energy metabolism (16). This also counts for post-surgical neonates. However, our research group has found that the metabolic stress responses in neonates are different from the responses in older children. We have found that the duration of the metabolic stress response in neonates is probably less than 24 hours (17). It should also be noted that post-surgical AA requirements have not been studied extensively. Currently, all AA are studied in the normal situation, the following step would be to evaluate post-surgical patients.

In both AA solutions threonine and cysteine levels were far above the upper limit of normal. Cysteine is important in that it reduces levels of methionine in parenteral nutrition, which is potentially hepatotoxic. It also reduces the pH of the solution, thereby increasing the calcium and phosphate solubility, and potentially improving bone mineralization (9). However, the pH of the AA solution is also adjusted by acidifiers. In GLN-AA, cysteine is replaced by N-acetyl-L-cysteine to increase stability of the concentration. The plasma concentration is closer to the upper limit of normal than that in Standard-AA.

GLN-AA contains a higher amount of taurine and this explains the higher plasma level in the GLN-AA patients. Taurine is considered as conditionally essential AA in neonates because the synthesis of taurine is limited in the earliest stage of life (18). Its supplementation improves cognitive development and possibly has positive effects on the development of the retina and middle ear (19, 20). Taurine may counteract cholestasis after long-term parenteral nutrition because taurine is involved in the formation of bile acid conjugates (21).

GLN-AA also contains a higher amount of arginine, which is crucial for the detoxification of ammonia, has a key function in the urea cycle, and has an advantageous effect on nitric oxide synthesis (22). Nitric oxide is a mediator that is crucial in maintaining mucosal integrity and intestinal barrier function. Supplementation of arginine has been shown to decrease the incidence of necrotizing enterocolitis in preterm infants (23). Therefore, GLN-AA is also a potentially interesting amino acid solution for preterm infants.

The rationale why dipeptides were chosen in the new GLN-AA, was the fact that for all chosen dipeptides the availability of the required AA is safe and efficient. Alanyl-glutamine has shown to provide a rapid provision of free glutamine (24-27). The same counts for glycyl-tyrosine, which is a good source tyrosine (25).

Glutamine is a newly added conditionally essential AA in GLN-AA. In adults it becomes essential under catabolic conditions such as trauma, major surgical procedures and severe infections (28). However, results in children are conflicting. Some premature evidence from parenteral and enteral studies suggests that glutamine decreases the rate of sepsis and nosocomial infections (29-31). Furthermore, glutamine possibly facilitates enterocyte proliferation, thereby maintaining the structural and functional integrity

and maturation of the gastrointestinal tract. This may help to prevent enterocolitis and improve feeding intolerance (29, 30, 32, 33). Recent evidence proves that most dietary glutamine is used as a first pass in splanchnic tissues of preterm infants (34). Poindexter et al. showed in that glutamine supplementation in extremely low-birth-weight infants increased the glutamine concentration without biochemical risk (35). However, in newborns and infants after major gastrointestinal surgery, glutamine supplementation of parenteral nutrition did not improve intestinal permeability, nitrogen balance, or outcome (36). More trials are needed to determine whether glutamine supplementation has significant benefits for infants with severe gastrointestinal abnormalities and to assess the difference between the parenteral and enteral effects of glutamine (8).

Due to the nature of this study (pilot study) the number of patients is relatively small. However, we were able to answer our aims. Also, only infants after surgery and older than 34 weeks gestational age were included. We can therefore not conclude whether this new solution would be suitable for other patient groups such as preterm infants. Unfortunately we did not find any differences in clinical outcomes. This should be one of the primary outcomes in further studies since this is clinical relevant.

In conclusion, this first-in-man pilot study showed that GLN-AA is safe. The AA profile in plasma is closer to the aimed reference values; however, no efficacy in terms of growth could be shown due to the relative short follow-up period.

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Additional table: Individual changes of amino acid values

	Low or normal pre-treatment and High at last measurement		High or normal pre-treatment and Low at last measurement	
	GLN-AA (N = 13)	**Standard-AA (N=6)**	**GLN-AA (N = 13)**	**Standard-AA (N=6)**
Alanine	0	0	1 (7.7)	0
Asparagine	0	0	3 (23.1)	5 (83.3)
Aspartic acid	1 (7.7)	2 (33.3)	1 (7.7)	0
Glutamic acid	1 (7.7)	1 (16.7)	0	0
Glutamine	2 (15.4)	0	0	0
Glycine	4 (30.8)	2 (33.3)	0	0
Isoleucine	0	0	1 (7.7)	0
Lysine	2 (15.4)	0	0	0
Methionine	1 (7.7)	0	0	0
Serine	6 (46.2)	1 (16.7)	0	0
Taurine	2 (15.4)	0	0	0
Threonine	10 (69.2)	6 (100.0)	0	0
Tryptophane	1 (7.7)	0	0	0
Tyrosine	0	0	0	1 (16.7)
Valine	6 (46.2)	6 (100.0)	0	0

Data are represented as n(%)

Low, normal or high are related to normal range values as mentioned in Additional table: Plasma amino acids at last measurement

Additional table: Plasma amino acids at last day of treatment

	Intervention group GLN-AA N=13	Control Group Standard-AA N=6	Normal range
Alanine	285.0 ± 62.4	298.5 ± 54.0	135-461
Arginine	76 ± 18.2	69.4 ± 26.1	17-121
Asparagine	29.1 ± 7.2	23.7 ± 4.7	30-189
Aspartic acid	9.7 ± 7.7	13.1 ± 5.2	8-15
Citrulline	10.8 ± 3.7	9.8 ±1.8	6-35
Cysteine	132.8 ± 31.7	223.4 ± 40.1	35-69
Glutamic acid	73.9 ± 34.9	89.7 ± 15.2	27-102
Glutamine	615.1 ± 167.7	535.1 ± 94.8	240-822
Glycine	313.0 ± 55.8	342.5 ± 58.8	107-253
Histidine	68.7 ± 16.2	73.4 ± 21.5	26-110
Isoleucine	49.1 ± 13.5	51.4 ± 9.1	31-84
Leucine	113.5 ± 24.6	124.0 ± 23.7	61-183
Lysine	215.6 ± 51.5	204.8 ± 29.0	68-267
Methionine	25.0 ± 6.4	27.3 ± 4.6	7-34
Ornithine	90.3 ± 34.0	60.8 ± 28.4	38-212
Phenylalanine	58.9 ± 12.9	65.7 ± 5.6	18-158
Proline	234.6 ± 42.9	237.3 ± 30.5	139-330
Serine	166.8 ± 41.4	183.3 ± 49.7	57-162
Taurine	120.2 ± 41.9	87.7 ± 40.2	24-160
Threonine	307.8 ± 152.8	311.9 ± 94.0	67-151
Tryptophane	40.3 ± 16.3	46.6 ± 10.4	15-69
Tyrosine	57.0 ± 17.3	40.3 ± 16.7	28-133
Valine	146.2 ± 28.3	164.5 ± 26.4	77-137

Values are in µmol/L. Data represented as mean ± SD
Normal ranges were defined by the analytical laboratory Medizinisches Labor, Bremen, Germany

Chapter 5

The timing of ostomy closure in infants with necrotizing enterocolitis: a systematic review

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ABSTRACT

Background: The optimal timing of ostomy closure is a matter of debate. We performed a systematic review of outcomes of early ostomy closure (EC, within 8 weeks) and late ostomy closure (LC, after 8 weeks) in infants with necrotizing enterocolitis.

Methods: PubMed, EMbase, Web-of-Science, and Cinahl were searched for studies that detailed time to ostomy closure, and time to full enteral nutrition (FEN) or complications after ostomy closure. Patients with Hirschsprung's disease or anorectal malformations were excluded. Analysis was performed using SPSS 17 and RevMan 5.

Results: Of 778 retrieved articles, 5 met the inclusion criteria. The median score for study quality was 9 (range 8-14 on a scale of 0 to 32 points, 11). One study described mean time to FEN: 19.1 days after EC (n=13) versus 7.2 days after LC (n=24; *P*=.027). Four studies reported complication rates after ostomy closure, complications occurred in 27% of the EC group versus 23% of the LC group. The combined odds ratio (LC vs. EC) was 1.1 [95% CI 0.5,2.5].

Conclusion: Evidence that supports early or late closure is scarce and the published articles are of poor quality. There is no significant difference between EC vs. LC in the complication rate. This systematic review supports neither early nor late ostomy closure.

INTRODUCTION

Ostomy formation is inevitable in certain cases, for example in almost half of patients operated on for necrotizing enterocolitis (NEC) (1). Unfortunately, in 15 to 68 percent of cases ostomy related complications may occur, such as stricture, parastomal hernia, prolapse, wound infection, wound fistula, wound dehiscence, and small bowel obstruction (2-5). Especially premature infants are at high risk; in patients with necrotizing enterocolitis, lower gestational age and birth weight were associated with greater risk of ostomy related complications (3). Subsequent ostomy closure carries a complication rate of about 20 percent, including wound infection, wound dehiscence, enterocutaneous fistula, bowel obstruction, anastomotic leak, and anastomotic stricture (2, 5, 6).

Following ostomy formation, surgeons tend to delay ostomy closure for at least 8 weeks or until the infant weighs two kilograms because of surgical aspects such as the postoperative abdominal adhesions and anesthetic aspects such as morbidity associated with ventilation anticipated in case of earlier closure (7-9). The timing of ostomy closure is very variable based on surgeon's preference or local protocols however universally without evidence based practice. Early closure could not only avoid ostomy related complications but it could also be favorable since having an ostomy is associated with diarrhea, severe fluid and electrolyte losses, and growth retardation (10). Moreover, ostomy closure during the same hospital admission is also favorable for parents and caregivers.

Therefore, we performed a systematic review to find an answer to the question whether early or late ostomy closure is preferred in infants with a history of NEC. The outcome measures were: time to full enteral nutrition and the complication rate.

METHODS

Search strategy

We conducted a systematic literature search in the PubMed, EMbase, Web-of-Science, and Cinahl databases from 1966 to October 2010. The following search terms were applied for the PubMed database: (stoma[tw] OR stomata[tw] OR stomas[tw] OR stomy[tw] OR ostom*[tw] OR enterostom*[tw] OR cecostom*[tw] OR coecostom*[tw] OR caecostom*[tw] OR colostom*[tw] OR duodenostom*[tw] OR ileostom*[tw] OR jejunostom*[tw]) AND (clos*[tw] OR seal*[tw] OR restor*[tw] OR repair*[tw] OR recover*[tw] OR re-establ*[tw] OR cure*[tw]) AND (infan*[tw] OR newborn*[tw] OR neonat*[tw]). The other databases were searched with the appropriate search terms concerning ostomy closure in infants less than 2 year of age. No limits were applied. All retrieved article titles and subsequently abstracts were screened for eligibility by two

independent reviewers (MCS and CEJS). Bibliographies of all selected abstracts were screened to identify any additional trials.

Selection criteria

All studies that compared early versus late ostomy closure in infants were eligible for inclusion in this study. Also, at least two thirds of included patients should be diagnosed with necrotizing enterocolitis and included studies needed to contain a description of either complication rate after ostomy closure and/or time to full enteral nutrition (FEN) after ostomy closure. Studies involving patients with Hirschsprung's disease or anorectal malformations were excluded, because the timing of ostomy closure is not related to the patient's recovery but to the moment of institutional determined surgical repair of either the Hirschsprung's disease or anorectal malformation.

Early ostomy closure (EC) was defined as ostomy closure within 8 weeks after ostomy formation; late ostomy closure (LC) as ostomy closure more than 8 weeks later than the ostomy formation. Reason being that in our hospital the 8 weeks time point is considered the cutoff point, without formal evidence from the literature.

Quality assessment

Study quality was assessed with a checklist as proposed by Downs et al. (11). This checklist contains 27 questions in 5 domains: reporting, external validity, internal validity-bias, internal validity-confounding, and power. Optimal study quality scores 32 points.

Data extraction

Two reviewers (MCS, CEJS), blinded for each other results, extracted the following predefined data: study design, study population, time to ostomy closure, complications following ostomy closure (including wound infection, fistula, wound dehiscence, wound evisceration, bowel obstruction, and anastomotic obstruction), and time to full enteral nutrition after ostomy closure. Discrepancies were resolved by consensus after discussion.

Statistical analysis

Data were analyzed using SPPS (version 17; SPSS, Chicago, IL) and Review Manager (RevMan) software version 5.0 (Copenhagen: The Nordic Cochrane Center, The Cochrane Collaboration, 2008) was used to pool data from the studies for the meta-analysis. Comparisons of dichotomous data were carried out using the Mantel-Haenszel statistical method under assumption of fixed effect analysis model, which derived from the fact that included studies entail similar therapies. Results for comparisons of dichotomous outcomes were expressed as odds ratio (OR) with 95% confidence interval (CI). Heterogeneity of the data was tested using a χ^2 statistic. All statistical tests were performed at the 5% significance level.

RESULTS

Trial flow for manuscript selection

The searches yielded 778 articles, of which 733 were found irrelevant based on the title. Of the 45 remaining articles, 33 were potentially eligible for inclusion in the meta-analysis (Figure 1). Of these, 5 articles met the selection criteria and were included in this study. Four studies compared complication rates after ostomy closure and only one study focused on mean time to full enteral nutrition after ostomy closure.

Characteristics of included studies

The characteristics of the five included studies are described in Table 1. Except for the study of Gertler et al. (14) which was a prospective cohort study, all studies described retrospective cohorts. Sample size ranged from 10 to 92 patients and the five studies concerned in total 253 patients, 160 of whom underwent EC; 93 underwent LC. The gestational age of the patients varied between 25-41 weeks. The main type of ostomy was ileostomy (between 54%-100%). Evaluation of the distal segment for strictures was done in 4 of 5 studies, either preoperative with contrast rontgenography or during ostomy

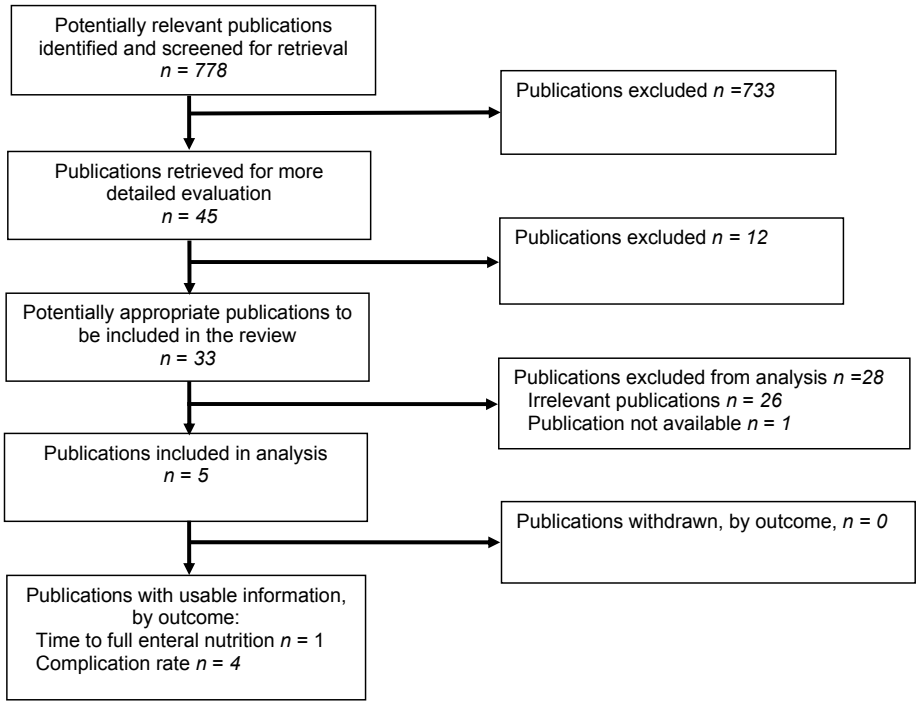

Figure 1 PRISMA flow chart presenting the selection of studies

Table 1 Included publications

Author	Journal of Publication	Year	Study design	n		% Diagnosis NEC	Type of ostomy n (%)			Study quality[a]
				EC	LC		J	I	C	
Al Hudhaif[12]	J Pediatr Surg	2009	Retrospective cohort study	13	24	100	4 (11)	28 (76)	5 (13)	13
Weber[13]	Arch Surg	1995	Retrospective cohort study	92	-	72*	29 (32)	50 (54)	13 (14)	9
Gertler[14]	J Pediatr Surg	1987	Prospective cohort study	3	7	100		10 (100)		8
Musemeche[15]	J Pediatr Surg	1987	Retrospective cohort study	39	50	100	10 (10)†	75 (75)†	15 (15)†	14
Cogbill[16]	Surg Gynecol Obstet	1985	Retrospective cohort study	13	12	100	3 (12)	16 (64)	6 (24)	9

EC indicates early ostomy closure; LC, late ostomy closure; J, jejunostomy; I, ileostomy; C, colostomy
*this number is an indication, 72% of 109 patients with ostomy had NEC, 17 infants died before ostomy closure. Separate number for total number of patients with NEC at ostomy closure were not provided
†in total 100 patients were included but time to ostomy closure was only provided for 89 patients, unfortunately no data were provided to separate these in type of ostomy
[a]study quality as measured by the checklist in Downs et al (optimal study quality scores 32 points)

Table 2 Complication rate in early and late ostomy closure group

	Early ostomy closure			Late ostomy closure		
Study	n	Mean time to closure (days)	Complications n (%)	n	Mean time to closure (days)	Complications n (%)
Weber[13]	92	40	36 (39)	-	-	-
Gertler[14]	3	37	0 (0)	7	131	0 (0)
Musemeche[15]	39	31	9 (23)	50	112	9 (18)
Cogbill[16]	13	56	6 (46)	12	154	7 (58)

closure. The poorest study scored 8 points for study quality; the best study 14 points (median 9 points).

Time to full enteral nutrition

The mean time to full enteral nutrition (FEN) was reported in one study. In the study by Al-Hudhaif et al. (12) FEN was 19.1 days (n=13) in the EC group versus 7.2 days (n=24) in the LC group (*P*=.027).

Complications

Weber et al. (13) only analyzed EC, and found a complication rate of 39% (Table 2); therefore this study could not be used in the meta-analysis. The other 3 studies could be used

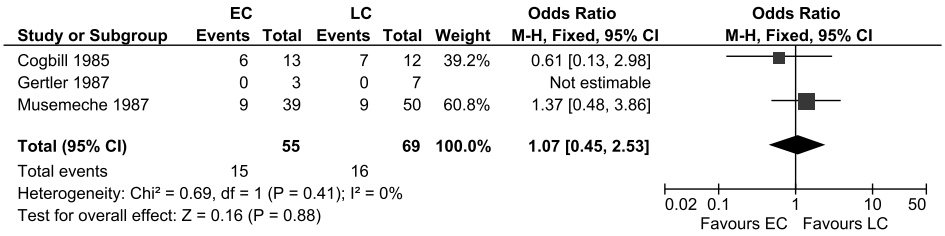

Figure 2 Forest plot comparison of postoperative complications in EC vs LC

for meta-analysis in the forest plot (Figure 2). Combining all 3 studies, the complication rate did not differ greatly between both groups, 27% (15/55) in the EC group versus 23% (16/69) in the LC group. The combined odds ratio (LC vs EC) was 1.1 [95% CI 0.5,2.5].

DISCUSSION

This systematic review showed that complication rate did not differ between early and late closure of ostomy in patients with necrotizing enterocolitis. Only one study provided data on enteral feeding after ostomy reversal favoring late closure. Al Hudhaif et al. found a longer duration to achieve full enteral nutrition in the EC group (19.1 days in the EC group vs 7.2 days in the LC group) (12). These results were not comparable with another study, which found that when ostomy closure occurred at a mean time of 30 days, the mean time to full enteral nutrition was 8 days (17). This study was excluded from the analysis because only 37% of included infants were diagnosed with necrotizing enterocolitis. Unfortunately, due to the limited number and relative low quality of the studies, a systematical analysis of the mean time to full enteral nutrition after ostomy closure was not possible. This systematic review did not bring conclusive evidence on the most favourable timing of ostomy closure in infants with a history of necrotizing enterocolitis.

Early closure is also associated with several other advantages. For one, maintaining a normal fluid and electrolyte balance is best helped by restoration of enterocolonic continuity as soon as possible. This was illustrated by six cases, as reported by Rothstein et al., in which an ileostomy for NEC was complicated by chronic diarrhoea, feeding difficulties, sepsis, rickets and developmental delay. These infants were all readmitted within the next three months due to severe acidosis and dehydration associated with a large-volume ileostomy output. This was resolved after reanastomosis, which illustrates the potential benefit of early ostomy closure (10). Another advantage of early ostomy closure was the possible prevention of distal strictures. The observed rate of distal strictures after ostomy formation was around 40% (2). Early closure of the ostomy might

lead to fewer strictures caused by feedings. This is speculative, however, and should be proven by a randomized controlled trial.

The results of our review should be interpreted with caution given the small sample sizes of individual studies and given the fact that not all studies included an early and late ostomy closure group. Also, the quality of the studies is generally low, mainly due to the mostly retrospective nature of the studies. The data for the meta-analysis regarding complications came from 3 studies only and full-fledged analysis for the time to full enteral nutrition was not even possible. It would also be interesting to construct a receiver-operator characteristic to obtain the most favourable timing of ostomy closure. Unfortunately this was not possible due to limited availability of data points. It is also clear that the type of ostomy has significant impact on the outcome and need for undoing. Since different types of ostomies were included in the studies, the interpretation of the data is more difficult. A jejunostomy is usually associated with extremely high output with electrolyte disturbances and poor absorption of nutrients and need for early undiversion. A well-managed distal ileostomy or colostomy is usually well tolerated with few metabolic consequences and no urgent need for ostomy closure. If the presented patient series were broken-up in different ostomy categories the numbers would have been too small to make any conclusions.

A randomized controlled trial (RCT) could bring conclusive evidence comparing early versus late ostomy closure in terms of time to full enteral feeding, weight gain, complication rate, and duration of hospital stay. Patients should be stratified according to ostomy type. Unfortunately no RCTs were available, and this is a problem encountered very often in pediatric surgery (18). Comparing laparotomy versus laparoscopy for pyloric stenosis has been the subject of many studies, a meta-analysis could even be performed for this topic (19-21). Same counts for different kinds of fundoplication in gastro-esophageal reflux disease (22, 23). In infants with necrotizing enterocolitis the main focus of the studies was peritoneal drain versus laparotomy (24-26). Unfortunately, there are no RCTs available regarding optimal timing of ostomy closure. Since no RCT is available in infants, we reviewed the data of adults regarding timing of ostomy closure. For comparison, in adults with temporary ostomy due to trauma or due to colorectal surgery it is considered safe to reverse ostomy on short time notice. Therefore, this could endorse the safety of earlier closure in infants too.

In conclusion, early closure (< 8 weeks) of an ostomy in infants did not lead to more surgery related complications. A recommendation for early or late ostomy closure cannot be given on the basis of the data from five studies of low quality. Other factors such as parent burden should also play a role in the strategy of timing of ostomy closure.

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Chapter 6

Late versus early ostomy closure for necrotizing enterocolitis: analysis of adhesion formation, resource consumption, and costs

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ABSTRACT

Background: Surgeons prefer to close ostomies at least 6 weeks after the primary operation, because of the anticipated postoperative abdominal adhesions. Limited data support this habit. Our aim was to evaluate adhesion formation – together with an analysis of resource consumption and costs – in patients with necrotizing enterocolitis (NEC) who underwent early closure (EC), compared to a group of patients who underwent late closure (LC).

Methods: Chart reviews and cost analyses were performed on all patients with NEC undergoing ostomy closure between 1997-2009. Operative reports were independently scored for adhesions by two surgeons.

Results: Thirteen patients underwent EC (median 39 days [32-40]), whereas 62 patients underwent LC (median 94 days [54-150]). Adhesion formation in the EC group (10/13 patients, 77%) was not significantly different (*P*=1.000) from the LC group (47/59 patients, 80%). No differences were found in the costs of hospital stay, surgical interventions, and outpatient clinic visits.

Conclusions: Ostomy closure within 6 weeks of the initial procedure was not associated with more adhesions, or with changes in direct medical costs. Therefore, following stabilization of the patient, ostomy closure can be considered within 6 weeks during the same admission as the initial laparotomy.

INTRODUCTION

Necrotizing enterocolitis (NEC) is a common and devastating neonatal gastrointestinal disorder. Others have demonstrated that surgery is warranted in 20-40% of neonates and the associated case fatality rate with surgical intervention is 50% (1-3). While there is considerable debate on optimal treatment of infants with NEC, little consensus exists on the most appropriate surgical intervention (4-8). Surgeons in favour of an ostomy are concerned that ongoing NEC causes breakdown of the anastomosis, that more bowel will be resected than necessary, and that strictures form in the remaining bowel. In contrast, advocates of performing a primary anastomosis try to avoid the complications associated with an ostomy, such as difficulties in achieving full enteral feeds and the potentially difficult access to the abdomen at the time of ostomy closure due to the formation of adhesions inside the abdomen (5, 9).

Adhesions are one of the main concerns at relaparotomy and/or ostomy closure. Adhesions are abnormal deposits of fibrous tissue within the peritoneal cavity and they are the most common cause of small bowel obstruction (10, 11) and underlie a high incidence of (re)admissions (12). Although adhesion formation is one of the main reasons surgeons tend to avoid a relaparotomy before 6 weeks postoperatively in our institution, no data is available in the literature to support this practice. However, early ostomy closure (defined as ostomy closure within 42 days after the ostomy formation) might potentially reduce medical costs, and improve the quality of life of the patients and their families. The aim of this study was to evaluate the presence of adhesion formation at early ostomy closure (before postoperative day 42) in patients with NEC and compare this with the presence of adhesions at late ostomy closure. In addition, a comparison of resource consumption and costs regarding early versus late ostomy closure was performed.

METHODS

Study design

Retrospective chart reviews were performed to identify all infants diagnosed with an acute episode of NEC undergoing lapatoromy and subsequent ostomy closure in our tertiary pediatric surgical centre between January 1997 and December 2009. The infants were divided into two groups: 1) early ostomy closure group (EC), indicating closure between 14-42 days after the first laparotomy; and 2) late ostomy closure group (LC), comprising all infants who underwent closure between 43-365 days after the first laparotomy. According to hospital practice we adopted 42 days as the cutoff point without formal evidence of the literature. To ensure a homogenous study population, infants

with only one primary laparotomy and relaparotomy for other reasons than ostomy closure were excluded from the analysis. All infants with a second procedure either within 14 days of the initial laparotomy or more than 1 year after the first procedure were also excluded. For all infants the following demographic, clinical and surgical characteristics were collected: gender, gestational age at birth, birth weight, co-morbidities, central venous line insertions, and septic episodes. Short bowel syndrome is defined > 70% resection of the small bowel and/or parenteral nutritional needed for longer than 42 days after bowel resection according to consensus guide lines of the Dutch pediatric association (13). In addition data on resource consumption were collected. Approval for this retrospective non-invasive study was waived by our institutional research ethics board.

Adhesion formation

Operative reports were independently scored for adhesion formation by two pediatric surgeons (RK, CM) blinded for the timing of surgery. Adhesion formation was evaluated on the basis of the following criteria: description of adhesions in the operative report was scored as positive if adhesions were described. If the operative report described no adhesions, this was scored as negative. If the operative report left room for interpretation, this was scored as adhesions unknown. In order to semiquantitatively validate the grading of adhesions, the presence of adhesions was correlated with the operative time at ostomy closure.

Cost analysis

Drawing on established methods for costing studies in health care (14), we set out to calculate the direct medical costs for both study groups. The costs that were taken into account compromised the costs of: 1) hospital days, both in ICU (intensive care unit) and non-ICU areas, including the period before the first laparotomy and the time spent in hospitals other than our own, 2) surgical interventions, including all interventions following ostomy closure related to the ostomy closure (this also included central venous catheter insertions), and 3) visits to the outpatient department, restricted to all visits to the paediatric surgery outpatient clinic. All costs were calculated for the year 2006 and reported in Euro (€).

The cost price of a hospital day included the costs of staff, materials, medications, and overheads (e.g., housing, utilities, cleaning, management, etc.). This method resulted in cost prices of € 1,375 per hospital day in an intensive care unit and € 538 for one hospital day in a medium care or high care unit. The cost prices of surgeries consisted both of fixed costs per intervention (e.g., costs for materials, sterilization, and the recovery room) and of variable costs, which depended on the duration of the intervention (e.g., costs for equipment, operating room assistants, anaesthesia nurses, surgeons, and anaesthetists).

Finally, the cost prices of outpatient visits were calculated on the basis of the costs of staff, material, and overheads (e.g., housing), as well as the costs of medical specialists. This resulted in cost prices of € 54 for a first visit to the outpatient department, € 42 for a follow-up visit, and € 35 for a consultation by telephone.

Statistical analysis

Data were analyzed using SPSS (version 15; SPSS, Chicago, IL) and a *P* value of < 0.05 was considered statistically significant. Chi-square test, Fisher's exact test, Mann-Whitney *U* test, or t-test, were used as appropriate to compare demographic data, surgical data, the formation of adhesions, and resource consumption and costs between the two groups. Cohen's kappa was calculated as a measure of agreement; a kappa >0.8 is generally considered good.

RESULTS

Study sample

A total of 198 infants underwent laparotomy for an acute episode of NEC during the study period. 75 infants fulfilled the inclusion criteria and were analyzed in this study (Figure 1). 13 infants underwent EC, median [IQR] time to ostomy closure was 39 days [32-40] and 62 infants had LC (median [IQR] time to ostomy closure 94 days [54-150]). Readmission for ostomy closure was necessary in 1/13 (8%) infants in the EC group and 39/62 (63%) in the LC group.

As demonstrated in Table 1, the groups were similar for demographic characteristics. Over 50% of the performed ostomies were end ileostomies with a mucous fistula. The end ileostomy and the mucous fistula were sutured close to each other in the same wound. Therefore, a limited laparotomy was most of the time sufficient to close the ostomy. However, even in these cases our surgeons would strive to release as many adhesions as possible while performing the laparotomy at the time of closure. The other types of ostomies performed were: double barrel ileostomy, end colostomy, double barrel colostomy, double barrel- and end jejunostomy.

Also, postoperative outcome with regards to central venous line insertions, sepsis due to central venous lines, neurodevelopmental outcome, rickets, short bowel syndrome, and survival one year after the first laparotomy were not different. The majority of septic episodes (13/31 cases, 42%) was caused by coagulase negative staphylococci of which 4 in the EC group and 9 in the LC group. The remainder of septic episodes were caused by S aureus, S epidermidis, Enterobacter cloacae, Klebsiella pneumoniae, S warneri, and bacillus cereus. Some septic episodes were caused by multiple organisms. The amount of blood loss during the primary laparotomy was mentioned in the operative reports

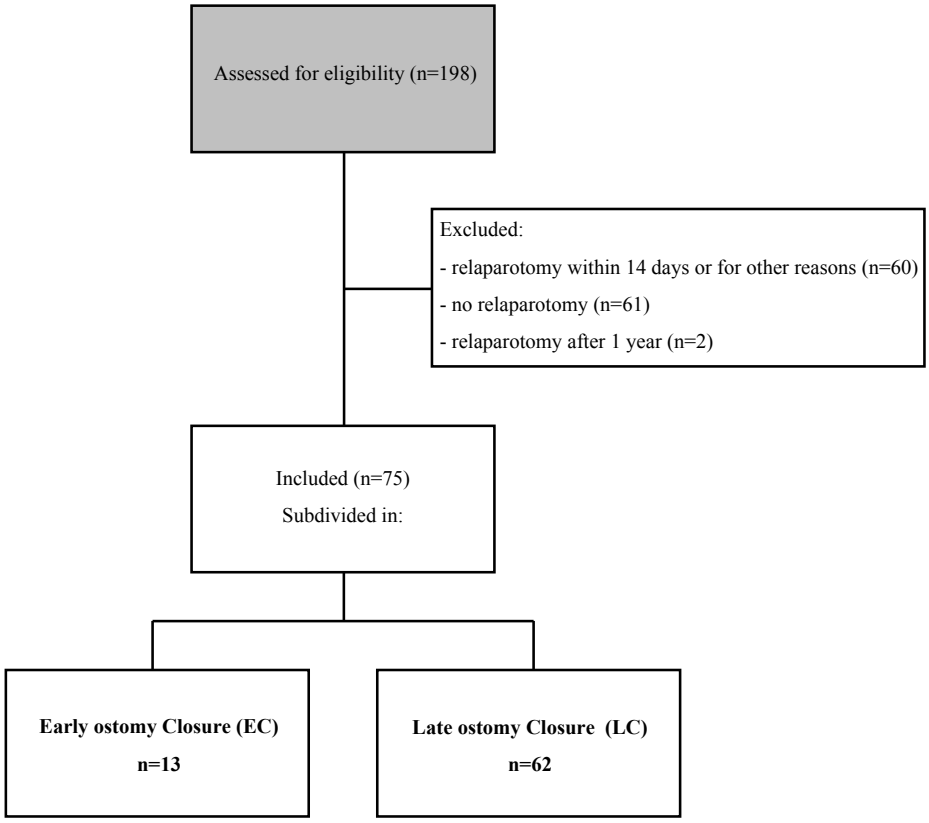

Figure 1 CONSORT diagram

of 13 patients, 12 of these patients needed a blood transfusion intra-operatively. All of these patients received one or more blood transfusions in the first week after surgery.

Adhesion formation

Adhesion formation could not be scored for three infants due to unavailability of surgical reports. Out of the 72 remaining infants whose operation reports could be scored, no comments regarding adhesions in the operative report were made in 5 cases (1 in the EC group) and in 13 cases (18%) only a small incision was necessary at the time of ostomy closure. Of the total group, 57/72 (79%) were found to have adhesions during ostomy closure. The proportion of infants with adhesions in the EC group did not differ significantly from the proportion of infants with adhesions in the LC group. In the EC group 10/13 infants (77%) had adhesions at time of ostomy closure versus 47/59 (80%) in the LC group (P=1.000). The kappa score for the scoring of adhesions between the two independent paediatric surgeons was 0.918.

Table 1 Characteristics

	Early Closure 14-42 days (n=13)	Late Closure 43-365 days (n=62)	*P*
Male sex	7 (54)	35 (56)	.863*
Gestational age at birth (weeks)	$29^{5}/_{7}$ [$27^{3}/_{7}$-$32^{6}/_{7}$]	$28^{5}/_{7}$ [$26^{1}/_{7}$-$31^{6}/_{7}$]	.320†
Birth weight (grams)	1335 [918-1725]	1010 [805-1554]	.272†
Patent ductus arteriosus	4 (31)	18 (29)	1.00‡
Infant respiratory distress syndrome	8 (62)	29 (50)[a]	.452*
Age at first laparotomy (days)	12 [7-19]	12 [8-22]	.585†
Type of ostomy			
End ileostomy plus mucous fistula	7 (54)	37 (60)	.638‡
Double-barrel ileostomy	5 (38)	13 (21)	
End colostomy		6 (10)	
End jejunostomy	1 (8)	4 (6)	
Other		2 (3)	
Number of central venous lines	0 [0-1]	1 [0-1]	.392†
Sepsis due to central venous line	7 (54)	24 (39)[c]	.336*
Rickets	0 (0)	8 (13)[b]	.336‡
Short bowel syndrome	2 (15)	5 (8)	.597‡
Normal neurodevelopmental outcome	11 (85)	48 (87)[d]	1.00‡
Survival 1 year after first laparotomy	12 (92)	60 (97)	.440‡

Values are expressed as n (%) or median [IQR]

*Chi-square, †Mann-Whitney U, ‡Fisher's exact [a]n=58, [b]n=60, [c]n=61, [d]n=55

Although the operative time at ostomy closure was not significantly prolonged when adhesions were present (*P*=.153), the median [IQR] operative time demonstrated a trend towards longer time when adhesions were present (95 [77-116] minutes in the patients with adhesions vs 81 [71-97] minutes in the patients with no adhesions at ostomy closure). Unfortunately, this analysis could only be performed for the entire group, since only 2 patients in the EC group did not have adhesions at ostomy closure. This group was too small to perform a statistical analysis.

A perforation of the bowel was diagnosed in 46 cases at primary surgery; in this group of patients 36 cases (78%) had adhesions at ostomy closure. Of all patients who did not have a perforation, 81% had adhesions at ostomy closure. This was not statistically different (*P*=.815).

Cost-analysis

Resource consumption and costs data are shown in Table 2. The mean number of hospital admissions was 1.2 in the EC group versus 1.8 in the LC group (*P*=<.001). This difference is a result of the fact that 85% (11 out of 13) of the infants in the EC group were only

Table 2 Resource consumption and costs

	Early Closure	Late Closure	*P*
Hospitalizations			
Readmission for ostomy closure	1 (8 %)	39 (63 %)	<.000*
Total length of stay (days)	100 [83-119]	96 [73-141]	.900†
Costs of hospital days (€)	€ 85,606 [€ 78,363-€ 119,930]	€ 102,721 [€ 64,353-€ 138,930]	.790†
Surgical procedures			
Operative time ostomy closure (minutes)	109 [65-118]	91 [77-115][a]	.901†
Relaparotomy after ostomy closure	3 (23)	11 (18)	.699‡
Number of relaparotomies	0 [0-1]	0 [0-0.25]	.550†
Costs of all surgical procedures (€)	€ 2,830 [€ 2,415-€ 5,370] [b]	€ 3,133 [€ 2,732-€ 4,082][c]	.889†
Visits to the surgical outpatient clinic			
Number of visits	2 [0.5-5.5]	3 [1-6.3]	.667†
Costs visits to outpatient clinic (€)	€ 96 [€ 27-€ 242]	€ 155 [€ 54-€ 295]	.583†
Total costs (€) of hospital days, surgeries, visits to outpatient clinic	€ 92,953 [€ 83,944-€ 128,740] [b]	€ 96,554 [€ 63,526-€ 141,420][c]	.875†

Values are expressed as n (%) or median [IQR]

*Chi-square, †Mann-Whitney U test, ‡Fisher's exact test. [a]n=59, [b]n=12, [c]n=51

admitted once and 29% (18 out of 62) of the LC group. Yet, the total length of stay was not different (*P*=.900) between the groups (a median of 100 days in the EC group versus 96 days in the LC group). No differences were observed for the number of days spent in an ICU (in both groups median of 52 days; *P*=.450). We observed a trend towards lower median total costs for hospital admissions in the EC group when compared to the LC group (€ 85,606 versus € 102,721) this did not reach statistical significance (*P*=.790).

Median operative time for ostomy closure was similar (*P*=.901) for both groups (109 minutes in the EC group vs 91 minutes in the LC group). In the EC group, 4 infants had one or more relaparotomies after ostomy closure (31%) versus 14 infants in the LC group (23%, *P*=.499). The main reasons for relaparotomy after ostomy closure were intestinal stenosis requiring additional resection (7/18 cases, 37%), followed by ostomy closure (a new ostomy was formed at time of ostomy closure) in 3/18 (17%), adhesiolysis in 2/18 cases (11%) and incisional hernia (2/18 cases, 11%). In the EC group a median [IQR] of 2 [2-3.5] surgical procedures were performed, including the initial ostomy formation, ostomy closure, and procedures for insertion of central venous line catheters. Median [IQR] of 2 [2-3] surgical procedures were performed for the LC group, this was not statistically significant (P=.809). The median total costs of all surgical procedures were comparable in the two groups (€ 2,830 versus € 3,133; *P*=.889).

As demonstrated in Table 2, the costs of outpatient visits were not different between the study groups. The median total direct medical costs calculated in this study were € 92,953 in the EC group, and € 96,554 in the LC group (*P*=.875).

DISCUSSION

This study demonstrates that patients with NEC and early ostomy closure do not have more adhesions than patients with NEC and late ostomy closure. We also observed similar operative times in the early ostomy closure group when compared to the late ostomy closure group. Furthermore, total costs of either early or late ostomy closure were the comparable. Thus our results do not support the assumption of a 'hostile' abdominal environment during early relaparotomy and ostomy closure could be considered at an earlier time during admission. This has potential benefits for the patients and parents.

To our knowledge, no data are currently available addressing the presence of adhesions during relaparotomy in infants. In one study in adults, 504 of 752 (67%) of patients demonstrated peritoneal adhesions at autopsy (15). Similar percentages of adhesions were observed in a study by Menzies et al., out of the 210 patients undergoing previous operations, 195 (93%) had adhesions at relaparotomy (16). Unfortunately, times between initial laparotomy and relaparotomy were not reported in this study. These numbers corroborate with the percentages observed in our study: in total 79% of all infants had adhesions at relaparotomy.

No consensus exists regarding timing of ostomy closure in NEC. Currently, most pediatric surgeons tend to wait for at least six weeks before a second laparotomy is considered, since this time is long enough for intestinal strictures to occur and be diagnosed either clinically or with a contrast enema prior to the second laparotomy. These strictures occur in 6-33% of the cases and can be treated at the same time during ostomy closure (17). Moreover, few studies have addressed the timing of ostomy closure and the available data are conflicting. Recently Al-Hudhaif et al. concluded that ostomy closure should be deferred until at least 10 weeks after ostomy formation. Their data demonstrated that ostomy closure within 10 weeks (13 patients) was associated with longer postoperative ventilation, longer time for total parenteral nutrition, and a longer length of stay when compared to ostomy closure after 10 weeks of ostomy formation (24 patients). Survival rates and anastomotic complications were equal (18). In comparison, other studies either advocated early ostomy closure or did not show any problems with early closure. Early restoration of continuity of the intestinal tract (defined as before 3 months of age or under a weight of 2.5 kg) did not make a difference in 100 patients with NEC (19). In a study conducted by Gertler et al. the same conclusion was drawn in 10 patients with NEC who underwent closure of ileostomy at a mean age of 18 weeks (range 5 to 36

weeks). However, the weight gain per week in this group significantly improved after closure (20). Elective restoration of intestinal continuity within 4 weeks in 27 neonates who had a temporary exteriorization of the small intestine after laparotomy, demonstrated no mortality, a leakage rate of 10% and full enteral nutrition after a mean of 8 days (21). None of these studies included an analysis of resource consumption or costs. To determine the optimal timing of ostomy closure in infants, a randomized controlled trial is warranted.

Another advantage of early ostomy closure is that morbidity associated with ostomies is avoided. Complication rates associated with an ostomy are known to vary from 24% to 68% (19, 22-26). Out of 109 patients, of whom 79 were diagnosed with NEC, 28% experienced a stricture, prolapse, or fistula (23). Early ostomy closure could improve intestinal adaptation. In one study, ostomy closure resolved the chronic diarrhoea in six infants with NEC who had an ileostomy which had resulted in prolonged hospitalization (27). In another study weight loss, severe fluid and electrolytes losses and metabolic acidosis were observed in 11 of 16 patients with an ileostomy after bowel resection for NEC (26). The potential benefit of a colon in continuity with the small intestine is subject of discussion. One study demonstrated improved survival with an intact colon (28). However, a recent multivariate analysis showed that the colon did not play a significant role in intestinal adaptation (29).

Although early closure seems to have a lot of advantages, not all patients are good candidates for early ostomy closure. For instance, the corrected gestational age and weight should be considered. Although there is little evidence in the existing literature, specific measures are required for low birth weight infants undergoing anaesthesia (30); also, anaesthesia below 1 year of age is associated with greater mortality and morbidity (31, 32). In view of these new insights, surgeons may consider to wait with ostomy closure until weight is above 1000 or 1500 grams. Other criteria for postponing ostomy closure to consider are current medical problems requiring treatment, such as an infection or neurological problems such as epileptic seizures and/or recent intracranial haemorrhages.

Cost-effectiveness in neonatal surgery has gained growing interest in the last decades (33-35). In the current era of cost containment, it is increasingly important to demonstrate whether treatments are cost-effective. We did not show a difference in hospital costs when the costs of early versus late closure of the ostomy were compared. This can in part be explained by the fact that most infants with NEC are born premature and have to stay in hospital as a result of conditions related to prematurity. The economic aspects of this study were restricted to the costs of health care treatments. Apart from possible differences in costs, it should be noted here that early ostomy closure may have an advantage in terms of parental burden, as it required less hospital admissions than were observed in the LC group. To get a more complete impression of the cost-

effectiveness of early versus late ostomy closure, an important aspect for future studies to include would be both patient outcomes (such as pain/discomfort, functioning, and quality of life, bearing in mind the young age of the patients) as well as outcomes in the parents (burden of care and quality of life), during a follow-up period as long as needed to capture all relevant outcomes.

The extent and type of adhesions were not scored in this study. Due to the retrospective character of our study, we were not able to score the number of adhesions and classify the type of adhesions as described by Swank (36). We expect that there is a difference in the type of adhesions between early and late relaparotomy, namely more filmy, less vascularized and easier to separate adhesions in the latter group. A prospective study would be able to address the extent and type of adhesions better. Unfortunately, because of the retrospective evaluation the number of patients in the early relaparotomy group was rather small, however both groups were comparable regarding demographics and clinical characteristics. Also, although in a small number of cases only a small incision was necessary at time of ostomy closure, the presence of adhesions was still reported in the operative report. Finally, we were unable to retrieve sufficient data to comment on time to transition to enteral feeds for the two groups, the blood loss during surgery and the amount of blood transfusions needed during and the first week after surgery. A few of our patients developed rickets which could be avoided by vitamin supplementation. All of our patients receive vitamin supplementation, however it is possible that we were dealing with a mixed picture of real rickets and so called 'osteoporosis of prematurity'. This is a frequently observed co-morbidity in this very vulnerable group of patients (37).

We conclude that adhesion formation during early ostomy closure (within 6 weeks of the initial laparotomy) was similar to adhesion formation during late ostomy closure (more than 6 weeks after initial laparotomy). Moreover, costs were comparable between the two groups. Therefore, after stabilization of the patient, ostomy closure should be considered within 6 weeks and possibly earlier during the same admission period as the initial laparotomy.

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Chapter 7

Does the colon play a role in intestinal adaptation in infants with short bowel syndrome?

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ABSTRACT

Purpose: We sought to examine in a multiple variable model the impact of residual colonic length on time to intestinal adaptation in a cohort of infants with Short Bowel Syndrome (SBS).

Method: Infants with a surgical diagnosis of SBS who underwent operation ≤90 days of age were included in this analysis. Univariate cox-proportional hazards models for time to full enteral feeds were developed. Predictors significant at the 0.2 level were entered into a stepwise multiple variable (MV) cox-proportional hazards model.

Results: 106 infants were included in the cohort (70 adapted). Predictors meeting criteria for the MV model were: Multidisciplinary management (p=0.045), STEP procedure (p=0.057), percent small bowel (p<0.001), percent large bowel (p<0.001), preserved ileocecal valve (p=0.001), number of septic (p<0.001) and central line complications (p<0.001). The final model included: Multidisciplinary management (HR [hazard ratio]: 1.932, 95% CI [confidence interval] 1.137-3.281), percent small bowel (HR: 1.028, 95% CI: 1.02-1.04) and septic events (HR: 0.695, 95% CI: 0.6-0.805).

Conclusions: The colon does not appear to play a significant role in intestinal adaptation. However, in addition to highlighting the importance of residual small bowel length, our model highlights the benefit of multidisciplinary intestinal rehabilitation and reduction of septic complications in achieving intestinal adaptation.

INTRODUCTION

Although there is no absolute relationship between the residual small intestinal length and the probability of adaptation and freedom from parenteral nutrition in children with Short Bowel Syndrome (SBS), the importance of the degree of small bowel loss is well established (1-3). Despite this, the independent role of the colon in achieving intestinal adaptation has not been well described in pediatric patients. The objective of this project was to examine the independent role of the colon in time to intestinal adaptation as evidenced by the time to weaning of parenteral nutrition (PN) and establishment of full enteral feeds.

METHODS

Subjects

This paper represents a retrospective analysis of prospectively collected data with the specific goals of determining the role of the large intestine in time to tolerance of full enteral feeds in infants with Short Bowel Syndrome (SBS). Data on children with SBS who underwent operation within the first 90 days of life, prior to 31 December 2007, were obtained from our intestinal failure registry. Accrual dates for our intestinal failure registry were from January 1, 2003 through December 31, 2007 and also include a historical cohort from January 1997 – December 1998. Enrollment criteria for our registry are all infants, managed on our quaternary pediatric surgical service, who met the Canadian Association of Pediatric Surgeons' case definition for SBS. This definition includes children who for congenital reasons or following a bowel resection have an intestinal length of less than the 25th percentile for gestational age, or remain on parenteral nutrition (PN) at 42 days following resection (4). There is at least 1-year of follow-up on all patients in the database. Data from the registry on subjects operated on prior to 1 January 2006 have previously been reported by us (4-6).

Since 2003, children meeting criteria for inclusion in the registry were managed by our multidisciplinary intestinal rehabilitation team, GIFT (Group for Improvement for Intestinal Function and Treatment), as has been described previously (6). Prior to formation of GIFT, children with SBS were managed by one of the pediatric surgeons at our institution. Detailed demographic, surgical, nutritional, biochemical, and therapeutic data were collected on all subjects in our SBS registry. Approval for this study was obtained from the research ethics board at our institution (REB # 1000012601).

Study design and analysis

All statistical analyses were performed using SPSS (version 14), and statistical significance was set as an alpha of 0.05 with a 0.1 being considered to be a trend.

In order to explore the role of the colon in the development of enteral tolerance, initially univariate cox proportionate hazard models with time to tolerance of 100% of feeds enterally were performed with a number of predictors. Predictors were chosen a priori and included: multi-disciplinary intestinal rehabilitation, age at surgery, gender, birth weight, gestational age, etiology of SBS, Serial Transverse Enteroplasty Procedure (STEP), percent small bowel remaining, percent large bowel remaining, resection of the ileocecal valve, total septic episodes, and number of central line complications. Predictors that were significant at the 0.2 level were entered into a backward stepwise multiple variable (MV) cox-proportional hazards model.

RESULTS

Study sample

One hundred and six patients were included in the present study. Median age at operation was 4 (range: 0 – 81) days of life. Sixty-two of the patients were male and the median gestational age was 33 (range: 24 – 41) weeks. The etiology of SBS is included in Table 1**,** the most common etiology was necrotizing enterocolitis (NEC) (31% of patients) followed by abdominal wall defects in 23%. Thirty six of the subjects were managed prior to the established of our GIFT team and 70 were managed by this multi-disciplinary intestinal rehabilitation team.

Table 1 Etiology of short bowel syndrome

Necrotizing enterocolitis	33	(31%)
Abdominal wall defect	24	(23%)
Intestinal atresia	18	(17%)
Meconium ileus	15	(14%)
Malrotation and volvulus	8	(8%)
Spontaneous perforation	5	(5%)
Hirschsprungs disease	3	(3%)

The median residual small bowel length was 78 (range 0 - 175) cm representing a median 78 percent length for gestational age according to norms established by Touloukian (7). The median residual percent colonic length for gestational age was 50% (range 0-100). Eighty-two (77%) of the subjects had preservation of their ileocecal valve

and 68 (64%) subjects were left with a stoma (30 ileostomy, 31 colostomy, and 6 sigmoid colostomy). Twelve patients underwent a STEP.

Overall, 70 (66%) of the subjects demonstrated intestinal adaptation after a median of 90 (range: 16- 680) days on parenteral nutrition by the end of the observation period. Median follow-up of the 36 patients who did not adapt was 232 (range 7 – 1021) days. During this follow-up period, 26 of the 36 patients died primarily from liver disease and sepsis (18 patients). There were 2 deaths in the group of patients who adapted.

Univariate predictors of adaptation

Table 2 shows the results of the univariate analyses for the time to intestinal adaptation. A hazard ratio greater than 1 suggests that the factor is associated with an increased probability of timely adaptation; factors associated with this outcome significant at the 0.05 level were a greater percent small and large bowel remaining, multi-disciplinary intestinal rehabilitation, and retention of the ileocecal valve. Number of septic episodes

Table 2 Univariate predictors of intestinal transplantation

Variable	Category	Hazard Ratio	**95% CI[1]**	***P*-value**
Intestinal rehabilitation	No	reference		
	Yes	1.69	1.01-2.81	0.045
Gender	Male	reference		
	Female	0.76	0.47-1.23	0.268
Age at surgery		0.99	0.98-1.01	0.912
Birth weight		1.00	1.00-1.00	0.732
Gestational age		0.97	0.91-1.02	0.248
Etiology of SBS	NEC[2]	reference		
	Atresia	1.22	0.59-2.54	0.595
	AWD[3]	1.00	0.51-1.96	0.996
	Volvulus	0.88	0.33-2.38	0.799
	Hirschsprungs	1.67	0.39-7.24	0.494
	Meconium ileus	1.62	0.77-3.38	0.202
	Spont perforation	3.18	1.06-9.56	0.040
STEP[4] procedure	No	reference		
	Yes	0.44	0.19-1.10	0.057
Percent small bowel remaining		1.04	1.02-1.05	<0.001
Percent colon remaining		1.01	1.01-1.02	<0.001
Ileocecal valve resected	Yes	reference		
	No	3.35	1.64-6.81	0.001
Total septic events		0.66	0.57-0.76	<0.001
Line complications		0.72	0.62-0.83	<0.001

[1] Confidence interval
[2] Necrotizing enterocolitis
[3] Abdominal wall defect
[4] Serial transverse enteroplasty

and line complications were both statistically significant univariate predictors associated with a decrease in the likelihood of intestinal adaptation. There was a trend for the requirement for a STEP procedure to be associated with a longer adaptation time.

Multiple variable predictors of adaptation

All variables with a p-value less than 0.2 on the univariate analyses were entered into a multiple variable cox proportional hazards with backward selection. The variables that were entered into this model included: multi-disciplinary intestinal rehabilitation, STEP, percent small bowel remaining, percent large bowel remaining, resection of the ileocecal valve, total number septic events, and number of central line complications. Variables not selected for the multiple variable model included: age at surgery, gestational age, gender, birth weight, and SBS etiology. The final multiple variable model (Table 3) included 3 terms, 2 that were associated with an increased probability of timely adaptation: Multidisciplinary management (HR [hazard ratio}: 1.86, 95% CI [confidence interval] 1.09-3.17) and percent small bowel remaining (HR: 1.03, 95% CI: 1.02-1.04) and one, septic events, that was associated with a decreased probability of adaptation (HR: 0.7, 95% CI: 0.6-0.81).

Further examination of the role of the colon in intestinal adaptation

Given that the primary objective of this study was to explore the role of the colon in intestinal adaptation, we performed a number of post-hoc analyses when we discovered that the colon was not included in our final multiple variable model. First, we "forced" the percent colon into a model that also contained multi-disciplinary management, small bowel length and septic events. This did not result in an appreciable change in the estimates for the 3 parameters in the original multiple variable model. The hazard ratio for percent colon in this model was 1.002 (95% confidence interval 0.995 – 1.009) (Table 3).

In a subsequent analysis we created a variable that categorized patients as having either some residual colon or no colon at all. This variable was also "forced" into a model that also contained multi-disciplinary management, small bowel length and septic events. Similar to the previous analysis, we did not demonstrate any evidence to support the notion that having colon in-continuity resulted in an increased probability of timely intestinal adaptation (Hazard Ratio: 1.66, 95% confidence interval 0.26 – 1.67). Similar to our previous analysis there was minimal impact on the remaining 3 terms in the model that remained statistically significant with similar parameter estimates relative to our primary analysis (Table 3).

Table 3 Multiple variable models of intestinal adaptation

Term	HR[i]	95% CI[ii]	*P*-value
Final multiple variable model			
Multi-disciplinary management	1.86	1.09-3.17	0.022
Percent small bowel	1.03	1.02-1.04	<0.001
Number of septic episodes	0.70	0.60-0.81	<0.001
Alternate models			
Model with colon percentile 'forced in'			
Multi-disciplinary management	1.85	1.09-3.15	0.023
Percent small bowel	1.03	1.02-1.04	<0.001
Number of septic episodes	0.70	0.60-0.81	<0.001
Percent colon	1.00	1.00-1.01	0.646
Model with presence of any colon 'forced in' (dichotomous)			
Multi-disciplinary management	3.37	1.48-9.44	0.005
Percent small bowel	1.04	1.02-1.06	<0.001
Number of septic episodes	0.66	0.54-0.82	<0.001
Any residual colon	0.66	0.26-1.67	0.383

[i] Hazard ratio

[ii] Confidence interval

DISCUSSION

Although, there is no absolute threshold for residual small intestinal length that determines whether an infant with Short Bowel Syndrome, will or will not demonstrate intestinal adaptation and the ability to be weaned from parenteral nutrition (PN), the degree of small intestinal loss is the most important predictor of intestinal adaptation (1-3). While the role of the small intestine is clear, there is less evidence to support the role of the colon in achieving adaptation. There are a number of theoretical reasons why residual colon length should be an important contributor to intestinal adaptation namely: improved absorption of fluid and electrolytes (8), bacterial metabolism of undigested carbohydrates and amino acids to produce short chain fatty acids (SCFAs) leading to improved caloric intake (9-11), the trophic effect of enteric hormones whose release is facilitated by the colon such as Glucagon-like Peptide 2 (GLP-2) (12-14) as well as release of Peptide YY which may result in slowing of gastric emptying (15).

In a study of adult patients mainly with Crohns disease, Nightingale et al. demonstrated that preservation of the colon was beneficial in terms of weaning from PN (16). The authors suggested that ½ of residual colon had the impact of up to 50cm of small intestine. In addition to improved weaning from PN, there was also a decreased need for electrolyte supplementation in those with a preserved colon. Similarly, Nordgaard

demonstrated a beneficial impact of the colon in terms of energy intake in adult patients with SBS (10). Examination of the impact of the colon in pediatric patients has been more mixed with Kauffman et al., failing to demonstrate any difference in time to PN weaning in those with and without a colon (17). Spencer et al., suggested that the colon was predictive of weaning from PN (1) and Goulet et al., suggested that although their sample size was too small for statistical analysis, it appeared that adaptation times were longer for children having resection of colon (2). Quiros-Tejeira et al suggested improved survival with an intact colon (18).

Although the role of colon in intestinal adaptation in infants has not been well studied, particularly when one considers degree of colon loss, rather than when considering colon as a dichotomous variable, a number of authors have examined the role of preservation of the ileocecal valve (ICV). We view the ICV to be of relevance when considering the role of the colon in intestinal adaptation as the ICV could be considered to represent a surrogate marker for preservation or loss of the right colon although it may also be argued as an important marker for loss of distal ileum. Overall, it appears that the preservation of the ICV is an important predictor of outcome (1, 19, 20), although not all series support this notion (3, 20). Even though we view the ICV to be of benefit in SBS, whether the beneficial impact of the ICV is primarily related to ileal preservation or preservation of the right colon is uncertain.

The present study was a retrospective analysis of data from our prospectively collected SBS registry with the goal of examining the impact of the degree of colon loss on time to weaning from PN. Of the 106 subjects enrolled in the registry, adaptation occurred one-third within a median of 90 days of operation. Although the degree of colon loss was an important predictor of the time to intestinal adaptation, this variable did not appear in our final multiple variable model that was built via a stepwise selection process that included all important univariate predictors. The variables included in the multiple variable model were residual small bowel length and management by our multi-disciplinary intestinal rehabilitation program which were associated with decreased time to adaptation and septic episodes which increased time to adaptation. Interestingly, although preservation of the ICV was a significant univariate predictor, this variable was not included in the final model.

Our initial hypothesis was that residual colon would be associated with improved intestinal adaptation, and we were surprised that our analysis did not demonstrate this to be the case. For this reason, we performed 2 additional post-hoc analyses. In the first of these analyses, we "forced" the colonic length into a model containing the 3 variables in our final model. In the second model we created a variable that specified whether the subject had any residual colon or total loss of their colon and "forced" this variable into a model containing the 3 variables in our final model. Neither of these post-hoc analyses demonstrated any evidence to support the role of the colon in terms of time to weaning

of PN support. Although it would have been optimal to examine the region of colon preserved or lost, these data were not available in the registry, but we view the analysis that dichotomized the subjects as to whether or not they had any residual colon to be the most extreme and since this analysis did not yield a positive result, we feel confident in our conclusion.

One can speculate on the reasons why colon was not a significant predictor of time to intestinal adaptation. First, it is possible that although degree of colon loss has been clearly demonstrated in adults to be associated with intestinal adaptation, neonatal SBS may be a fundamentally different pathophysiologic process. Second, it is possible that the small intestine is the primary predictor of intestinal failure whereby the setting of substantial macronutrient malabsorption by the small bowel, the resultant negative osmotic effects on the colon would negate any theoretical benefit in terms of enhanced absorption. It may be that the inherent adaptive and growth potential of the newborn and infant small intestine is the driving force of adaptation (21). In addition to the typical adaptive responses to bowel loss in the infant, young children are also experiencing normal developmental changes. In adults where small intestinal growth and development are limited, the colon may plan a more important role in ultimate weaning from parenteral nutrition. Finally, although we stated enhanced GLP-2 release as a theoretical benefit for the colon, Sigalet et al. have challenged the importance of the colon in GLP-2 release in infants (14).

In conclusion although we set out to demonstrate the importance of the colon in intestinal adaptation, our data suggest that the colon does not appear to play a significant role in intestinal adaptation. However, our data do confirm the importance of residual small bowel length. As well, our model highlights the benefit of multidisciplinary intestinal rehabilitation including the importance of reducing septic complications in achieving intestinal adaptation in infants.

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Chapter 8

The gap in referral criteria for pediatric intestinal transplantation

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Accepted Transplantation

ABSTRACT

Background: Advancement in treatment of children with intestinal failure did not lead to change in generally accepted referral criteria for intestinal transplantation. Therefore, a study was conducted to evaluate the current referral criteria and to identify potential new criteria for pediatric intestinal transplantation among transplant centers in Europe, and USA/Canada.

Methods: The literature was searched to identify discussion points regarding current referral criteria and potential needs for extension. Questionnaires were sent to 50 centers performing pediatric intestinal transplantation. Closed ended questions were analyzed with descriptive statistics. Open ended questions were analyzed by 2 reviewers using the thematic analysis method. Data were analyzed with SPSS 17.

Results: 18 questionnaires were completed (36% response rate; 14 European centers and 4 American/Canadian). 77% of respondents considered referral of children as too late and suggested that education of referring hospitals could improve this. The current referral criteria were considered too general in 50%. More specifically, respondents suggested that 'persistent hyperbilirubinemia' must be defined by a time and value limit and the list of referral criteria should include recurrent septic episodes and fluid/electrolyte disturbances.

Conclusions: Referral criteria for pediatric intestinal transplantation can be improved by defining more specified decision moments and by educating referring hospitals.

INTRODUCTION

About 15% of children with intestinal failure develop life-threatening complications despite optimal medical and surgical treatment (1). For this group of patients intestinal transplantation either isolated or as combined liver-intestinal transplantation, may be an option.

To achieve long-term survival in children with intestinal failure early referral and listing for intestinal transplantation is essential. This pre-transplant assessment is crucial because the survival rate for patients who are transplanted while still waiting at home is 15% higher than for those who are transplanted while staying in the hospital waiting for a transplant (2).

The referral criteria for intestinal transplantation, as described by Kaufman et al. in 2001 (3), were hardly modified when compared with the referral criteria as described in a recent update by Avitzur et al. in 2010 (4). Ten years of development in medical and surgical techniques were hardly reflected in adjustments of the referral criteria. Changes in the criteria consisted of omitting recurring sepsis out of the list and adding the request of the patient or family to the referral criteria (Table 1).

In the literature different analyses are available regarding the outcome of children who had an intestinal transplant. In commenting on the results it was suggested that there may be an unidentified 'grey area' in the referral criteria for intestinal transplantation. Moreover, since not all transplant centers have their referral and listing criteria published online, they may have adjusted these criteria to suit their local practices. This could lead to differences between centers when selecting patients for intestinal transplantation.

Table 1 Referral criteria in Kaufman (2001) and Avitzur (2010)

Kaufman et al, 2001	Avitzur et al, 2010
Intestinal failure with impending life-threatening complications - Liver disease - Recurring sepsis - Impending loss of central venous access	Children with massive intestine resection
	Children with severely diseased bowel and unacceptable morbidity
	Microvillous inclusion disease or intestinal epithelial dysplasia
	Persistent hyperbilirubinemia (>6 mg/dL)
	Thrombosis of 2 of 4 upper body central veins
Intestinal failure that virtually always results in early death despite optimal parenteral nutrition - Extreme short bowel syndrome - Congenital intractable epithelial (mucosal) disorders	Continuing prognostic or diagnostic uncertainty
	Request of the patient or family
Intestinal failure with high morbidity and poor life quality	

The aim of this questionnaire based study was to evaluate and compare the current referral criteria as described in the literature (4) with the referral criteria used in pediatric transplant centers in Europe, United States of America, and Canada. In addition, we investigated whether changes to the current referral criteria were considered desirable and should be introduced or added to the current list of referral criteria.

METHODS

Study design

The referral criteria as described by Avitzur et al. (4) were used as a guideline to create the questionnaire. PubMed was used to identify all articles, which discuss referral criteria and recent results regarding pediatric intestinal transplantation. Bibliographies of all selected articles were screened to identify any additional articles. The selected articles were used to identify discussion points and pitfalls regarding the current referral criteria and potential new referral criteria (short summary of a few points in Table 2).

Table 2 Short summary of discussion points

- The natural history of parenteral nutrition-induced liver disease is that of progressive liver failure and death 6 to 12 months after the onset of cholestasis (defined as bilirubin >100 µmol/l (5).
- Prolongation of prothrombin time is a sign of parenteral nutrition-associated liver disease, this usually occurs in a later phase of the disease (5).
- Early listing can increase life expectancy and quality-adjusted life years as determined by a Markov analytic model (6-8).
- The residual small intestinal length is an important predictor to achieve autonomy from parenteral nutrition (9).
- Not only is the length of the intestine important in patients with intestinal failure but also assessment of the function of the intestine. Serum citrulline (non-essential amino acid produced primarily in the enterocyte) is a potential biomarker of functional enterocyte mass; this could be correlated to the absorptive capacity (10).

A short summary of recent papers (1997-2011) handling the referral criteria for pediatric intestinal transplant centers

Questionnaire

The questionnaire was developed by a research fellow (MCS), and an experienced transplant surgeon (JIJ). An expert in the field of pediatric intestinal transplantation reviewed the questionnaire (DG), and gave comments and suggestions to improve the questionnaire (available online; SDC, Material and Methods).

The questionnaire consisted of two parts. Part one included general queries of the respondent and questions regarding the center's experience. When centers were currently not performing any intestinal transplants, they were asked to continue to section two.

Part two focused on the referral criteria as published in Avitzur et al. (4) and included detailed questions regarding current referral criteria and potential new referral criteria. For this manuscript we have not used the results of the cases which were part three of the questionnaire. The conclusions which were drawn from these cases were similar to the results from the questionnaire.

Questions were either closed (tick boxes) or open ended. Most questions consisted of the format yes – no. When multiple boxes could be ticked, this was mentioned as a comment ('multiple options are possible'). Respondents could also choose to answer the question with 'other' if they thought the appropriate answer was not in the list of options. If necessary, respondents could place comments if they wanted to elaborate on the answer they had chosen.

Study centers

Pediatric transplant centers were identified using the following website: www.intestinal-transplant.org. In total 50 centers were identified in Europe (24 centers), United States of America (USA, 23 centers) and Canada (3 centers). Respondents could complete the questionnaire either online (created via SurveyMonkey using a unique email account), or they could fill in the paper version and return it in a prepaid return envelope. Two reminders were sent to all centers by email, the first after two weeks and the second after 6 weeks. All responses were dealt with anonymously.

Statistical analysis

Data were analyzed using SPSS (version 17; SPSS, Chicago, IL). Questions with tick boxes were represented by numbers and percentages. Analysis of the open ended questions was done by 2 independent reviewers and was analyzed using the thematic analysis method (11).

RESULTS

Study centers

In total 18 out of 50 centers completed the questionnaire (response rate 36%) of which 17 centers fully completed the questionnaire (Table 3). Of the 18 respondents 14 had an active pediatric intestinal transplant program, and these centers accounted for 12% of the yearly performed pediatric intestinal transplants.

The program for pediatric intestinal transplantation was initiated in the different centers between 1987-2010. Most of the pediatric intestinal transplant programs were started before 2003, although a small number also started recently (2009-2010: 3 centers). Besides isolated intestinal transplantation all centers also performed liver

Table 3 Characteristics of the respondents

Geographic location of transplant center	
Europe	14 (88)
USA/ Canada	4 (22)
Respondent	
Pediatric surgeon	13 (72)
Pediatric gastroenterologist	4 (22)
Pediatric hepatologist	1 (6)
Gender of the respondent	
Male	17 (94)
Female	1 (6)
Work environment of the respondent	
Pediatric transplant center	9 (50)
Both pediatric and adult transplant center	9 (50)
Age of the respondent (years)	48 [45-55]
Experience in the field of pediatric intestinal transplantation (years)	17 [9-25]

Data are expressed as n (%) or median [IQR]

transplantation and multivisceral transplantations. There were major differences in the number of intestinal transplantations performed in various centers. In the last 5 years, median 3.5 isolated pediatric intestinal transplants were performed with a range varying from 0 to 20 procedures. Even larger differences were found in the number of liver transplants, varying from 0 to 180 procedures in the last 5 years. The centers that were the first to start pediatric transplantation programs also had the highest volume.

Role of intestinal transplantation in the treatment of intestinal failure

Of all patients that were referred to a transplant center, about half were sent with the intention for assessment for intestinal transplantation and half were sent for a comprehensive treatment of intestinal failure. Of all patients undergoing an intestinal transplant; 62% of patients had short bowel syndrome, 30% of transplants were performed due to motility disorders, and the remainder (8%) were because of mucosal disorders.

All respondents stated that there continues to be a role for pediatric intestinal transplantation even with advancements in medical and surgical treatment options. More than half (62%) expected an increase of intestinal transplantation, whereas 23% expected a decrease; the remainder (15%) expected no change in the number of intestinal transplants.

Although medical and surgical care has improved over the last years, 31% of the centers noticed a decrease in the number of patients referred for intestinal transplantation. 31% of the centers noticed an increase in the referral of patients for assessment for intestinal transplant. They considered more awareness among gastroenterologists

regarding the treatment options in a transplant center and the better outcome after intestinal transplant the main reasons why referral for intestinal transplant has increased.

Timing of referral and availability of a document with referral criteria

About half of the transplant centers experienced that referred patients had no indication for intestinal transplant in 50-80% of the cases. The reasons why these patients had no indication were: incorrect use of the referral criteria, insufficient communication between referring doctor and transplant center, the presence of contra-indications, and the main reason for referral was not assessment for transplant but comprehensive assessment of options for the treatment of the intestinal failure. Only 5 centers had a very low percentage of patients which were not considered suitable (between 1-15%).

In contrast, when patients were suitable for intestinal transplant, 77% of respondents thought patients should be referred for intestinal transplant at an earlier time. The best way to achieve earlier referral was to educate the referring hospitals in the area of the transplant center according to 88% of the centers. Other options were more information on the internet (supported by 61% of the centers), and educating parents who have a child with intestinal failure (supported by 56% of the centers). The volumes of the centers did not change the results for this statement or any of the following results.

In 10/18 centers (56%) a document which lists the referral and listing criteria for pediatric intestinal transplantation was available. However, only 5 centers (50%) had either published this document on the internet and/or sent this document to referring hospitals in the region.

Current referral criteria

Four centers (22%) did not use the criteria as described by Avitzur et al. (4), they either used the criteria by Kaufman et al. (3) or adjusted the criteria to their own preference. Two thirds of the centers stated that the current referral criteria were adequate, whereas 50% stated that the criteria should be more specific.

The most commonly used criterion for referral was 'children with severely diseased bowel and unacceptable morbidity', followed by 'children with massive intestine resection' and 'persistent hyperbilirubinemia'. However, further questioning revealed that persistent hyperbilirubinemia was considered the most pivotal criterion when patients are referred for intestinal transplantation (Table 4). Continuing prognostic or diagnostic uncertainty and request of the patient or family were considered the least important referral criteria. Table 4 shows which referral criteria should remain in the list with referral criteria and which ones should be reconsidered or even omitted according to the different centers. All respondents agreed upon the statement that persistent hyperbilirubinemia was the only referral criterion that should remain in the list.

Table 4 Evaluation of current referral criteria

	Pivotal	IN	OUT
Children with massive intestine resection	10 (56)	16 (89)	2 (11)
Children with severely diseased bowel and unacceptable morbidity	12 (67)	16 (89)	2 (11)
Microvillous inclusion disease or intestinal epithelial dysplasia	8 (44)	16 (89)	2 (11)
Persistent hyperbilirubinemia (>6 mg/dL)	13 (72)	18 (100)	0 (0)
Thrombosis of 2 of 4 upper body central veins	10 (56)	17 (94)	1 (6)
Continuing prognostic or diagnostic uncertainty	3 (17)	12 (67)	6 (33)
Request of the patient or family	2 (11)	8 (44)	10 (56)

Data are expressed as n (%)
This table describes the number of centers that think the criterion is pivotal for referral for intestinal transplantation (grey area) and the percentage of centers that think the criterion should remain in the list of referral criteria or should go out.

Although 'request of the patient or family' was not considered as a pivotal criterion in the decision making process to refer a patient 7 centers (39%) would refer the patient for intestinal transplant if the family would make a request, even when the patient did not fulfill referral criteria. Table 5 shows the percentages of centers that agreed to the

Table 5 Suggested changes to current referral criteria and potential referral criteria

	AGREE
CHANGES TO CURRENT REFERRAL CRITERIA	
Persistent hyperbilirubinemia	
- Criterion should be present when patient is referred for intestinal transplantation	2 (11)
- Inclusion of time limit in the current definition	
- Changes to the upper limit for bilirubin	10 (56)
- Inclusion of the prothrombin time in the current definition	8 (47)[a]
	6 (33)
Children with massive intestine resection	
- Incorporation of small intestinal length <30 cm with no ileocecal valve in the current criterion	9 (50)
- Inclusion of enterocolonic discontinuity in current criterion	6 (33)
- Referral for intestinal transplant when dependent on parenteral nutrition for more than 75% after 6 weeks	5 (28)
POTENTIAL REFERRAL CRITERIA	
Recurrent life-threatening septic episodes	17 (94)
Refractory fluid and electrolyte disorders	15 (83)
Enterocolonic discontinuity	7 (39)
Prothrombin time	6 (33)
Use of biomarkers for intestinal function, e.g. citrulline	3 (17)
Minimum age for referral for transplant	0 (0)

Data are expressed as n (%); [a] n=17

suggested changes to the referral criteria persistent hyperbilirubinemia and children with massive intestine resection.

Potential referral criteria

Recurrent life-threatening septic episodes and refractory fluid and electrolyte disorders were two criteria that could be considered for addition to the current referral criteria (Table 5).

Septic episodes and fluid and electrolyte disorders were also the most mentioned suggestions to include in the current referral criteria using the thematic analysis. The other suggestions included impossible to discharge from the hospital, impossible to provide home total parenteral nutrition, liver dysfunction, irreversible shal D syndrome, survival better with intestinal transplantation than with total parenteral nutrition, renal dysfunction, central thrombosis, poor quality of life, full enteral nutrition disturbances, and coagulopathy.

Another suggestion was to adjust the referral criteria to different age categories. Of all centers, 67% considered this idea feasible. However, none of the centers believed there should be a minimum age for referral for transplant. The creation of a flow chart for referral for assessment was considered an interesting idea by 78% of the centers (14/18).

DISCUSSION

In this study we investigated the current 'state-of-the-art' referral criteria for pediatric intestinal transplantation and we evaluated potential new referral criteria. We showed that half of the centers stated that the current referral criteria for assessment for pediatric intestinal transplantation should be defined more specifically and 75% preferred earlier referral of patients. 'persistent hyperbilirubinemia' was established as the most pivotal referral criterion, however, only 2 centers thought this criterion should definitely be present when a patient is referred to their transplant center for intestinal transplant. About 50% of the centers would like to change the referral criterion, by adding a time limit and lowering the level of acceptable bilirubin.

New potential referral criteria were recurrent life-threatening septic episodes and refractory fluid and electrolyte disorders. Other favourable changes were to develop age categories and to create a flow chart. This was supported by Beath et al. who focused on risk factors that should alert physicians to refer children with chronic intestinal failure to an intestinal rehabilitation center. This included young age, poor mucosal integrity, lack of ileocecal valve with < 25 cm residual small bowel, intractable diarrhea, early central venous line infections (before 3 months), more than 3 central venous line infections, administration of excess lipid (>3.5 g/kg/d), lack of enteral feeding, and lack of specialist

staff. An international expert meeting at the 2007 international small bowel symposium also incorporated the findings of the study in a table describing criteria for referral to an intestinal failure rehabilitation unit. These results are consistent with our findings and should therefore be combined with our improvements to the referral criteria for intestinal transplantation (12). Ideally, intestinal failure rehabilitation units should always be in the same center as where the intestinal transplants are performed.

Some studies have considered the length of the remaining intestine in relation to weaning from parenteral nutrition. Children achieving autonomy had a mean residual small intestinal length of 91 ± 12 cm compared to patients remaining on parenteral nutrition who had a mean small intestinal length of 34 ± 6 cm (9). Comparable results were found in neonates with short bowel syndrome; those with a mean residual small intestinal length of 31 ± 30 cm without ileocecal valve were destined to remain on parenteral nutrition, compared to those who successfully weaned who had a length of 81 ± 65 cm (13). Goulet et al. found that when the residual small intestinal length was less than 40 cm without an ileocecal valve, infants had a 40% probability of remaining of parenteral nutrition (14). Regarding intestinal length, it is probably even more important to express residual length compared to the expected length in percentages then to express the residual length in absolute numbers (15). This supports our conclusion that the current referral criterion 'children with massive intestine resection' should be for example adjusted to 'children with massive intestine resection: less than 25% remaining intestine of the expected small intestinal length' or 'children with massive intestine resection with <30cm of small intestinal length with no ileocecal valve.

Since 75% of centers put forward that patients were referred too late for intestinal transplant, it is surprising that only half of the centers have published their referral criteria online. To achieve earlier referral, regional pediatric intestinal failure programs may be developed which will be leading in education of the participating hospitals and early identification potential candidates for intestinal, liver and multivisceral transplantation. In this approach multidisciplinary meetings with referring hospitals could help to improve successful enteral feeding advancement, preserve liver function and overall survival (16-20). Regular multidisciplinary meetings at the referring hospitals might be preferred in the management of such an educational and surveillance program aiming to realize earlier referral and increase of quality-adjusted life years. Early listing was associated with 0.27 additional life years, and 0.76 quality-adjusted life years, as determined by a Markov analysis (7). Liver failure at time of referral is a main factor of mortality. Fecteau et al. showed that when patients were younger and had advanced liver disease at the time of listing, they were more prone to die while being on the waiting list for intestinal transplant (6). Moreover, the current limit of bilirubin for referral is set at > 6 mg/dL. The level of bilirubin is important for the prognosis since patients have a better prognosis when the serum bilirubin is less than 3 mg/dL compared to the patients who

had a bilirubin of greater than 3 mg/dL at the time of referral (8). The recent literature is very clear regarding this level of unacceptable bilirubin, but only 50% of the centers in this study thought it was necessary to lower the limit for referral. This is especially surprising when we consider the natural course of cholestasis (defined as bilirubin > 6 mg/dL) leading to progressive liver disease and death after 6 to 12 months after the onset. Therefore bilirubin level from 3-6 mg/dL could count as a minor criterion, but when another referral criterion is present, referral to an intestinal transplant center is preferred.

Despite medical and surgical advances to treat intestinal failure in children the role for intestinal transplantation is expected to increase over time according to more than half of the centers. An explanation for this thought could be that survival after intestinal transplantation is improving even after failure of the conservative treatment (21-23). This was confirmed in a study by Sauvat et al. They concluded that intestinal transplantation should evolve from being a 'rescue' procedure to becoming a 'true' therapeutic

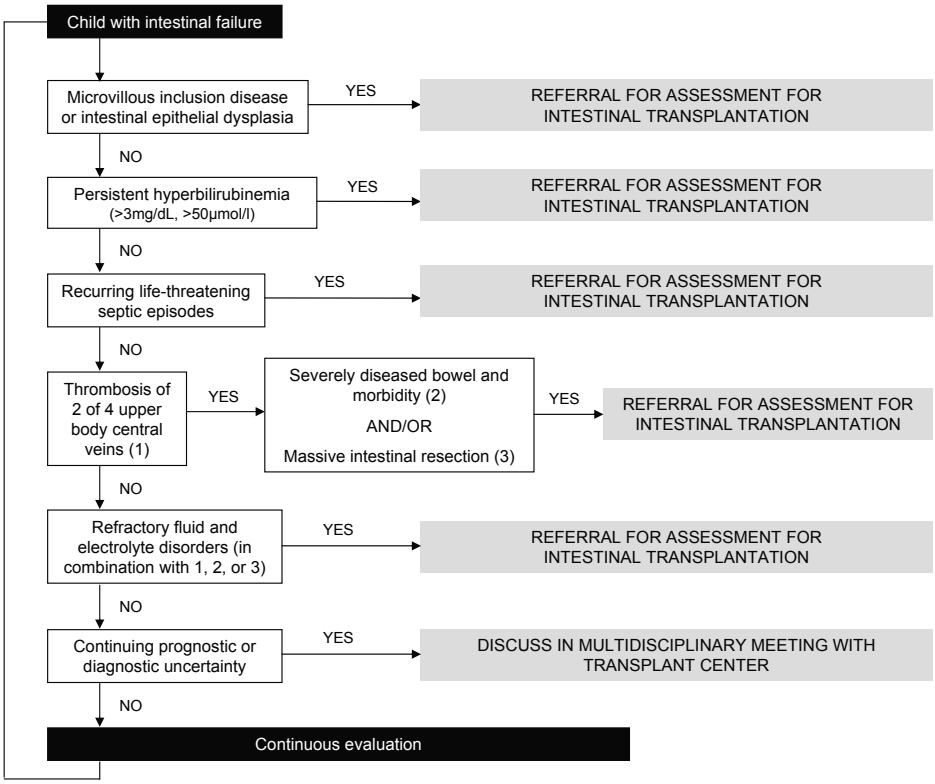

Figure 1 Flow chart for referral of children for pediatric intestinal transplantation

option (24). More adequate referral of children with intestinal failure could improve the outcome of intestinal transplantation.

In our study we found that 62% of patients undergoing intestinal transplants was due to short bowel syndrome. This was consistent with the literature where short bowel syndrome is the main reason for intestinal transplant in 68% of the cases. Motility disorders are 14-17% of all cases, this was not consistent with the number we found in this study which is almost double (2, 4, 25). In the years 2004-2008 around 115 intestinal transplantations were performed each year (2).

The strength of this study is that we specifically paid attention to the intestinal transplantation programs in children and we critically evaluated the current referral criteria, and suggested potential referral criteria. The results of this study should be interpreted with caution, since only 18 of 50 centers completed the questionnaire. This raises concerns regarding the commitment of the centers; the centers that did respond were slightly negative towards the use of the current referral criteria. There could be a selection bias; maybe only the centers that are very open minded to questionnaires, and are well-organized responded. While analyzing the questionnaire, we found a question that was asked twice (although in different words). This is inaccurate, however the answers to these questions were consistent, and this shows the questionnaire is reproducible. Also, since the results of the questionnaires were anonymous, we could not consider differences between responders and non-responders. It would also have been very interesting to compare the European versus the USA/Canadian centers, as well as a comparison of the responses of gastroenterologists with the surgeons. A next step would be to develop a flow chart (see Figure 1) and to send the questionnaire to all hospitals which have patients with intestinal failure. It would be interesting to get their opinion regarding the current and potential referral criteria.

In conclusion, this interview-based study showed that intestinal transplant centers judge the current referral criteria for intestinal transplantation are too general and adjustments should be made. The acceptable level for bilirubin is too high and should be lowered. New criteria such as recurring life-threatening septic episodes and refractory fluid and electrolyte disturbances could be added. We also suggest to develop referral criteria according to age categories and to create a flow chart for the referral for assessment for intestinal transplantation. Referral is considered too late in the majority of cases, therefore referring hospitals should be educated regarding the interpretation of the referral criteria and should be provided with a document with the referral criteria either online or in paper. We recommend to dedicate a session on a world-wide meeting to update the current referral criteria for pediatric intestinal transplantation.

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Pediatric Intestinal Transplantation

Questionnaire

Dear colleague,

This questionnaire will mainly focus on the referral criteria for pediatric intestinal transplantation in children (0-18 years). It evaluates the considerations when children are referred for intestinal transplantation.
It consists of three parts:

Part I	General inquiries	(consists of 18 questions)
Part II	Referral criteria	(consists of 16 questions)
Part III	Cases	(consists of 3 cases)

It will take about 30 min to fill in this questionnaire.

All information will be dealt with anonymously and discretely.

There are three possibilities to fill in this questionnaire:
- Fill in online: a link has been sent to your email address. If you would like to receive your link again, please send an email to the email address below.
- Fill in this paper version of the questionnaire and return it in the included envelope, **NO** stamp is required. If you have not received a paper version, and if you prefer the paper version, please send an email to the email address below.

This questionnaire is part of a Masters' thesis for the MSc in Surgical Sciences at the University of Edinburgh. We would greatly appreciate it, since this thesis is restricted to time limits, if you could complete this questionnaire by: **April 8, 2011**.

In case you have any questions, please contact Marie-Chantal Struijs, either by email: a.e.c.j.m.struijs@erasmusmc.nl or phone: +31610897076.

Thank you very much in advance for your cooperation!

With kind regards,

Prof JNM IJzermans, MD, PhD
Transplant Surgeon
Director of Surgical Training

Marie-Chantal Struijs, MD
Research fellow
Erasmus MC, Rotterdam

Inquiries about the person filling in this questionnaire:

I am a
- ☐ Gastroenterologist
- ☐ Surgeon
- ☐ Nurse practitioner
- ☐ Other, _____

I work at a
- ☐ Pediatric transplant center
- ☐ Adult transplant center
- ☐ Both

This center is situated in:
- ☐ USA, Canada
- ☐ Europe

I work at different pediatric transplant centers:

☐ No

☐ Yes

My age is: _____years

My gender is

☐ Male

☐ Female

I have _____years of experience in this field

Please start the questionnaire

PART I

GENERAL INQUIRIES

Question 1

In which year was the first pediatric **intestinal** transplantation (0-18 years) performed in your center?

☐ _____→ continue to question 3

☐ NA → continue to question 2

Question 2

This question is only applicable if you do **not** perform pediatric **intestinal** transplants. What is the reason no intestinal transplants are performed?

(multiple options are possible)

☐ Marketing

☐ Patient population too small

☐ Procedure too difficult

☐ Not a program priority

☐ Financial

☐ Other _____

_____

_____

If you do not perform pediatric intestinal transplants, please continue to Part II Referral criteria

Question 3

Which kind of pediatric visceral transplants are performed in your center?
(*multiple options are possible*)

☐ Isolated intestinal transplantation
☐ Isolated liver transplantation
☐ Combined intestinal-liver transplantation
☐ Multivisceral transplantation (any combination)

Question 4

Regarding question 3, please indicate the number of transplants which were performed in your center in patients under 18 years in the last 5 years (2006-2010)

_____ number of isolated intestinal transplants
_____ number of isolated liver transplants
_____ number of combined intestinal-liver transplants
_____ number of multivisceral transplants

Question 5

How many **surgeons** are performing pediatric visceral transplants in your center?

_____ surgeons

Question 6

The majority of patients are referred to our center for:

☐ Assessment for transplant
☐ Comprehensive treatment of intestinal failure
☐ Both options equal number of patients

Question 7

What are the **3 main reasons/diagnosis** patients are referred for transplant to your center (column 1) and what are the main diagnosis for patients undergoing intestinal transplantation at your center (column 2)?

Please put 1, 2, and 3 behind the 3 main reasons, with 1 being the most common reason, 2 the 2nd most common and 3 for the 3rd most common reason

	Column 1 Referral for assessment for intestinal transplant	**Column 2 Diagnosis at intestinal transplantation**
Short bowel syndrome due to volvulus		
Short bowel syndrome due to gastroschisis		
Short bowel syndrome due to necrotizing enterocolitis		
Short bowel syndrome due to atresia		
Short bowel syndrome due to other reasons		
Motility disorders (Hirschsprung, pseudo-obstruction)		
Mucosal disorders (i.e. microvillus inclusion disease)		
Retransplantation		
Other:		

Question 8

With the improvement in medical (ie better forms of TPN) and surgical care (ie intestinal lengthening procedures) do you think there continues to be a **role** for intestinal transplantation?

☐ YES

☐ NO

Question 9

Regarding question 8, do you think the role for intestinal transplantation will:

☐ Increase over time

☐ Decrease over time

☐ Remain the same

Question 10

Also, with the improvement in medical and surgical care, do you notice a **difference** in the **number** of patients referred for intestinal transplantation?

☐ Increased
☐ Decreased
☐ Remains the same

If you have answered "remains the same" skip question 11

Question 11

What do you think is the **reason** the number of patients referred for intestinal transplantation has changed (either increased or decreased)?

☐ Improvement in medical care (i.e. TPN)
☐ Improvement in surgical procedures (i.e. intestinal lengthening procedures)
☐ Both
☐ Other, _____

_____

_____

Question 12

How many patients have been referred to you the last 5 years (2006-2010) for intestinal transplantation?

± _____ patients

Question 13

What do you think of this statement:

I believe patients should be referred for intestinal transplant at an earlier time than patients are referred at this moment

☐ Strongly disagree
☐ Disagree
☐ Neither agree or disagree
☐ Agree
☐ Strongly agree

Question 14

What percentage of patients that are referred are **not suitable** for intestinal transplantation for medical and/or surgical reasons?

_____%

Question 15

What do you think the **reason** is that these patients are not suitable?

☐ Incorrect use of the referral criteria
☐ Bad communication between referral hospital and transplant center
☐ Referring doctors are not aware of indications for referrals
☐ Referring center wanted a comprehensive assessment of intestinal failure treatment options, not just a transplant
☐ Other, _____

_____

_____

Question 16

What percentage of patients that are referred to your center, should have visited your center **earlier** in your opinion?

_____%

Question 17

Do you believe intestinal lengthening procedures should be performed at the referral center, if possible, before the patient is referred for intestinal transplantation?

☐ YES
☐ NO

Please explain your answer: _____

_____

_____

Question 18

Do you believe intestinal lengthening procedures should **solely** be performed in transplant centers?

☐ YES, only in transplant centers
☐ NO, also possible in the center that refer the patient
☐ NO, only in high-volume centers

Please explain your answer: _____

_____

_____

PART II

REFERRAL CRITERIA

Question 1

Is there a document available in your center which lists the criteria for referral/listing for intestinal transplantation in children (0-18 years)?

☐ YES (if yes, please attach this document to this questionnaire, please continue to question 2)

☐ NO (please continue to question 5)

Question 2

Is this document available online (on the internet)?

☐ YES

☐ NO

If no, please explain why this is not available online:_____

_____

_____

Question 3

Did you sent this document to hospitals in your region which refer patients to your hospital?

☐ YES

☐ NO

Question 4

Are these criteria adjusted to the wishes of the surgeons and gastroenterologists in your center or are these the same as the criteria from the literature/American Society of Transplantation?

☐ Criteria are adjusted to the preferences of the surgeons and gastroenterologists

☐ Criteria are taken from the literature/ American Society of transplantation

☐ Other, _____

_____

_____

Question 5 Referral Criteria

In the literature (Avitzur et al. 2010) the following criteria are listed as the recommended pediatric referral criteria for intestinal transplantation:

- Children with massive intestinal resection
- Children with severely diseased bowel and unacceptable morbidity
- Microvillous inclusion disease or intestinal epithelial dysplasia
- Persistent hyperbilirubinemia (>6 mg/dl or > 100 µmol/l)
- Thrombosis of 2 of 4 upper body central veins
- Continuing prognostic or diagnostic uncertainty
- Request of the patient and family

The following questions will consider these criteria.

5a

Are these the criteria that are used in your center?

☐ YES

☐ NO

If no, please elaborate on why these referral criteria are not used in your center: _____

_____

_____

5b

What are the top 3 referral criteria that are used in patients referred to your center?

Please put 1, 2, 3 for the top three referral criteria

Children with massive intestinal resection	
Children with severely diseased bowel and unacceptable morbidity	
Microvillous inclusion disease or intestinal epithelial dysplasia	
Persistent hyperbilirubinemia (>6 mg/dl or > 100 µmol/l)	
Thrombosis of 2 of 4 upper body central veins	
Continuing prognostic or diagnostic uncertainty	
Request of the patient and family	

5c

Do you think these criteria are sufficient/ comprehensive?

☐ YES

☐ NO

Please explain your answer: _____

_____

_____

5d

Do you believe these criteria are specific enough?

☐ YES, no changes are necessary

☐ NO, the criteria are too general: additions should be made to make them more specific

5e

Do you think all of these criteria should be present before the patients are referred?

☐ YES

☐ NO

5f

Do you think the referral should be adjusted to different age categories? For example, criteria for children <1 year, 1-6 years of age, and > 6years?

☐ YES

☐ NO

5g

Which criteria should **definitely** be present/ are pivotal when the patient is referred for intestinal transplant to your center?

Please put an X in the answer of your choice

Children with massive intestinal resection	
Children with severely diseased bowel and unacceptable morbidity	
Microvillous inclusion disease or intestinal epithelial dysplasia	
Persistent hyperbilirubinemia (>6 mg/dl or > 100 µmol/l)	
Thrombosis of 2 of 4 upper body central veins	
Continuing prognostic or diagnostic uncertainty	
Request of the patient and family	

5h

Please grade the criteria for importance, 1 being the most important criterion and 7 the least important criterion from the list (fill in 1, 2, 3, 4, 5, 6, and 7)

Children with massive intestinal resection	
Children with severely diseased bowel and unacceptable morbidity	
Microvillous inclusion disease or intestinal epithelial dysplasia	
Persistent hyperbilirubinemia (>6 mg/dl or > 100 µmol/l)	
Thrombosis of 2 of 4 upper body central veins	
Continuing prognostic or diagnostic uncertainty	
Request of the patient and family	

5i

Which criteria should remain in the referral list and which ones should go out?

Please put an X in the answer of your choice and explain your answer

	IN	OUT
1. Children with massive intestinal resection		

	IN	OUT
2. Children with severely diseased bowel and unacceptable morbidity		

	IN	OUT
3. Microvillous inclusion disease or intestinal epithelial dysplasia		

	IN	OUT
4. Persistent hyperbilirubinemia (>6 mg/dl or > 100 µmol/l)		

	IN	OUT
5. Thrombosis of 2 of 4 upper body central veins		

	IN	OUT
6. Continuing prognostic or diagnostic uncertainty		

	IN	OUT
7. Request of the patient and family		

5j

Which criteria would you like to add to the referral list and **why**?

If you don't have any criterion you would like to add, please fill in write on line 1

1 _____

_____

2 _____

_____

3 _____

_____

For example, prolongation of prothrombin time, recurrent septic episodes, severe fluid and electrolyte disturbances

Question 6

The following questions will consider the referral criterion:
Persistent hyperbilirubinaemia (>6 mg/dl or > 100 µmol/l)

6a

Do you think this is an **important** referral criterion for intestinal transplantation?

☐ YES
☐ NO

Please explain your answer: _____

_____

_____

6b

Should this criterion be present in **every patient** referred for intestinal transplantation?

☐ YES
☐ NO

Please explain your answer: _____

_____

_____

6c

The term "**persistent**" is not really defined. It is known from the literature that parenteral nutrition-induced liver disease leads to liver failure and death usually within 1 year. So should the referral criterion be changed to include time limit?

☐ YES
☐ NO

6d

Do you think changes should be made to the **upper limit**? For example change the >6mg/dl (> 100 µmol/l) into >4mg/dl (>67 µmol/l)?

☐ YES, it should be changed into: >_____mg/dl or >_____ µmol/l
☐ NO

6e

It is known the prothrombin time becomes prolonged in the later stages of parenteral nutrition-associated liver disease. Do you think the prothrombin time should be included in the criteria?

☐ YES
☐ NO

6f

Do you think the criterion should be **changed into**: Intestinal failure-associated liver disease (IFALD), with conjugated bilirubin ≥2mg/dl (34.2 µmol/l)?

☐ YES

☐ NO

Question 7

The following questions will consider the referral criterion:
Thrombosis of 2 of 4 upper body central veins.

7a

Do you think this is a **pivotal** referral criterion?

☐ YES

☐ NO

Please explain your answer: _____

_____

_____

7b

One of the referral criteria is: Loss of more than 50% of the standard central venous access site; however one of the contraindications for transplant is insufficient vascular patency to guarantee vascular access.
Do you think this is **contradictive**?

☐ YES

☐ NO

Question 8

The following questions will consider the referral criterion:
Request of the patient and family

8a

Do you think this is an **pivotal** referral criterion in the decision-making process to refer a patient for intestinal transplantation?

☐ YES

☐ NO

Please explain your answer: _____

_____

_____

8b

If the family would request an intestinal transplant but the patient does not fulfill all your criteria, would you **still** consider referral of the patient?

☐ YES

☐ NO

Please explain your answer: _____

_____

_____

Question 9

Microvillous inclusion disease or intestinal epithelial dysplasia

These rare disorders almost never resolve or improve sufficiently with medical treatment. In most cases, death from liver disease or sepsis occurs within first 1-2 years of life.

Considering this information, do you think that having a congenital epithelial (mucosal) disorders without other criteria is sufficient for referral?

☐ YES, all patients should be referred as soon as the patient is diagnosed

☐ NO, more referral criteria should be present

Question 10

Small intestinal length < 30 cm with no ileocecal valve is mentioned as a risk factor for transplant.

10a

Do you think this should be **incorporated** into the referral criterion: *Children with massive intestine resection*?

☐ YES

☐ NO

10b

Do you agree with the following: children with less than 25% remaining intestine of the expected small intestinal length for age should be referred for transplantation when on TPN for more than 6 weeks?

☐ YES

☐ NO

10c

It is suggested the colon in continuity plays a role in intestinal adaptation. Moreover, enterocolonic discontinuity is suggested to be a risk factor for transplantation.
Do you think the following criterion should be added to the referral criteria: *Enterocolonic discontinuity*

☐ YES
☐ NO

10d

Some articles define irreversible intestinal failure as to be dependent on TPN for >75% for more than 6 weeks **and** suggest to refer patients for transplantation.
Do you insist on referral of these patients as early as 6 weeks after being dependent on TPN?

☐ YES
☐ NO

Question 11

Although recurring life-threatening sepsis are mentioned in the listing criteria, do you think physicians should insist on referral when children have recurring septic episodes (as was suggested in the article by Kaufman et al in 2001)?

☐ YES
☐ NO

Question 12

Refractory fluid and electrolyte disorders are mentioned as a risk factor for transplantation.
Do you think this criterion should be added to the referral criteria?

☐ YES
☐ NO

Question 13

Outcome data show that mortality and morbidity associated with transplantation is higher when children are younger than 1 year of age. Do you think there should be a **minimum age** for referral for transplant?

☐ YES, it should be _____years
☐ NO

Question 14

The literature shows that children who are transplanted while still staying at home have a higher survival. Do you think children in general are referred **too late** for intestinal transplant?

☐ YES

☐ NO

What do you think should be done to change this (to achieve earlier referral)? (multiple options possible)

☐ Educate hospitals in your area

☐ More information on the internet

☐ Educate parents who have a child with intestinal failure

☐ Other, _____

_____

_____

Question 15

Citrulline is identified as a potential biomarker of functional enterocyte mass. Do you think there should be a referral criterion considering biomarkers for intestinal function?

☐ YES

☐ NO

Question 16

Do you think it is useful to create a flow chart for the decision making process whether a patient should be referred for intestinal transplant?

☐ YES

☐ NO

PART III

CASES

CASE 1

Girl, 2nd of monochorial-diamniotic gemelli, born at 37 weeks, birth weight 1350 grams. Six days after birth, midgutvolvulus, 35cm ileal resection and jejunostomy. Six weeks later, ileocecal resection due to stenosis, and restoration of continuity using the remaining 45cm jejunum and colon. Other medical problems: hypothyreoidism requiring replacement therapy, muscle hypertonicity, developmentally delayed in motor skills, chronic anemia, and deficiencies of vitamin A and D, and zinc. Unable to discharge, in the hospital since birth. Parents also have the care for the patient's twin sister and an older brother who is regularly admitted to the hospital. They are hesitant against prolonged admission in a transplantation center since they will not have the opportunity to visit often.

Current status: 2 ½ years old, weight 11.2 kg (0 SD) and height 72 cm (<-2.5 SD). Partially dependent on parenteral nutrition (50% enterally via drip feeding); Omegaven® as lipid solution because of cholestasis. Thrombosis of one internal jugular vein; right and left subclavian vein have an "irregular" aspect. Multiple septic episodes (18x) and placement of 13 central venous catheters. Medication: questran, fraxiparine, vitamin supplements, imodium, thyrax, ranitidine. Last laboratory results: total bilirubin 5 µmol/l (0.3 mg/dl), direct bilirubin <1 µmol/l (<0.05 mg/dl), alkaline phosphatase 754 U/l, γGT 76 U/l, AST 64 U/l, ALT 147 U/l, triglycerides 1.00 mmol/l.

QUESTIONS CASE 1

Question 1

Is there medical information missing you need to evaluate this case for transplant?

☐ NO

☐ YES, namely_____

_____

_____

Question 2

Would you refer this patient for assessment for intestinal transplantation?

☐ YES (please continue to question 3)

☐ NO (please continue to question 4)

Question 3

What are the reasons you would refer this patient?

Please put an X in the box of the referral criteria applicable to this patient

Children with massive intestinal resection	
Children with severely diseased bowel and unacceptable morbidity	
Microvillous inclusion disease or intestinal epithelial dysplasia	
Persistent hyperbilirubinemia (>6 mg/dl or > 100 µmol/l)	
Thrombosis of 2 of 4 upper body central veins	
Continuing prognostic or diagnostic uncertainty	
Request of the patient and family	

Other reasons: _____

_____

_____

Please continue to question 5

Question 4

What are the reasons you would not refer this patient?

☐ Not enough criteria to refer for transplant

☐ Still enough options for treatment

☐ Other, _____

_____

_____

Question 5

What would be your treatment proposal?

☐ Medical treatment: try to increase enteral feeding

☐ Intestinal lengthening procedure

☐ Placement on waiting list for intestinal transplantation

☐ Other, _____

_____

_____

Question 6

In case this patient would be medically suitable for intestinal transplantation, would the social situation (concerns of the parents) be of influence on your decision?

☐ YES, I would not place her on the waiting list

☐ NO, not of influence at all

Question 7

Would you list this patient for intestinal transplantation?

☐ NO

☐ YES, based on the following criteria (please put an X in the boxes):

Small bowel length of <25 cm without an ileocecal valve	
Intestinal failure with high morbidity and poor quality of life	
Congenital intractable mucosal disorder	
Persistent hyperbilirubinemia and signs portal HT or liver dysfunction	
Loss of >50% of standard central venous access sites	
Recurrent life-threatening episodes of sepsis	

CASE 2

Boy, born at term, no problems until 6 weeks after birth. Patient presented with intestinal cramps, vomiting and no defaecation for 5 days. At laparotomy lymphocele, volvulus and ileal stenosis were seen. Lymphocele and stenosis were resected. At second look formation of 2 enterostomies, and at the third look necrotic bowel was resected, leaving 8 cm jejunum, 1cm terminal ileum, and the colon (with ileocecal valve) in situ. The case was discussed in a multidisciplinary meeting and a somber prognosis was concluded. They proposed to withdraw medical treatment but parents disagreed, so treatment continued. One month later, intestinal continuity was restored. At 3 years of age cholecystecomy was performed.

Current status, 7 year old boy, weight 24,5 kg (0.8 SD) and height 120.9 cm (-1.5 SD) with gastrostomy. Partially TPN dependent: enteral feeding 51 ml/kg/day and parenteral feeding 35 ml/kg/day. Medication: zinc sulfate, sodium bicarbonate, Fragmin. Multiple septic episodes, 30 placements of central venous catheters. Occlusion of the left jugular vein. Number of hospital admissions: 57. No elevated liver parameters or other elevated laboratory parameters.

In conclusion, 7 year old boy with total of 9 cm small bowel and colon in situ after intestinal resection for volvulus and lymphocele. Partially TPN dependent, multiple septic episodes and multiple placements of central venous catheters.

QUESTIONS CASE 2

Question 1

Is there medical information missing you need to evaluate this case?

☐ NO

☐ YES, namely _____

_____

_____

Question 2

Would you refer this patient for intestinal transplantation?

☐ YES (please continue to question 3)

☐ NO (please continue to question 4)

Question 3

What are the reasons you would refer this patient?

Please put an X in the box the referral criteria applicable to this patient

Children with massive intestinal resection	
Children with severely diseased bowel and unacceptable morbidity	
Microvillous inclusion disease or intestinal epithelial dysplasia	
Persistent hyperbilirubinemia (>6 mg/dl or > 100 µmol/l)	
Thrombosis of 2 of 4 upper body central veins	
Continuing prognostic or diagnostic uncertainty	
Request of the patient and family	

Other reasons: _____

_____

Please continue to question 5

Question 4

What are the reasons you would not refer this patient?

☐ Not enough criteria to refer for transplant

☐ Still enough options for treatment

☐ Other,_____

_____

Question 5

What would be your treatment proposal?

☐ Medical treatment: try to increase enteral feeding

☐ Intestinal lengthening procedure

☐ Placement on waiting list for intestinal transplantation

☐ Other, _____

_____

Question 6

Would you list this patient for intestinal transplantation?

☐ NO

☐ YES, based on the following criteria (please put an X in the boxes):

Small bowel length of <25 cm without an ileocecal valve	
Intestinal failure with high morbidity and poor quality of life	
Congenital intractable mucosal disorder	
Persistent hyperbilirubinemia and signs portal HT or liver dysfunction	
Loss of >50% of standard central venous access sites	
Recurrent life-threatening episodes of sepsis	

CASE 3

Boy, born at 37 3/7 week gestational age, birth weight 2350 gr with prenatally diagnosed gastroschisis. Primary closure of the abdominal wall was performed shortly after birth, but on day 1 after primary closure, there were signs of abdominal compartment syndrome. Relaparotomy showed signs of intestinal ischaemia, and the abdomen was closed with a large patch. On day 2 all intestines showed necrosis and all surgical and medical options were discussed in a multidisciplinary session and with the parents. Conclusion was that intestinal transplant was an option. Resection of all bowel from Papilla of Vater up to left part of the colon followed, with 10 cm colon remaining. A gastrostomy was placed concurrently. The boy has been completely dependent on TPN. Six weeks after birth, total bilirubin concentration increased to 187 µmol/l (10.9 mg/dl), and conjugated bilirubin was 140 µmol/l (18.2 mg/dl), caused by incomplete drainage (leading to sepsis and cholangitis). MRCP showed scarring of Papilla of Vater, consequently multiple drains were placed which led to adequate drainage of bile. Neurological development was normal. Parents are very involved in the caretaking and are willing to enter the screening process of possible (combined liver-) intestine transplantation.

In conclusion, 4 month old boy, primary closure of gastroschisis, complicated by necrotic bowel, leaving 10 cm of colon in situ and no small bowel. Completely dependent on TPN, weight 5400 gr (-2 SD). Course complicated by cholangitis for which drainage and 3 septic episodes.

QUESTIONS CASE 3

Question 1

Is there medical information missing you need to evaluate this case?

☐ NO

☐ YES, namely _____

_____

_____

Question 2

Would you refer this patient for:

☐ Isolated intestinal transplantation (please continue to question 3)
☐ Isolated liver transplantation (please continue to question 3)
☐ Combined liver/intestine transplantation (please continue to question 3)
☐ I would not refer this patient (please continue to question 4)

Please explain your answer: _____

_____

_____

Question 3

What are the reasons you would refer this patient? Please put an X in the boxes

Children with massive intestine resection	
Children with severely diseased bowel and unacceptable morbidity	
Microvillous inclusion disease or intestinal epithelial dysplasia	
Persistent hyperbilirubinemia (>6 mg/dl or > 100 µmol/l)	
Thrombosis of 2 of 4 upper body central veins	
Continuing prognostic or diagnostic uncertainty	
Request of the patient and family	

Other reasons _____

_____

_____

Please continue to question 5

Question 4

If you would not refer this patient, what are the reasons you would not refer this patient?

☐ Not enough criteria to refer for transplant
☐ Still enough options for treatment
☐ Other,_____

_____

_____

Continue to question 5

Question 5

What would be your treatment proposal?

☐ Medical treatment

☐ Intestinal lengthening procedure

☐ Placement on waiting list for transplantation

☐ Other, _____

_____

_____

Question 6

If you would list this patient, what are the listing criteria applicable to this patient?

Please put an X in the boxes

Small bowel length of <25 cm without an ileocecal valve	
Intestinal failure with high morbidity and poor quality of life	
Congenital intractable mucosal disorder	
Persistent hyperbilirubinemia and signs portal HT or liver dysfunction	
Loss of >50% of standard central venous access sites	
Recurrent life-threatening episodes of sepsis	

Case continued

Current status: 5 ½ years old, history of primary closure of gastroschisis, complicated by necrotic bowel, leaving 10 cm of colon in situ. Weight 14.95 kg (0.1 SD) and height 96.3 cm (-3.0 SD), medication: ranitidine, somatostatine, addamel (trace elements). Still completely dependent on TPN. Multiple septic episodes and central venous catheter placements (7). However on ultrasound, no problems with the major veins.
Chronic pancreatitis with cholecystectomy at 2 years of age. Liver pathology, started 5 months after birth. At the moment septal liver fibrosis, possibly cirrhosis with cholestasis. Laboratory parameters: total bilirubin 66 µmol/l (3.8 mg/dl), conj bilirubin 46 µmol/l (2.7 mg/dl), alkaline phosphatase 457 U/l, γGT 212 U/l, AST 155 U/l, ALT 142 U/l, triglycerides 1.12 mmol/l and PT 12.5 sec.

Question 7

Does this additional information change your decision regarding referral of this patient?

☐ NO, I would still not refer this patient for transplantation → Continue to question 8

☐ YES, I would still refer this patient for transplantation → Continue to question 9

Question 8

I would still not refer this patient for the following reasons:

☐ Not enough criteria to refer for transplant

☐ Still enough options for treatment

☐ Other, _____

_____

_____

Please continue directly to last page

Question 9

What are the reasons you would refer this patient? Please put an X in the boxes

Children with massive intestine resection	
Children with severely diseased bowel and unacceptable morbidity	
Microvillous inclusion disease or intestinal epithelial dysplasia	
Persistent hyperbilirubinemia (>6 mg/dl or > 100 µmol/l)	
Thrombosis of 2 of 4 upper body central veins	
Continuing prognostic or diagnostic uncertainty	
Request of the patient and family	

Other reasons _____

_____

_____

Question 10

I would refer this patient for:

☐ Isolated intestinal transplantation
☐ Isolated liver transplantation
☐ Combined liver/intestine transplantation
☐ Combined liver/intestine/pancreas transplantation
☐ Other_____

Question 11

If you would list this patient, what are the listing criteria applicable to this patient?
Please put an X in the boxes

Small bowel length of <25 cm without an ileocecal valve	
Intestinal failure with high morbidity and poor quality of life	
Congenital intractable mucosal disorder	
Persistent hyperbilirubinemia and signs portal HT or liver dysfunction	
Loss of >50% of standard central venous access sites	
Recurrent life-threatening episodes of sepsis	

You have reached the end of the questionnaire!
We greatly appreciate it that you have taken the time to fill in this questionnaire.

If you have any comments or items that you have missed in this questionnaire:

We would like to thank you again very much for filling in all the questions, we really appreciate your cooperation.

THANK YOU!!!

PART IV
GENERAL DISCUSSION & SUMMARY

imperfection is beauty,
madness is genius and it's
better to be absolutely ridiculous
than absolutely boring.

Marilyn Monroe

Chapter 9

General discussion

NECROTIZING ENTEROCOLITIS

NEC is the most common gastrointestinal disorder affecting mainly premature neonates (90% of the cases). Among infants with a low birth weight (500-1500 grams) the mean prevalence of NEC is 7%. The pathogenesis remains poorly understood and is probably multifactorial. Intestinal immaturity (e.g. circulatory regulation, barrier function, innate immunity, motility, and digestion), genetic predisposition, feeding with formula milk, abnormal bacterial colonization, and hypoxic-ischaemic injury have all been suggested, alone or in combination, to play a role in the pathogenesis of NEC. An estimated 20-40% of babies with NEC require surgical intervention, and the associated case fatality rate still approaches 50% (1, 2).

Previous research has already attempted to identify the exact role of the hypoxic-ischemic insult and whether it is primary or secondary in the pathogenesis of NEC (3). So far no conclusive results have been obtained. Based on the histopathology of NEC (coagulation necrosis), the role of the microcirculation is still a hot topic (4-6).

A number of questions remain unanswered so far such as:

- The origin of local outbreaks in neonatal intensive care units.
- Changing prevalence over the years in individual units.
- The variable phenotype with regards to the extent of intestinal involvement.
- The optimal non-invasive biomarker both in high risk patients to predict NEC and/or as a marker of the natural history.
- Optimal preventive strategies.
- The optimal surgical approach.

BIOMARKERS FOR NECROTIZING ENTEROCOLITIS?

Diagnosing NEC is a challenging problem. At present, there is no definitive diagnostic instrument, either laboratory and/or radiological, available to accurately diagnose NEC and to follow its course, unless NEC is in its end stage. Diagnosis is mainly based on clinical findings, supported by laboratory measurements (such as a decrease in platelet count and increase in lactate levels), imaging techniques such as plain abdominal films, abdominal ultrasound and/or explorative laparoscopy/laparotomy (2). The mostly used radiological staging system in NEC are the modified Bell staging criteria, which consist of 3 stages and are based on a combination of systemic, abdominal and radiographic signs (7).

Recent research has focused on the use of biomarkers to evaluate infants at risk for NEC or to evaluate the severity of NEC (8). Most importantly, good biomarkers should be

non-invasive and the results should be readily available. Due to the multifactorial origin of NEC, a single biomarker will probably not be available to detect NEC; most likely a combination of biomarkers will be used to diagnose NEC. Laboratory biomarkers are C-reactive protein (CRP), hemoglobin, leukocytes, platelets, lactate, glucose level, electrolyte levels, and acid-base balance. Due to the use of these biomarkers in diagnosis and monitoring of all kind of disease processes, it might lack specificity to diagnose NEC as was demonstrated in a meta-analysis by Evennett et al. (9).

One of the most promising laboratory biomarkers is CRP, although this might be not discriminative since it is also increased in infants with sepsis alone. In infants with suspected NEC the odds ratio to predict NEC was 5.82 for CRP (9). CRP can also be instrumental in monitoring the course of the disease after medical management is initiated or whether infants require a surgical intervention (10). However, additional studies are required to determine a threshold CRP level above which neonates with NEC need surgery. Another non-specific biomarker for intestinal ischemia is lactate. Lactate may be used both to predict the need for surgery (11) and to determine the prognosis. Neonates with a pre-operative lactate concentration > 1.6 mmol/l had a positive predictive value for mortality of 66.7% (12). Thrombocytopenia has been shown to occur later in the course of NEC, therefore the disease has probably progressed to a more severe stage if the platelets are low (13-16). It might be more useful to use the platelet count as a prognostic marker for survival as described by Hutter et al. (17). One recent study has investigated the platelet count in the days prior to the onset of NEC. They demonstrated that the platelet count was significantly lower in infants who developed NEC compared to the platelet counts in control infants (18). These studies have several limitations. First of all, several of them are retrospective studies. Also, the studies lack sufficient power and as mentioned before, CRP, lactate, and platelets are also markers for disease processes other than NEC and therefore lack specificity.

Based on the histology of NEC, ischemia is probably still one of the factors strongly associated with the start and progression of NEC. Interesting markers to study ischemia are fatty acid binding proteins (FABP). These are tissue-specific inflammatory markers usually elevated during episodes of ischemia. Two FABP are more relevant in NEC: liver FABP (L-FABP) and intestinal FABP (I-FABP). At the onset of NEC, plasma concentrations of L-FABP have been demonstrated to be significantly elevated (19). Therefore, L-FABP might be a sensitive marker for the early detection of the disease (even for stage I of NEC). In contrast, I-FABP levels were significantly higher in the infants who later developed severe NEC (stage III of NEC), and therefore I-FABP levels are not useful for screening purposes (19, 20). A major disadvantage of these markers when measured in plasma is the need for repeated blood sampling, which potentially might result in multiple blood transfusions and associated problems. As such, urinary I-FABP levels are more promising since urine is an excretion product. In a sample of 226 neonates (of whom 6 developed

NEC), urinary I-FABP was not shown to be a useful tool for screening for NEC. Urinary I-FABP levels, as in plasma levels, were significantly higher in neonates with NEC needing surgery (21-23). Other promising markers are urinary claudin-3 and fecal calprotectin (21, 24). The next step is to evaluate urinary I-FABP, urinary claudin-3, plasma L-FABP, and fecal calprotectin in a larger cohort of neonates admitted to a neonatal intensive care unit to determine whether one or more of these markers are a sensitive tool to diagnose NEC in a very early stage or maybe even before any symptoms occur.

In the past, other markers (lactulose and rhamnose) have also been suggested based on passive and active intestinal transport of sugar in patients with NEC. After a thorough analyis they turned out to be of limited value especially as an indicator to resume feeding in the early phases after NEC (25). The same holds true for gut hormone profiles as a marker of intestinal integrity and response to treatment (26).

Another way to evaluate intestinal ischemia is by monitoring the microcirculation and tissue perfusion. Sidestream darkfield imaging (SDF) is a non-invasive method assessing the microcirculation using concentrically green light emitting diodes (LEDs) surrounding a central light guide to provide sidestream dark field illumination. This light will be scattered in the tissue and will be reflected by hemoglobin and produce an image of the microvasculature where the erythrocytes can be observed in the vessels (27-30). Until now research has mainly focused on infants with sepsis, infection, congenital diaphragmatic hernia, or infants on extracorporeal membrane oxygenation (31-36). Only Top et al. have studied NEC patients intra-operatively using orthogonal polarization spectral imaging (precursor of SDF). They observed a decreased functional capillary density (0.4 cm/cm^2) on the mesentery of necrotic bowel compared to a functional capillary density of 2.7 cm/cm^2 on the mesentery of vital bowel (37). Our intra-operative measurements did not confirm these results, but we observed that the vessel density of non-affected intestinal tissue in infants with NEC was lower than the non-affected intestinal tissue of infants with other gastrointestinal disorders. This could be due to age related differences (since the infants with NEC were significantly younger), it could also indicate that although macroscopically the intestine does not seem affected, the blood flow is diminished. Maybe more intestinal tissue should be resected at primary surgery to prevent a second laparotomy for progression of NEC or stenosis. However, this should be evaluated in a larger cohort of patients. Pre-operatively we evaluated infants with suspected NEC by SDF, and compared these to observations in control infants. No statistically significant differences were found for vessel density and blood flow between both groups. When analyzing individual NEC patients we did observe that vessel density decreased during the course of suspected NEC, but no differences were found between infants with NEC who eventually required surgery and infants who did not require surgery (38, Chapter 2). This could be due to the fact that vessel density decreases in the first month of life anyway (39). To be able to use SDF as a biomarker more studies evaluating vessel density

at an individual level from the first day of life are mandatory. It should be noted that SDF measurements are influenced by different factors such as edema, skin color, and movements of the infants. Also, formal analysis is time-consuming, and experience is needed to be able to evaluate the images at the bedside. On the other hand to be able to perform bedside measurements is one of the major advantages, as well as the fact that it is real-time. Other suggestions for improvement to the current studies are to obtain normal values for standardized measuring points, for example the armpit, greater curvature of the stomach, ileum and colon. In chapter 2, the armpit was used to evaluate the intestinal microcirculation. This might not be the correct location since this is not in line with the gastrointestinal tract. Maybe the rectum would better reflect the microcirculation in the jejunum, ileum and colon. The same holds true for the mesenteric measurements as performed in this study. This might not reflect the intraluminal microcirculation, so our results will not be representative of the area we are most interested in. One other explanation for the absence of differences between the vessel density and blood flow in necrotic and non-affected tissue is the fact that vascular reactivity is not represented by morphological changes as no-reflow phenomena might occur. In this respect detailed in-vitro analysis of the different branches of the mesenteric vasculature is the preferred method of investigation. It could be that different periods of vasoconstriction eventually lead to intestinal ischemia.

Methods evaluating tissue perfusion are near infrared spectroscopy (NIRS) and visible light spectroscopy (T-stat). NIRS is a non-invasive, bedside method that measures the relative light absorption by hemoglobin. Currently, it is mainly used to monitor cerebral oxygenation or during cardiac surgery, but it could also be used to monitor organ or muscle tissue perfusion (40-45). It would be ideal to be able to evaluate abdominal saturations, however few studies are available. Petros et al. showed that recovery of the abdominal saturation after episodes of apnea and bradycardia lasted twice as long as the recovery of the peripheral saturation. These episodes, if frequent, could lead to chronic ischemia in infants who are susceptible to periods of low oxygenation such as preterm infants (46). The cerebro-splanchnic oxygenation ratio (CSOR) was investigated in 39 neonates. A group of 10 patients had abdominal problems (5 patients with NEC). This group had a significantly lower median CSOR of 0.66 versus 0.96 in the control group; the sensitivity of these measurements were 90% (56-100%) (47). T-stat measures microvascular haemoglobin oxygen saturation ($SgvO_2$) with shallow-penetrating visible light in small, thin-tissue subsurface volumes, whereas NIRS measures larger, deeper volumes of tissue (48-50). Both techniques should be more widely evaluated in the evaluation of infants with NEC. A problem with abdominal NIRS measurement is the fact that the values could be false-positive or false-negative. Intestines have peristaltic movements, therefore non-affected intestinal tissue could be measured while necrotic intestinal tissue is not in the field of the infrared light.

Finding the ideal set of biomarkers for the detection of NEC might lead to a suitable scoring system (as summarized in Table 1). Different scoring systems have been evaluated to guide therapy in NEC. SNAPPE-II does not seem to be a good tool to decide whether surgery is needed in infants with NEC (51). Higher SNAPPE-II scores did correlate with increased mortality (52). Another mortality score consisted of gestational age, Bell stage, platelets, and lactate level (53). Although this scoring system might be useful to determine the prognosis, it would be more useful to have a scoring system to guide medical therapy or even a scoring system that evaluates neonates at risk to develop NEC. This has already been attempted (54, 55), however it did not seem very useful in the prediction of NEC probably due to the variable and sometimes unpredictable course in every individual patient.

Table 1 Suggested (bio)markers for NEC

Clinical symptoms	Laboratory biomarkers	Microcirculatory biomarkers	Radiographic findings
General Temperature instability, apnea, bradycardia, lethargy, hypotension	*Plasma* CRP, platelets, lactate, metabolic acidosis, I-FABP, L-FABP	*Sidestream darkfield imaging* vessel density, blood flow	Intestinal dilation, pneumatosis intestinalis, increased thickness intestinal wall, ascites, pneumoperitoneum
Abdominal gastric retention, abdominal distension, hemepositive/bloody stool, abdominal tenderness	*Urine* I-FABP, claudin-3 *Feces* calprotectin	*Near infrared spectroscopy* abdominal tissue perfusion	

CRP: C-reactive protein; I-FABP: intestinal fatty acid binding protein; L-FABP: liver fatty acid binding protein

SURGICAL CHALLENGES IN PATIENTS WITH NECROTIZING ENTEROCOLITIS

Initial management consists of bowel rest, decompression with a gastric tube, and broad-spectrum antibiotics. When the clinical condition deteriorates or intestinal perforation occurs, surgical treatment is mandatory (1, 56). The only absolute indication for surgery in NEC is intestinal perforation (57). Once the decision to perform surgery has been made, it depends on the surgeons' preference and experience whether peritoneal drainage or primary laparotomy is performed. In general, there is a paucity of randomized controlled trials (RCT) being performed in pediatric surgery making evidence based practice challenging (58, 59). Two RCTs and one meta-analysis have been performed to determine if peritoneal drainage or primary laparotomy is the best surgical technique to treat NEC. One RCT did not find any differences between both groups (60), however the other 2 studies showed that peritoneal drainage is associated with delayed laparotomy

and excess mortality (61, 62). Therefore peritoneal drainage as treatment option has been abandoned in most centers.

The interpretation of the results of these RCTs is difficult since infants with NEC are a very heterogeneous population. Until now, it is unclear why one patient experiences only a mild episode of NEC, which can be treated non-operatively while the other patient develops pan necrosis warranting surgical intervention. A typical NEC patient does not exist, and this explains the difficulty in performing RCTs in infants with NEC. More international multicenter studies are needed to optimize treatment strategies for infants with NEC, which should include a detailed documentation of standardized supportive care. Especially the use of vasopressive agents pre-operatively is not investigated in detail, which might have a major impact on the splanchnic circulation in a dose dependent manner and as a result influence the development of NEC.

Several options are available at laparotomy: resection and primary anastomosis, resection and ostomy formation, proximal diversion, "clip and drop" technique, and "patch, drain, and wait" (63, 64). The first two options are the most widely used, however no formal evidence is available regarding which option is preferred. Surgeons favouring the ostomy are afraid that their anastomosis might not heal due to the presence of peritonitis, and inflammation (65). Also, anastomoses are associated with high rates of breakdown, and stricture formation (66). In contrast, ostomies have a high incidence of complications, have difficulties achieving adequate enteral feeding, are associated with fluid/electrolyte losses, and require a second laparotomy for ostomy closure (63, 67). Currently, a RCT is investigating which treatment option is superior and our institution is participating in this trial.

Once the infant has an ostomy, this is associated with a high rate of complications such as stricture formation, parastomal hernia, prolapse, wound infection, wound fistula, wound dehiscence, and small bowel obstruction (68-71). Subsequent ostomy closure is also associated with a complication rate of 21 percent, including wound infection, wound dehiscence, enterocutaneous fistula, bowel obstruction, anastomotic leak, and anastomotic stricture (68-70). The timing of ostomy closure is very variable and based on the surgeons' preference and clinical state of the infant. No evidence-based guidelines exist regarding the optimal timing of ostomy closure. A systematic review evaluating the evidence regarding the best time for ostomy closure did not result in data to develop guidelines. The study quality was poor and almost all included studies were retrospective (72, Chapter 5). Early closure is also usually not preferred due to the possibility of abdominal adhesions. In a retrospective cohort study we did not find any significant differences between the percentage of adhesions at early closure (<6 weeks) and late closure. A concomitant analysis of resource consumption and costs did not show any differences between both groups either (73, Chapter 6). However, these studies consisted of a small number of patients. An RCT could bring conclusive evidence comparing

early versus late ostomy closure in terms of time to full enteral feeding, weight gain, complication rate, and duration of hospital stay. Patients should be stratified according to ostomy type.

NON-SURGICAL CHALLENGES IN NECROTIZING ENTEROCOLITIS

After surgery for NEC, optimal intestinal adaptation is very important. Especially adequate nutrition is essential since infants after surgery have a higher metabolic rate and energy requirement per unit body weight than older infants and children (74, 75). When the composition of amino acids is not ideal, recovery after surgery and growth will be suboptimal (75-77). Glutamine has been the subject of a number of studies since it possibly facilitates enterocyte proliferation, thereby maintaining the structural and functional integrity and maturation of the gastrointestinal tract. This may help to prevent enterocolitis and improve feeding intolerance (78, 79). Current results regarding glutamine supplementation are conflicting and further trials are needed (80, 81). Recently, a new amino acids composition specifically developed for infants was tested. The composition contains alanyl-glutamine as a precursor for glutamine, glycyl-tyrosine as a precursor for tyrosine, and acetyl-cysteine as a source of cysteine. A pilot RCT demonstrated that it is as safe and well tolerated as current amino acids solutions. Compared to reference values, it better reflected the amino acid requirements of the infant (82, Chapter 4). This is a very interesting observation and should be further evaluated in a larger cohort of infants. Within this concept, additives to total parenteral nutrition (TPN) have also been investigated in NEC, in particular glutamine. Glutamine has been shown to have different mechanisms of action. These include tissue protection, immune modulation, preservation of glutathione and antioxidant capacity, preservation of metabolism, decreased intestinal apoptosis, and enhancement of heat shock proteins (83). The level of glutamine was decreased in infants with NEC (84). However, a Cochrane review could not provide concluding evidence whether glutamine supplementation has an effect on the outcome of NEC (85). A recent trial evaluating the effect of oral glutamine supplementation did show a decrease in NEC (86).

So far, we have only discussed the natural course of NEC and the question remains if and how NEC can be prevented. The only proven effective preventive measure is using donor human milk instead of formula feeding (87-89). Several multicenter trials are currently conducted to further focus on the use of donor human milk and the prevention of NEC. Recent studies have mainly focused on probiotics. Probiotics are life microorganisms, which are thought to be beneficial to the host organism. Several trials have been performed evaluating probiotic agents to prevent NEC. A meta-analysis showed that enteral supplementation of probiotics seems to reduce the incidence of severe

NEC (90, 91). These studies have not evaluated the individual probiotics, therefore their results should be interpreted with caution. A meta-analysis evaluating Bifidobacterium animalis subsp lactis did not show that supplementation had an effect on the risk of necrotizing enterocolitis (92). Overall, although results are encouraging more evidence is needed before probiotic supplementation can be recommended for the standard care of all preterm neonates.

Infants with a large resection of intestinal tissue or no intestinal adaptation after surgery are dependent on parenteral nutrition for a certain period of time or even until intestinal transplantation has been performed. Until recently, parenteral nutrition associated liver disease (PNALD) has been a major problem in this patient category. Omega-6 lipids have been suggested to play a role in the advancement of PNALD (93). Therefore, research has focused on reversal of PNALD and prevention of PNALD by changing the lipid concentration in parenteral nutrition. Reducing or even completely replacing the amount of omega-6 lipids with omega-3 lipids has improved outcome in infants with PNALD and can even prevent PNALD (94-98). These results are very promising and together with the improvement in amino acid solutions, postoperative care regarding parenteral nutrition for the surgical neonate could be improved. In general the period of TPN should be as short as possible as the duration of TPN is a well known predictor of outcome (99). In many clinics expressed breast milk has become the enteral substrate of first choice but adequately powered studies are lacking.

Independent factors that are predictive of successful intestinal adaptation are: length of the remaining intestine, and presence of the ileocecal valve or colon (100-102). Regarding remaining intestinal length, >15-40cm of residual intestinal length or >10% of initial intestinal length are associated with a higher rate of successful adaptation (99, 103, 104). We propose to use a percentage of remaining intestinal length since this is more illustrative than an absolute number, and this was also supported by a study by Wales et al. (105). Recently, we reported the first data on normal values for intestinal length in infants from 0-5 years of age (106, Chapter 3). Currently we are performing a validation study to evaluate height as the best predictor for normal small and large intestinal length. We propose to use our normal values after intestinal resection to calculate the percentage of intestine remaining. This is important in the definition of short bowel syndrome, since one of the definitions is residual intestinal length of <25% of expected intestinal length. Regarding the role of the colon in continuity and outcome conflicting data are available. Some authors have suggested that the colon is essential for intestinal adaptation, others and we have failed to demonstrate the importance of the colon (Chapter 7; 100, 103, 107, 108).

When intestinal adaptation fails, it is important that early referral to a pediatric intestinal transplantation center is pursued. Based on our evaluation, referral is considered too late in 77% of the cases. This could be improved by educating referring hospitals,

making referral criteria available on the internet, and by re-evaluating the current referral criteria since these are considered too general in 50% of the cases. The referral criterion 'persistent hyperbilirubinemia' does not have a value and time limit. Recurrent septic episodes and fluid/electrolyte disturbances are not included in the current referral criteria. Other suggestions for improvement include referral criteria for different age categories and the creation of an algorithm (109, Chapter 8).

After the initial recovery, the long-term effects become very important. To date, it is unknown whether NEC affects long-term bowel function and whether the nutritional status in adolescence/ adulthood of neonates who experienced an episode is different from neonates who did not have an NEC episode. In 10% of the infants surviving NEC, gastrointestinal tract dysfunction was reported in childhood (110-112). A recent study investigating the outcome of surgically managed NEC (median follow-up 36 months) showed that 39% of all long-term survivors experienced gastrointestinal tract symptoms. The most common problems were constipation, encopresis, gastroesophageal reflux disease and subacute bowel obstruction (113). Kurscheid et al found similar results: 28% of the children had more than 4 stools/day, 25% showed some food intolerance, 16% had recurrent diarrhea and 13% had obstipation (114). Survivors of NEC were also more often below the 10th and 3rd percentiles for weight, height, and head circumference than controls (110, 114, 115). It should however be emphasized that prematurity is a very important variable which determines outcome in many domains. About 33-50% of NEC patients experience neurodevelopmental impairment at the age of 2-7 years, such as hypotonia, cerebral palsy (spastic diplegia or quadriplegia), hydrocephalus, or severe visual/hearing impairment (112, 116). Although these studies show significant morbidity, Abbasi et al found no differences between NEC survivors and controls. At the age of 1 year, anthropometric measurements, biochemical values and gastrointestinal tract function did not differ either (117). Unfortunately, no contemporary data are available.

Deficiency in vitamin B12 and a decreased bone mineral density are also frequent findings in gastrointestinal disease. When more than 20 cm of terminal ileum has been resected, vitamin B12 malabsorption and deficiency was more likely (118-120). Also, bone resorption markers in premature infants after an episode of NEC were significantly higher compared to premature infants who developed sepsis and premature infants who received parenteral nutrition (121). Decreased bone mineral density is a frequent finding (2-11x more than expected by chance) in gastrointestinal disease. Possible contributing factors are the malabsorption of vitamin D, calcium, and maybe other nutrients (122). However, these studies did not evaluate possible abnormalities in laboratory parameters and bone mineral density at adolescent/adult age. Acknowledgement of these problems could be of benefit for recovery and also useful when evaluating these patients in the adult surgical/general practice. Therefore, we are currently performing a study in adolescents/adults aged 15-30 years who experienced an episode of NEC at the

neonatal age to evaluate their gastrointestinal function (by a questionnaire, laboratory evaluation and DEXA scan), the prevalence of irritable bowel syndrome, and the quality of life.

OVERALL CONCLUSIONS

Taken together, we conclude that:

- Body height is the best predictor for expected small and large intestinal length; a prospective validation study is currently being performed.
- Sidestream darkfield imaging is a new interesting non-invasive, realtime, bedside biomarker. Further studies are needed to determine the optimal location, which reflects the gastrointestinal circulation, as well as normal values for standardized measurement locations along the gastrointestinal tract.
- There is still improvement warranted for the composition of pediatric amino acid solutions. Addition of glutamine and tyrosine to a new amino acid solution has already led to an improvement, and better reflects the need of the neonate and infant.
- Early ostomy closure is as safe as late ostomy closure and might even be favorable considering ostomy related complications. A randomized controlled trial is needed to evaluate whether early or late ostomy closure is the preferred option.
- Infants should be referred earlier to a pediatric intestinal transplantation center.
- Current referral criteria for pediatric intestinal transplantation should be adjusted to include a time and value limit for the criterion 'persistent hyperbilirubinemia', and new criteria as recurrent septic episodes and fluid/electrolyte disturbances should be included in the referral criteria list.

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Chapter 10

English summary

Nederlandse samenvatting

ENGLISH SUMMARY

Necrotizing enterocolitis (NEC) is the most common gastrointestinal disorder affecting mainly premature neonates (90% of the cases). Among infants with a low birth weight (500-1500 grams) the mean prevalence of NEC is 7%. The pathogenesis remains poorly understood. Currently, a combination of intestinal immaturity, abnormal bacterial colonization, feeding with formula milk and hypoxic-ischemic injury is thought to play a role in the pathogenesis. Making the diagnosis is challenging, as well as determining the optimal timing of surgical intervention. An estimated 20-40% of neonates with NEC require surgical intervention, and the associated case fatality rate still approaches 50%.

PRE- AND INTRA-OPERATIVE EVALUATION

To determine the role of the microcirculation in the pathogenesis of NEC we evaluated the role of vascular accidents in the pathogenesis of NEC and other gastrointestinal disorders using a new non-invasive biomarker: sidestream darkfield imaging (SDF) in **chapter 2**. Infants with suspected NEC (5 patients) were measured in the armpit and these measurements failed to predict which infants eventually needed surgery. Intra-operatively, the mesenteric circulation in infants with NEC (7 patients) and other gastrointestinal pathology (17 patients) was measured at standardized regions of the intestines (necrotic and non-affected intestinal tissue). We observed no significant differences in the vessel density and blood flow of affected (necrotic) and non-affected intestinal tissue. We did observe a lower vessel density in the non-affected tissues of the NEC group (4.4 mm/mm^2) compared with the gastrointestinal disorders group (7.8 mm/mm^2). This might be due to age differences at surgery because the gestational age of the NEC group was significantly lower than the gestational age of the gastrointestinal disorders group. Atresia patients had a decreased vessel density and blood flow in the atretic part of the intestine compared with the non-affected tissue.

In **chapter 3** we measured intestinal length in 108 patients varying from 24 weeks gestational age to 5 years of age. Until now, only post-mortem values were available, therefore we performed this study in children undergoing a laparotomy. Curve fitting was applied to determine the best model for small intestinal and large intestinal length according to post-conceptional age, weight, and body height at surgery. Body height was eventually the best predictor for expected small and large intestinal length. The equation for small intestinal length was: $\ln(SBL) = 6.741 - 80.409/height$ and for large intestinal length: $CL = 0.111 * height^{1.521}$.

POST-OPERATIVE CARE & CONSIDERATIONS

The optimal composition of pediatric amino acids infusion fluids has been a continuous focus of study. In **chapter 4** we performed a randomized, double-blind, multicentre clinical trial comparing a newly developed parenteral amino acids solution containing alanyl-glutamine with a standard amino acids solution. In total 23 infants were included; all had surgery for a congenital gastrointestinal disorder. 17 received the new composition and 6 the standard composition. The safety and efficacy for the new composition was as good as the tolerance for the standard composition. Compared to reference values, the new composition reflects the amino acid requirements of the infant even better.

In most pediatric surgical departments, the timing of ostomy closure is very variable primarily based on the surgeons' preference or local protocols and currently without any evidence-based guidelines. Therefore we systematically reviewed the literature and performed a meta-analysis to determine the optimal timing of ostomy closure in infants with NEC in **chapter 5**. Only 5 articles were eligible for inclusion of which three articles could be used for a forest plot regarding complication rate after ostomy closure. These 3 articles consisted all of the necessary data to create a forest plot; the other articles were used for descriptive purposes. Infants with early closure (within 8 weeks) had a complication rate of 27% versus 23% in infants with late ostomy closure (after 8 weeks); the combined odds ratio was 1.1. Due to the lack of well-designed studies, we could recommend either one of the approaches.

Chapter 6 retrospectively evaluated our own patient population with NEC in regard to the optimal timing of ostomy closure. The study focused on intra-abdominal adhesion formation and an analysis of resource consumption and costs. Between 1997-2009, 13 patients underwent early ostomy closure and 62 patients late ostomy closure. Adhesion formation was not different between both groups. In addition, the costs of hospital stay, surgical interventions, and outpatient clinic visits were similar.

The role of the residual colonic length on intestinal adaptation in 106 infants with short bowel syndrome was the focus of study in **chapter 7**. A multiple variable cox-proportional hazards model showed that multidisciplinary management, percentage of remaining small intestinal length, and the number of central line complications were more important factors for intestinal adaptation than the residual colonic length.

In **chapter 8** we evaluated the current and new potential referral criteria for pediatric intestinal transplantation. In total 18 transplant centers (36% response rate) from Europe and USA/Canada participated in the study via a questionnaire. Referral for intestinal

transplantation was considered to be too late by 77% of the respondents; educating referring hospitals could improve this. Also, current referral criteria were considered too general by 50% of the respondents. The referral criterion 'persistent hyperbilirubinemia' must be more specific by including a time and value limit. New referral criteria include recurrent septic episodes and fluid/electrolyte disturbances.

MAJOR FINDINGS

Chapter 9 discusses the results from the different studies and implications for the future. The major findings and recommendations of this thesis are the following:

- Sidestream darkfield imaging is a new interesting technique, which could be used as a biomarker to detect viable intestinal tissue at surgery for gastrointestinal disorders.
- Small and large intestinal length should be described as a percentage of expected small and large intestinal length according to body height at surgery.
- A new amino acid composition containing alanyl-glutamine better reflects the amino acid requirements of infants after surgery for gastrointestinal disorders.
- Regarding post-operative complication rate, early ostomy closure is as favorable as late ostomy closure.
- There are no differences in adhesion formation at early versus late ostomy closure.
- Early ostomy closure did not result in a reduction in resource consumption and costs compared with late ostomy closure.
- The colon does not play a role in intestinal adaptation in infants with short bowel syndrome.
- Multidisciplinary management, the percentage of remaining small intestinal length, and the number of central line complications are of importance for intestinal adaptation in infants with short bowel syndrome.
- The referral criterion 'persistent hyperbilirubinemia' for pediatric intestinal transplantation should be more defined by adding a time and value limit.
- The referral criteria 'recurrent septic episodes' and 'fluid/electrolyte disturbances' should be added to the list of referral criteria for pediatric intestinal transplantation.

NEDERLANDSE SAMENVATTING

Necrotiserende enterocolitis (NEC) is een van de meest voorkomende darmziekten bij premature neonaten (90% van de gevallen). De prevalentie van NEC is 7% onder neonaten met een laag geboortegewicht (500-1500 gram). De pathogenese blijft een mysterie. Een combinatie van intestinale immaturiteit, abnormale bacteriële kolonisatie, flesvoeding en hypoxisch-ischemische incidenten lijken alle een rol te spelen bij het ontstaan van NEC. Het is een uitdaging om de diagnose te stellen, evenals het bepalen van de optimale tijd van chirurgische interventie. Ongeveer 20-40% van de neonaten met NEC wordt uiteindelijk geopereerd, en de geassocieerde mortaliteit benadert 50%.

PRE- AND INTRA-OPERATIEVE EVALUATIE

Om de rol van de microcirculatie in de pathogenese van NEC te bepalen, hebben we in **hoofdstuk 2** de rol van vasculaire incidenten in NEC en andere gastrointestinale aandoeningen bepaald met behulp van een nieuwe non-invasieve biomarker: sidestream darkfield imaging (SDF). Neonaten met vermoeden NEC (5 patiënten) zijn gemeten in de oksel. Deze metingen waren niet voorspellend voor welke neonaten uiteindelijk een operatie nodig hadden. Intra-operatieve metingen werden verricht in neonaten met NEC (7 patiënten) en neonaten met andere gastrointestinale pathologie (17 patiënten) op gestandaardiseerde plekken op de darmen (necrotisch en niet aangedaan darmweefsel). Er werden geen significante verschillen gevonden tussen de dichtheid van de vaten en de stroomsnelheid van het bloed tussen necrotisch en niet aangedaan darmweefsel. De vaatdichtheid van het niet aangedane darmweefsel van neonaten met NEC (4.4 mm/mm^2) is lager dan dat van neonaten met een gastrointestinale aandoening (7.8 mm/mm^2). Dit kan mogelijk verklaard worden door de significant kortere zwangerschapsduur bij geboorte van de NEC groep vergeleken met de gastrointestinale aandoeningen groep. Neonaten met een atresie hadden een verminderde vaatdichtheid en bloedstroomsnelheid in het atretische deel vergeleken met het niet aangedane deel van het darmweefsel.

In **hoofdstuk 3** hebben we de darmlengte gemeten van 108 patiënten in de leeftijd van 24 weken zwangerschapsduur tot 5 jaar oud die een laparotomie ondergingen. Tot op heden waren er alleen postmortale metingen beschikbaar. Het beste model voor dunne- en dikke darmlengte werd bepaald aan de hand van post-conceptionele leeftijd, gewicht en lichaamslengte. Lichaamslengte bleek uiteindelijk de beste voorspeller voor de darmlengte. De formule voor dunne darmlengte is: ln(dunne darmlengte) = 6.741 – 80.409/lichaamslengte en voor dikke darmlengte: dikke darmlengte = 0.111 * lichaamslengte$^{1.521}$.

POST-OPERATIEVE ZORG EN OVERWEGINGEN

De optimale samenstelling van pediatrische aminozurenoplossingen is een veel bestudeerd onderwerp. In **hoofdstuk 4** hebben we een gerandomiseerde, dubbelblinde, multicenter klinische studie verricht waarin we een nieuw ontwikkelde aminozurenoplossing die alanyl-glutamine bevat vergeleken met de huidige standaard samenstelling. 23 Kinderen die een operatie hebben ondergaan in verband met een congenitale gastrointestinale aandoening waren geïncludeerd van wie 17 kinderen de nieuwe samenstelling hebben ontvangen en 6 de standaard samenstelling. De nieuwe samenstelling voldeed beter aan de aminozurenbehoefte van de kinderen dan de standaard samenstelling.

In de meeste kinderchirurgische klinieken is de timing van het sluiten van een stoma erg variabel. Dit is gebaseerd op de voorkeur en ervaring van de kinderchirurgen of lokale protocollen zonder evidence-based richtlijnen. Daarom hebben we in **hoofdstuk 5** de literatuur systematisch bekeken en een meta-analyse verricht om de optimale timing van stoma sluiten in kinderen met NEC te bepalen. Slechts 5 artikelen voldeden aan de inclusiecriteria waarvan 3 artikelen gebruikt zijn om een forest plot te creëren dat het aantal complicaties bekeek na het sluiten van een stoma. De andere artikelen zijn gebruikt voor descriptieve doeleinden. Er werden geen verschillen gevonden in het aantal complicaties tussen het vroeg sluiten van het stoma (binnen 8 weken, 27%) en het laat sluiten van het stoma (na 8 weken, 23%). De gecombineerde odds ratio was 1.1. Tot er betere studies verschijnen, kan voor geen van beide benaderingen een voorkeur worden uitgesproken.

In **hoofdstuk 6** hebben we een retrospectieve evaluatie beschreven van alle kinderen met NEC die sluiten van het stoma hebben ondergaan. De studie richtte zich op intra-abdominale vorming van adhesies en een analyse van de kosten-effectiviteit. Tussen 1997-2009 ondergingen 13 patiënten vroeg sluiten van het stoma (binnen 6 weken) en 62 patiënten laat sluiten van het stoma (tussen 6 weken en 1 jaar). Aantal adhesies verschilde niet tussen beide groepen. Ook de kosten, chirurgische interventies en bezoeken aan de polikliniek verschilden niet tussen beide groepen.

De rol van het colon in intestinale adaptatie in 106 kinderen met het korte darm syndroom stond centraal in **hoofdstuk 7**. Analyse toonde aan dat een multidisciplinaire aanpak, het percentage resterende dunne darm en het aantal complicaties door een centrale lijn van groter belang waren als factoren bij intestinale adaptatie dan het colon in continuïteit.

Hoofdstuk 8 richtte zich op de huidige en potentiële criteria voor verwijzing voor darmtransplantatie bij kinderen. 18 Transplantatiecentra (36% van alle centra) uit Europa en USA/Canada hebben de vragenlijst ingevuld. Verwijzing voor darmtransplantatie gebeurt te laat in 77% van de gevallen. Dit zou verbeterd kunnen worden door de ziekenhuizen die verwijzen, beter te informeren. De criteria voor verwijzing waren weinig specifiek volgens 50% van de respondenten. Het criterium 'persisterende hyperbilirubinemie' kan specifieker worden gedefinieerd door het toevoegen van een tijds- en waardelimiet. Nieuwe criteria voor de lijst zijn recidiverende septische episoden en water/elektrolyten stoornissen.

BEVINDINGEN

Hoofdstuk 9 bediscussieert de resultaten van de studies en geeft aanbevelingen voor de toekomst.

De belangrijkste bevindingen en aanbevelingen van dit proefschrift zijn:

- Sidestream darkfield imaging is een nieuwe interessante techniek die gebruikt zou kunnen gaan worden om darmweefsel tijdens een operatie te beoordelen.
- Dunne en dikke darmlengte moeten beschreven worden als een percentage van verwachte lengte naar lichaamslengte bij operatie.
- Een nieuwe aminozurenoplossing die analyl-glutamine bevat, voldoet beter aan de aminozurenbehoefte van kinderen na chirurgie voor gastrointestinale aandoeningen dan de huidige oplossingen.
- Vroeg versus laat sluiten van het stoma geeft geen verschil in post-operatieve complicaties.
- Er zijn geen verschillen in het aantal adhesies bij het vroeg en laat sluiten van het stoma.
- Het vroeg sluiten van het stoma leidde niet tot een reductie in de kosten in vergelijking met laat sluiten.
- Het colon speelt geen rol in intestinale adaptatie in kinderen met het korte darm syndroom.
- Een multidisciplinaire benadering, percentage resterende dunne darmlengte en het aantal complicaties ten gevolge van een centrale lijn zijn belangrijk bij intestinale adaptatie in kinderen met het korte darm syndroom.
- Het criterium 'persisterende hyperbilirubinemie' voor darmtransplantatie bij kinderen dient specifieker gedefinieerd te worden; dit kan door het toevoegen van een maximale acceptabele waarde van bilirubine en een tijdafhankelijke variabele.
- 'Recidiverende septische episoden' en 'water/elektrolyten stoornissen' zijn criteria die toegevoegd moeten worden aan de lijst criteria voor verwijzing voor darmtransplantatie bij kinderen.

PART V
APPENDICES

Nothing is as important as passion. No matter what you want to do with your life, be passionate

Jon Bon Jovi

About the author

List of publications

PhD portfolio

Dankwoord

ABOUT THE AUTHOR

Adriana Elisabeth Catharina Johanna Maria (Marie-Chantal) Struijs was born on June 6, 1982 in Breda. She grew up in Etten-Leur, where she completed her secondary school education (Gymnasium) in 2000 at the Katholieke Scholengemeenschap Etten-Leur (KSE). Before studying medicine, she obtained her 'propedeuse' in Health Sciences at Maastricht University in 2001. From 2001 to 2007, she went on to study Medicine at Maastricht University (doctoral phase cum laude). For her scientific research project she spent 5 months in the Department of Lung Biology (supervisor Dr. Belik) at The Hospital for Sick Children in Toronto, Canada. After completing her medical studies, she returned to The Hospital for Sick Children to work on various research projects in the Department of Pediatric Surgery (Head Dr. Langer, supervisors Dr. Wales and Dr. Chiu). In September 2008 she began the work on this thesis in the Department of Pediatric Surgery of the Sophia Children's Hospital in Rotterdam, The Netherlands (supervisors Prof.dr. Tibboel and Prof.dr. Van Goudoever). In July 2011, she completed an MSc with distinction in Surgical Sciences through the University of Edinburgh. From May 2011 to September 2011 she went on to work as a surgical resident in the Department of General Surgery at the Erasmus MC, Rotterdam (Head Prof.dr. Van Lanschot). In January 2012, she began her general surgical training at the Sint Franciscus Gasthuis in Rotterdam, The Netherlands (supervisors Dr. Mannaerts and Prof.dr. IJzermans).

LIST OF PUBLICATIONS

1. **Struijs M-C**, Buijs EAB, Vlot J, Hop WCJ, Goudoever van JB, Keijzer R, Tibboel D. Microcirculatory evaluation of the surgical newborn: a new biomarker? Submitted.
2. **Struijs M-C**, Schaible T, Elburg van RM, Debauche C, Beest te H, Tibboel D. Efficacy and safety of GLN-AA versus Standard-AA in infants: a first-in-man randomized double-blind trial. Submitted.
3. **Struijs M-C**, Sloots CEJ, Hop WCJ, Tibboel D, Wijnen RMH. The timing of ostomy closure in infants with necrotizing enterocolitis: a systematic review. Accepted Pediatr Surg Int.
4. **Struijs M-C**, Sloots CEJ, Tibboel D, IJzermans JNM. The gap in referral criteria for pediatric intestinal transplantation. Accepted Transplantation.
5. **Struijs M-C**, Poley MJ, Meeussen CJHM, Madern GC, Tibboel D, Keijzer R. Late versus early ostomy closure for necrotizing enterocolitis: analysis of adhesion formation, resource consumption, and costs. J Pediatr Surg 2012;47:658-664.
6. Diamond IR, **Struijs M-C**, Silva de N, Wales PW. Does the colon play a role in intestinal adaptation in infants with short bowel syndrome? J Pediatr Surg 2010;45:975-979.
7. **Struijs M-C**, Somme S, Lasko D, Chiu PPL. Gastric emptying scans: unnecessary pre-operative testing for fundoplications? J Pediatr Surg 2010;45:350-354.
8. Nasr A, **Struijs M-C**, Ein S, Chiu PPL. Outcomes after muscle flap vs prosthetic patch repair for large congenital diaphragmatic hernias. J Pediatr Surg 2010;45:151-154.
9. **Struijs M-C**, Diamond IR, Silva de N, Wales PW. Establishing norms for intestinal length in children. J Pediatr Surg 2009;44:933-938.
10. **Struijs M-C**, Diamond IR, Pencharz P, Chang K, Viero S, Langer JC, Wales PW. Absence of the interstitial cells of Cajal leading to intestinal pseudo-obstruction. J Pediatr Surg 2008,43:E25-E29.

PhD PORTFOLIO

Name PhD student	AECJM Struijs
Erasmus MC department	Pediatric Surgery
PhD period	September 2008 – December 2011
Promotoren	Prof.dr. D. Tibboel
	Prof.dr. J.B. van Goudoever
Copromotor	Dr. R. Keijzer

	Year	Workload (ECTS)
General academic skills		
English biomedical writing and communication	2010	4
Good clinical practice (BROK)	2009	1.1
Minicursus methodologie en patiëntgebonden onderzoek	2009	0.3
Research skills		
Classical methods for data analysis	2009	5.7
Introduction to clinical research	2009	0.9
In-depth courses		
MSc Surgical Sciences, University of Edinburgh	2008-2011	90
Presentations		
EUPSA/BAPS: oral (2x)	2012	0.8
EUPSA: oral, poster (2x)	2011	1.0
Chirurgendagen: poster	2010	0.3
EUPSA/BAPS: oral presentation	2009	0.4
CAPS: oral presentation	2008	0.4
International conferences		
EUPSA/BAPS, Rome, Italy	2012	0.9
EUPSA, Barcelona, Spain	2011	0.9
EUPSA/BAPS, Graz, Austria	2009	0.9
CAPS, Toronto, Canada	2008	0.9

Seminars and workshops

Chirurgendagen	2010	0.6
Symposium: Monitoring cerebral (and somatic) oxygenation: does it have clinical relevance	2010	0.3
Research day pediatrics	2009	0.3
Chirurgendagen	2009	0.6
Minisymposium: let it flow	2009	0.1
Scientific writing course	2008	0.6
Gallie day, Toronto	2008	0.3
Lab courses	2007	0.5

Teaching activities

Supervision medical student (research project)	2010	1.5
Presentations for medical students	2009-2010	0.3
Presentations nursing staff	2009	0.3

DANKWOORD

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