UNDERSTANDING COMPLICATIONS OF SURGERY IN INFANCY

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A thesis submitted to University College London
for the degree of Doctor of Philosophy

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January 2017

Declaration

I, Mark Bishay, confirm that the work presented in this thesis is my own.

Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signature:  Date: 31st January 2017

Abstract

This thesis investigates complications of surgery in infants, particularly infections and liver disease in infants receiving parenteral nutrition (PN) following gastrointestinal surgery, and intraoperative hypercapnia and acidosis in surgery for congenital diaphragmatic hernia (CDH) and oesophageal atresia with tracheo-oesophageal fistula (OA/TOF), using a series of clinical studies.

A pilot randomised controlled trial comparing open versus thoracoscopic surgery in neonates with CDH and OA/TOF showed that neonatal thoracoscopy resulted in more severe intraoperative hypercapnia and acidosis than open surgery, particularly in patients with CDH. This highlights a need for studies assessing neurodevelopmental outcomes following neonatal thoracoscopy.

In surgical infants receiving PN, chlorhexidine antisepsis to clean central venous catheter connectors was associated with a significant reduction in the rate of septicaemia (particularly staphylococcal). In such infants, septicaemia due to bowel organisms occurred later than septicaemia due to coagulase-negative staphylococci.

In congenital duodenal obstruction, while avoidance of initial PN was successful for two thirds of cases in which it was attempted, one third subsequently required PN, and this group showed poorer growth than children who commenced PN soon after surgery.

One third of surgical infants with intestinal failure develop intestinal failure associated liver disease (IFALD), and 61% developed septicaemia. I found no association between septicaemia and IFALD.

In a randomised controlled trial to investigate whether glutamine supplementation affects the incidence of microbial invasion in surgical infants receiving PN, microbial invasion was detected by blood cultures, broad-range and targeted PCR for bacterial DNA, and assays of endotoxin, and lipopolysaccharide binding protein. Monocyte HLA-DR expression was measured by flow cytometry. Glutamine had no effect on microbial invasion, which was detected in 60% of patients (half of which was detected by blood culture). Glutamine supplementation significantly enhanced recovery of monocyte function. Among patients with low monocyte function at enrolment, glutamine was protective against microbial invasion.

Acknowledgements

This work would not have been possible without the support of many people. I want to thank my supervisors Dr Simon Eaton, Prof Agostino Pierro, and Prof Nigel Klein for their guidance, time, ideas, feedback and support. I am grateful to colleagues in the lab, in particular Hannah Jones and Marianne Jacobsen for teaching me the flow cytometry techniques, and Kathryn Harris and colleagues in the microbiology lab for teaching me DNA extraction and PCR techniques and providing advice and guidance. Thanks to the pharmacists and dieticians who supervised the administration of trial supplementation and randomised patients, especially Venetia Horn, Dominika Gayda-Pimlott, Sarah MacDonald, Marlene Ellmer and Danielle Hesketh. I am also grateful to my clinical and nursing colleagues for their assistance. I am particularly grateful to all the parents who agreed for their children to take part in these studies.

I thank Sparks for the project grant that financially made this work possible. I am deeply thankful to my research colleagues for their friendship, camaraderie, advice and collaboration, which made my time at the Institute of Child Health so enjoyable and memorable – thanks especially to Shireen Nah, Luca Giacomello, Giuseppe Retrosi, Massimo Garriboli and Mandela Thyoka. I thank Prof Paolo de Coppi for his warmth, support and advice, and Mr Ed Kiely for his wisdom and inspirational example. Finally, thanks to my family and friends, and most of all my wife Helen, for supporting me through this long journey with unfailing encouragement, love and prayers.

Statement of Personal Contribution

The work in this thesis is all my own, except where I have acknowledged the contributions of others, detailed below. In particular, I co-ordinated and ran the prospective randomised controlled trials described in chapter 6 and 7, and I performed the laboratory analyses for the MIGS study described in chapter 6, including molecular biology techniques (PCR) for detection of bacterial DNA, and immunological and biochemical assays including flow cytometry for measurements of monocyte activation, and measurements of endotoxin, lipopolysaccharide binding protein and soluble CD14 levels.

I am grateful to the following for assistance with data collection for various studies: Giuseppe Retrosi, Mandela Thyoka, Massimo Garriboli, Judith Pichler, Luca Giacomello, Bhanumathi Lakshminarayanan, Alexis Arnaud, Shireen Nah, Stavros Loukogeorgakis, and Sonia Basson. Both randomised controlled trials had been designed and obtained ethical approval prior to my involvement (this was done by my supervisors together with Nicholas Alexander for the MIGS trial and Luca Giacomello for the CO_2 trial). I amended the trial protocols and obtained ethical approvals for the amendments. My supervisor Dr Simon Eaton kindly performed the mass spectrometry of exhaled CO_2, as well as the multilevel modelling, binary logistic, ordinal and Poisson regression analyses.

Table of Contents

List of Tables

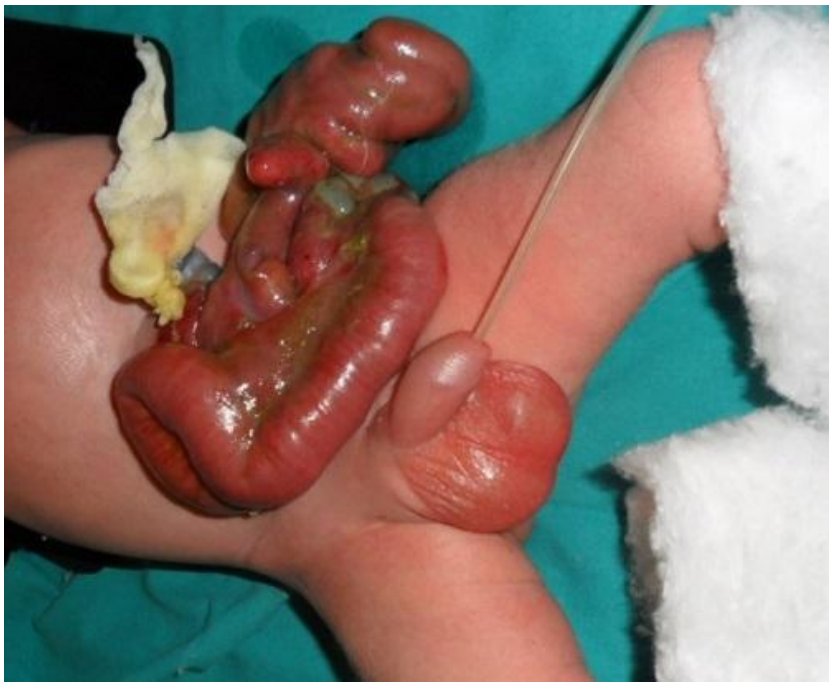
List of Illustrations

Chapter 1. Introduction

1.1 Background

Figure 1.1 shows a newborn baby with gastroschisis. This striking, and distressing, condition is a congenital defect of the anterior abdominal wall with herniation of the intestines. Fifty years ago, this condition carried a very poor prognosis – for example, Schwaitzberg et al. (Schwaitzberg et al. 1982) from Texas reported a 60% mortality for children with gastroschisis treated in the period 1960-1972.

Figure 1.1 A newborn baby with gastroschisis.



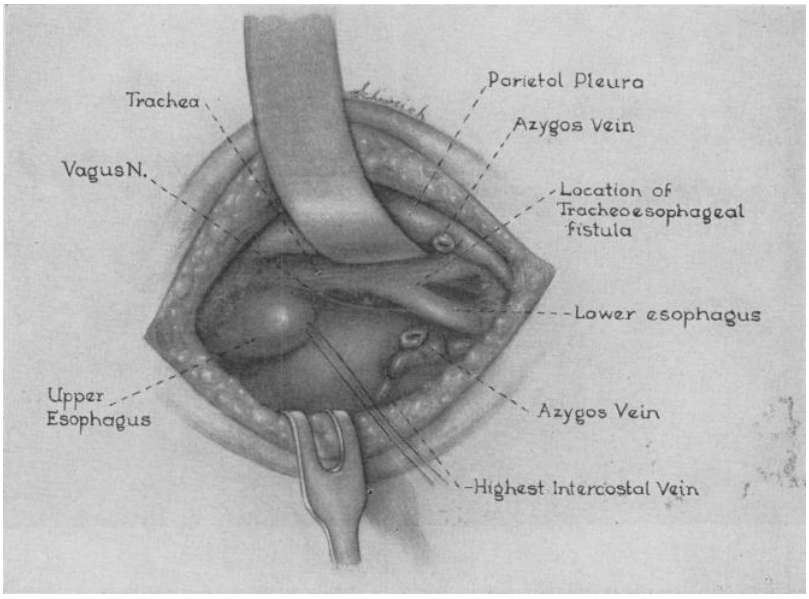
A congenital anomaly whose outcome has been transformed by the introduction of parenteral nutrition. Reproduced from (Zvizdic 2016) - Creative Commons License. https://creativecommons.org/licenses/by/4.0/legalcode

The outlook for these babies has been transformed, and survival is now over 90% in developed healthcare systems (Fillingham and Rankin 2008), with the vast majority growing up to lead a normal independent life. This improvement was relatively sudden and dramatic - Schwaitzberg et al. (Schwaitzberg et al. 1982) themselves report 93% survival for the period 1973-1981, and the main reason for the improvement ("the most significant advance", according to the authors) was the introduction of intravenous, or parenteral nutrition (PN), which allows children to receive essential nutrition (including water, carbohydrate, lipid, protein, electrolytes, vitamins and trace elements) directly into the veins, while the intestines are unable to absorb enteral feed.

Gastroschisis is only one of a group of conditions, including other abdominal wall defects, congenital intestinal obstruction due to various causes, and necrotising enterocolitis (NEC), in which PN has come to play a key role following gastrointestinal surgery in infancy. Section 1.2 gives a brief outline of these conditions.

Through the course of the twentieth century, developments in paediatric surgical technique and perioperative care of infants have led to similar dramatic improvements in survival for a number of other congenital anomalies. Take the example of oesophageal atresia with trachea-oesophageal fistula (OA/TOF; see Figure 1.2). Mortality for this condition was 100% in the early part of the twentieth century. Various surgeons began to attempt primary surgical repair of oesophageal atresia with ligation of trachea-oesophageal fistula in the 1930s. Thomas Lanman of Boston reported in 1940 a series of 30 neonates with oesophageal atresia and trachea-oesophageal fistula who had been submitted to surgery: none survived (Lanman 1940). Lanman had performed four attempted repairs by an extrapleural approach from 1936 onwards.

Figure 1.2 Operative view of oesophageal atresia and tracheo-oesophageal fistula



Reproduced with permission from (Haight 1944). This is an example of a congenital anomaly for which the outcome has been transformed by advances in neonatal surgical and perioperative care.

The fourth survived nine days and was found at post mortem to have died of overhydration. His remarkable conclusion was that: “In spite of the fatal outcome in all the 30 operative cases, it is felt that considerable progress along rational lines is being made. The successful operative treatment of a patient with this anomaly is only a question of time.”

This optimistic prediction was proved correct when Cameron Haight (Figure 1.3) of Ann Arbor, Michigan, performed the first successful primary repair of oesophageal atresia and tracheo-oesophageal fistula in March 1941 (at his sixth attempt) in an ‘unusually robust’ 12 day old girl (Haight and Towsley 1943). There was a leak from the anastomosis, managed conservatively, and subsequent stricture, managed with a single dilatation at age

Figure 1.3 Cameron Haight, MD

Dr Haight performed the first successful primary repair of oesophageal atresia with tracheo-oesophageal fistula. Reproduced with permission from (Ailawadi et al. 2010).

17 months. The girl was discharged after 18 months and was reported to be alive and well in 2005 (Holcomb et al. 2005). Haight subsequently managed 284 infants with oesophageal atresia over a 30-year period and reported a 52% survival rate. At least half of infants with oesophageal atresia have associated congenital anomalies, and outcomes are strongly influenced by birth weight and congenital heart disease. Waterston in 1962 reported his experience of 218 infants with oesophageal atresia (Waterston et al. 1962). Overall, half survived (i.e. were discharged from hospital able to take feeds well). Over the study period (1946 to 1959), the mortality steadily declined, as the number of operations performed increased (Figure 1.4). He classified them into three risk categories according to birth weight and comorbidity (associated anomalies and pneumonia).

Figure 1.4. Operations performed and mortality rates for oesophageal atresia 1946 - 1959

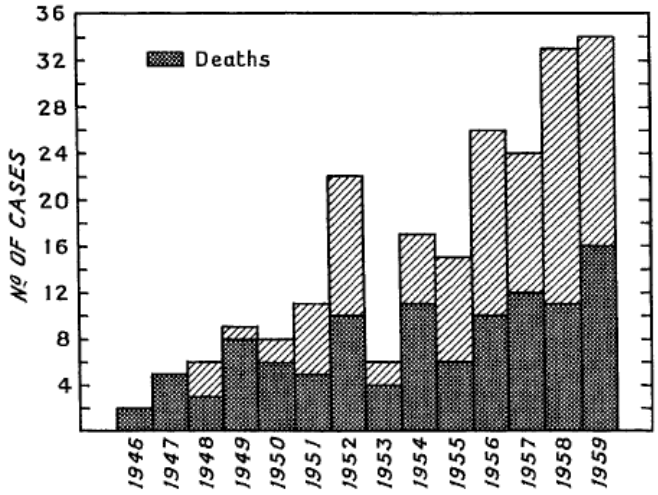


Fig. 2—Number of cases operated on each year and related deaths.

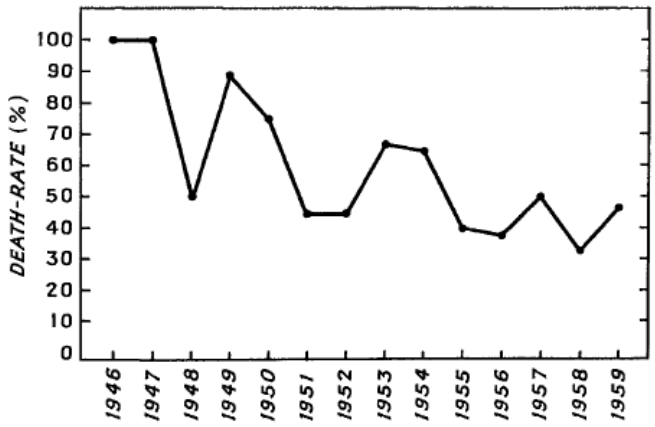


Fig. 3—Death-rate each year.

Waterston's data demonstrate increasing numbers of operations performed and decreasing mortality rates for oesophageal atresia in the period 1946 – 1959. Reproduced with permission from (Waterston et al. 1962).

Survival rates ranged from 95% in the most favourable category down to 6% in the least favourable. Over the years, with shorter time to diagnosis and operation, and improved perioperative and intraoperative care, and increased surgical experience, survival rates continued to improve, at least in well-resourced healthcare settings. By 2006, survival in the least favourable (amended) category was reported to be 50% (Figure 1.5). Similar findings were observed in the most recently reported cohort (Malakounides et al. 2016).

A congenital anomaly which still has a relatively high rate of mortality, despite advances in antenatal diagnosis and neonatal care, is congenital diaphragmatic hernia (CDH), which has an incidence of around 1 in 2500 (Colvin et al. 2005). In this condition, there is a developmental defect in the diaphragm which allows abdominal viscera including bowel/stomach/liver/spleen to move into the thorax (Figure 1.6). Surgical correction involves restoring the abdominal viscera and repairing the diaphragmatic defect, either by primary suturing, or using a patch. Surgery has most often been performed via a laparotomy. Complications of surgery include recurrence of the diaphragmatic hernia - reported in around 3% to 20% of cases (Lansdale et al. 2010; Tsao et al. 2011), and bowel obstruction due to intra-abdominal adhesions.

However, CDH is associated with pulmonary hypoplasia: the ipsilateral lung does not develop normally, leading to pulmonary hypertension (Areechon and Reid 1963). The degree of pulmonary hypoplasia is the major determinant of mortality in infants with CDH. Reviews of series of infants with CDH from paediatric surgical centres report mortality rates in the region of 80% (Garriboli et al. 2012).

Figure 1.5 Survival of neonates with oesophageal atresia at Great Ormond Street Hospital, 1980 to 2004

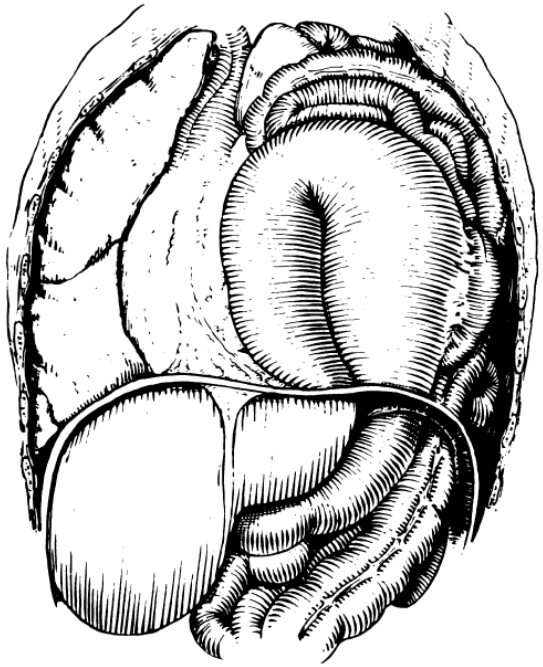
Table 4 Spitz classification comparison of group between 2 studied decades of neonates presented with OA

Group	Definition	1980-1992 (n = 372)	1993-2004 (n = 188)	*P*
I	Birth weight >1500 g, no major cardiac anomaly	283/293 (97%)	130/132 (98.5%)	.44
II	Birth weight <1500 g or major cardiac anomaly	41/70 (59%)	41/50 (82%)	.01
III	Birth weight <1500 g and major cardiac anomaly	2/9 (22%)	3/6 (50%)	.57

These data show continued improvements in survival for the period up to 2004, particularly for those neonates with birth weight <1500g and/or major cardiac anomalies. Reproduced with permission from (Lopez et al. 2006).

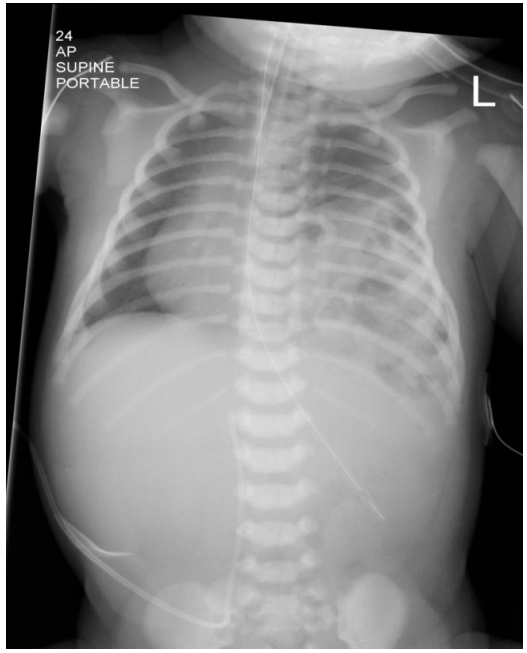
Figure 1.6 Diagrammatic illustration and imaging of congenital diaphragmatic hernia

A



A. Diagrammatic illustration of congenital diaphragmatic hernia; reproduced with permission from (Spitz and Coran 2013); original appears as Figure 1 on page 186.

B



B. X-ray of an infant with left CDH showing bowel herniating into the left side of the chest.

However, such series are only reporting the subgroup of patients who survive to reach surgical centres: population based studies show overall mortality in the region of 50% of liveborn infants, with around 35% of liveborn infants dying before referral or transport to a surgical centre (Colvin et al. 2005). This mortality rate remains significant despite changes in the management of CDH over the last few decades including the use of extracorporeal membrane oxygenation (ECMO) (Morini et al. 2006), inhaled nitric oxide (Finer et al. 1997), high-frequency oscillatory ventilation (HFOV) (Snoek et al. 2016), gentle ventilation with permissive hypercapnia (Guidry et al. 2012), and delayed operative repair (de la Hunt et al. 1996; Reyes et al. 1998).

Long-term follow up of children with CDH demonstrates a broad range of long-term morbidity. Follow up studies report lung function impairment (obstructive and restrictive), abnormality of the pulmonary vasculature with pulmonary hypertension and reduced pulmonary perfusion, gastro-oesophageal reflux disease, failure to thrive, neurocognitive impairment, and musculoskeletal deformity (including scoliosis and chest wall deformities such as pectus excavatum) (Colvin et al. 2005; Jaillard et al. 2003; Peetsold et al. 2007; Peetsold et al. 2009a; Peetsold et al. 2009b; Peetsold et al. 2010). It is difficult to determine to what extent these problems are related to the developmental anomaly and to what extent they may be partly attributable to complications of surgery or perioperative management.

All surgical intervention carries a risk of complications, both intraoperative and postoperative. Intra-operative complications of surgery in infants include blood loss - which frequently necessitates transfusion, hypothermia, dehydration and acidosis. Postoperative complications applicable to a wide range of neonatal surgery include

infections, pain, recurrent bowel obstruction due to intra-abdominal adhesions, and anastomotic leaks or strictures.

In the last three decades, minimally invasive surgery has been increasingly applied in children, infants and neonates (Ponsky and Rothenberg 2008; Ure et al. 2005), with the goals of reducing operative trauma and hence promoting a quicker recovery with less postoperative pain. It also results in more subtle scars (a cosmetic advantage) and aims to reduce the risk of future bowel obstruction due to adhesions. Minimally invasive techniques have been applied to a wide range of surgical problems in infancy, including laparoscopy for the diagnosis and/or treatment of conditions including inguinal hernia, gastro-oesophageal reflux disease, Hirschsprung's disease, malrotation, intussusception, necrotising enterocolitis and duodenal atresia. Thoracoscopy has been applied in neonates for the repair of CDH and OA/TOF. In the latter case this aims to avoid a thoracotomy and thus reduce the risk of chest wall deformity and scoliosis. However, the application of these techniques may bring its own complications. I shall examine this further in Chapter 7.

Parenteral nutrition, although it has revolutionised outcomes for infants requiring gastrointestinal surgery, also has complications of its own. It has been associated with the development of liver disease, complications related to vascular access devices, and metabolic complications (related to imbalance in administration and metabolism of nutrients) (Pierro 2003). However the most commonly encountered complications in surgical infants receiving PN are infections – with bloodstream infections found in 15% of such infants with an incidence rate of 12.6 episodes of septicaemia per 1000 PN days (Donnell et al. 2002), and 56% of surgical infants receiving PN for 28 days or more

(Pichler et al. 2010). These infections can lead to impaired liver function (Beath et al. 1996), removal of central venous catheters, critical illness, or even death. Clearly it is of vital importance that those looking after infants requiring surgery do everything possible to minimise the risks of significant complications.

1.2 Surgical Conditions Requiring Parenteral Nutrition in Infants

Infants with gastrointestinal disease requiring surgery will almost all have a period postoperatively in which they are not fed, as the bowel is not ready to function normally and absorb enteral feeds (postoperative ileus). This maybe a very short period, less than 48 hours even, in, for example an infant undergoing a laparoscopic-assisted transanal operation for Hirschsprung's disease. Historically, surgeons have been very cautious about introducing enteral feeds postoperatively, fearing that early feeding may increase the likelihood of complications such as anastomotic leakage, or that it would lead to overfilling of a dilated loop of bowel which is in ileus, leading to vomiting, or even to a dilated loop of bowel kinking over to cause obstruction. More recently, some surgeons have adopted a policy of feeding more promptly, even immediately postoperatively, in, for example an infant who has undergone colostomy closure having previously had surgery for an anorectal malformation. At the other extreme, some infants with extremely dysmotile bowel (for example due to gastroschisis), or with a very short length of remaining small bowel (for example due to necrotising enterocolitis), may need parenteral nutrition for many months and even be discharged home with PN. The decision to commence PN is made based on the expected duration of postoperative ileus (although this can be difficult to predict) and other factors including the infant's preoperative nutritional status. In general, an infant who is not expected to tolerate enteral feeds for at least 5-7 days, should be commenced on parenteral nutrition. Such infants will have a nasogastric tube in situ to drain the stomach to prevent vomiting and minimise the risk of aspiration. As bowel function starts to return, the volume of gastric aspirates will reduce and the aspirates will become less bilious, and the infant will start to stool.

This will guide the introduction and gradual increase of enteral feeds as tolerated. As enteral feeds are gradually increased, PN is gradually decreased and eventually stopped when the infant is tolerating sufficient enteral feed.

1.2.1 Congenital Bowel Obstruction

1.2.1.1 Duodenal and jejunoileal atresias

Congenital intestinal obstruction in the form of an atresia (a complete blockage in the bowel) or a stenosis (a narrowing causing a partial blockage, for example due to a fenestrated membrane across the bowel lumen) is one of the most common causes of intestinal obstruction in the newborn, with an overall incidence of around 1 in 2500 to 1 in 5000 live births (Best et al. 2012; Torfs and Christianson 1998). Duodenal atresia or stenosis has an incidence of around 1 in 4000 to 1 in 11000 live births (Best et al. 2012; Torfs & Christianson 1998). It is frequently associated with other anomalies including trisomy 21 and cardiac malformations (Choudhry et al. 2009). It is generally repaired by an operation to bypass the obstruction (a duodenoduodenostomy or a duodenojejunostomy). Different surgeons and centres have varying policies as to whether they routinely commence parenteral nutrition post operatively in these infants (I shall explore this further in Chapter 4).

Jejunoileal atresia (Figure 1.7) occurs in around 1 in 6000 to 1 in 14000 newborns (Best et al. 2012; Torfs & Christianson 1998), with multiple atresias found in around one fifth of cases (Grosfeld et al. 1979). The aetiology is thought to be antenatal vascular disruption: experiments in canine models showed that intrauterine ligation of mesenteric vessels produces similar anomalies (Louw and Barnard 1955).

Figure 1.7 Different types of jejunoileal atresia

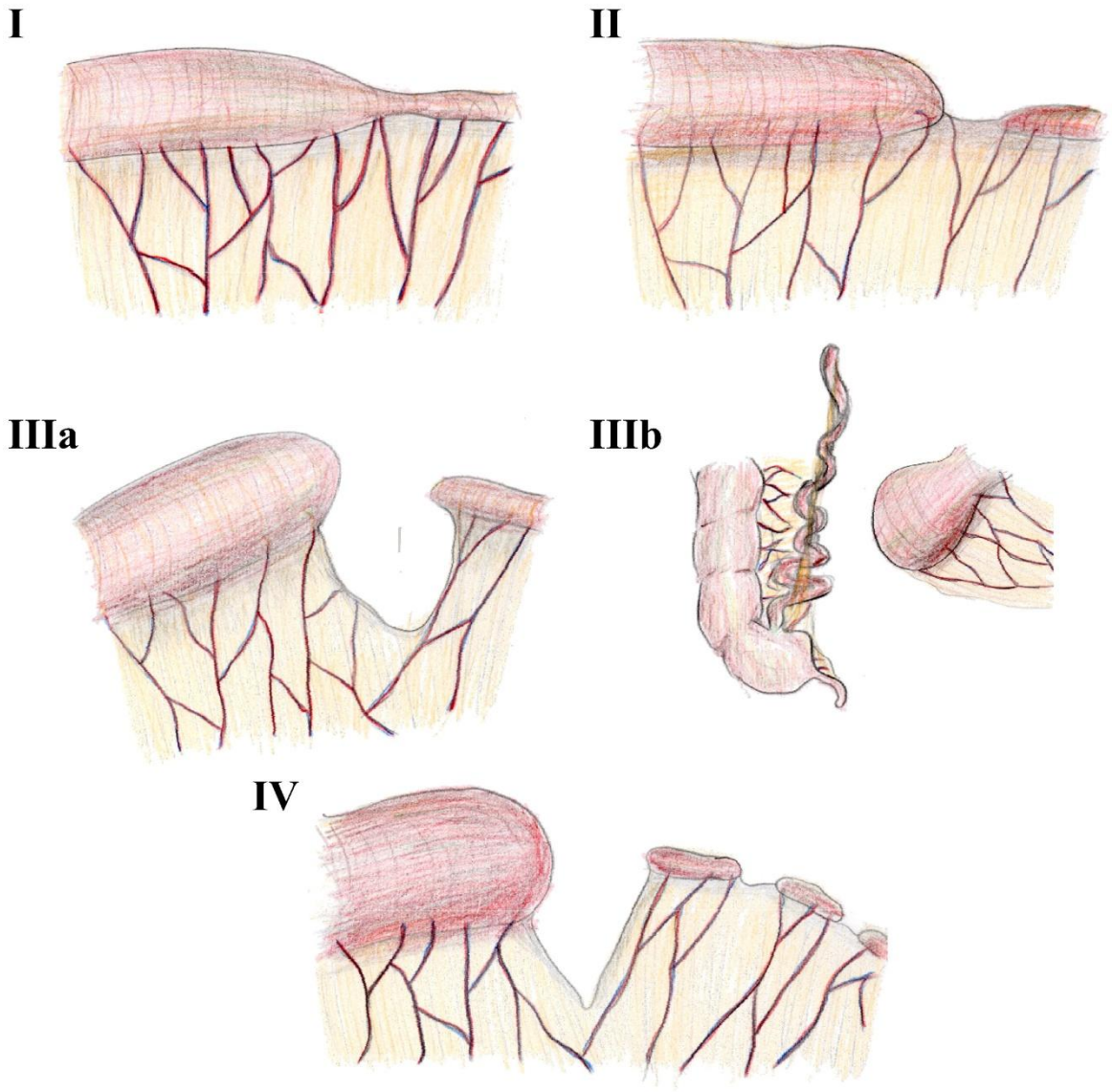


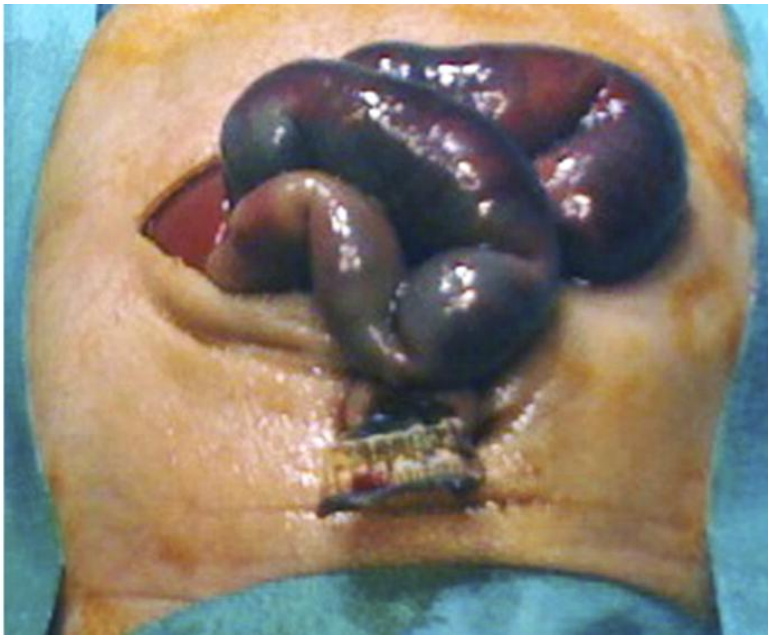
Illustration of different types of jejunoileal atresia encountered, reproduced with permission from (Adams and Stanton 2014), adapted from (Grosfeld et al. 1979).

Jejunoileal atresia can be associated with gastroschisis, or with cystic fibrosis. Surgery typically involves resection of the distended proximal bowel (which will otherwise be dysmotile) and end-to-end anastomosis, with or without some tapering of the proximal bowel. Like gastroschisis, this is a condition where the introduction of parenteral nutrition has transformed the prognosis, with survival rates improved from around 50% before the use of PN (Pollock and Bergin 1961), to 90% or more thereafter (Grosfeld et al. 1979). The duration of PN required varies greatly, influenced to some extent by factors including the remaining length of small bowel, the degree of proximal bowel dilatation, the number of anastomoses, and the site of the original obstruction.

1.2.1.2 Malrotation

Another congenital anomaly which causes neonatal intestinal obstruction is malrotation, in which the bowel has not gone through the normal process of rotation, folding and fixation in the abdominal cavity as it grows antenatally (and as it returns to the abdominal cavity following herniation within the cord during embryological development), with the result that the midgut (the section of bowel supplied by the superior mesenteric artery) has an abnormally narrow mesentery and is at risk of twisting on its blood supply (midgut volvulus). This can have catastrophic consequences, leading to loss of almost the entire small bowel, leaving insufficient remaining bowel to absorb nutrients. Intestinal obstruction in the context of malrotation may result from either volvulus or extrinsic compression of the duodenum by peritoneal attachments (known as Ladd's bands) running between the abnormally sited caecum and the right side of the abdominal cavity.

Figure 1.8 A neonate with malrotation and volvulus



Intraoperative photograph demonstrating ischaemic small bowel in a neonate with malrotation and volvulus. Reproduced with permission from (Kiely et al. 2012).

Malrotation presents clinically with obstruction in around 1 in 3000 infants (Forrester and Merz 2003; Torfs & Christianson 1998). Post mortem studies are reported to suggest the condition may be present in up to 1% of the population (with the vast majority apparently asymptomatic) (Adams & Stanton 2014).

Malrotation may be associated with duodenal or jejunoileal atresias, anorectal malformations, cardiac anomalies, or trisomy 21 (Forrester & Merz 2003; Torfs & Christianson 1998). Surgery may involve detorsion of volvulus, excision of necrotic bowel (if bowel is of dubious viability relook surgery may be planned around 48 hours later to give the opportunity for bowel to recover following detorsion), and steps to minimise the future risk of volvulus, including division of Ladd's bands, broadening the

mesentery, and placing the small bowel on the right and the colon on the left. Again, the duration of postoperative PN required will vary, and will be influenced by factors including the remaining length of small bowel, whether there was any bowel resection (and if so whether an anastomosis or a stoma was formed), and the physiological condition of the infant at presentation.

1.2.1.3 Meconium ileus

Another important cause of neonatal intestinal obstruction is meconium ileus, where abnormally viscous bowel contents (often described as inspissated meconium) cause bowel obstruction. This condition is almost always associated with cystic fibrosis (Burge 2016). In these infants, abnormal exocrine mucus secretion and pancreatic enzyme deficiency result in intestinal contents with lower water content and decreased enzyme levels, with more viscous mucus. The resulting firm concretions of meconium tend to cause obstruction in the terminal ileum. Meconium ileus may be described as simple (uncomplicated intestinal obstruction), or as complicated. Complications of meconium ileus may include segmental volvulus, bowel necrosis, intestinal atresia, and antenatal bowel perforation which may lead to formation of a meconium pseudocyst. Surgical management may include enterotomy and washout of inspissated meconium, bowel resection and anastomosis or stoma formation. Postoperatively, when enteral feeds are reintroduced, infants with cystic fibrosis are given pancreatic enzyme supplements. Simple cases with no bowel resection may not require parenteral nutrition, while infants with complicated meconium ileus are likely to have a longer period of postoperative ileus.

1.2.1.4 Hirschsprung's disease

This is a condition affecting around 1 in 5000 to 1 in 9000 infants (Best et al. 2014; Chia et al. 2016; Suita et al. 2005; Torfs & Christianson 1998) where the nerve supply to the bowel has not developed normally, resulting in a segment of bowel which does not relax and dilate normally and acts as an obstruction (although the lumen is physically patent).

The condition is named after Harald Hirschsprung, a Danish paediatrician who described two cases at a conference of the German Society of Paediatrics in March 1886 (Hirschsprung H 1888; Skaba 2007). He described two infants who died of complications of bowel obstruction and were found at autopsy to have marked dilatation and hypertrophy of the colon, while the rectum appeared normal or narrow. He named the condition congenital megacolon and went on to describe another ten cases before his death in 1916 (Roed-Petersen and Erichsen 1988; Skaba 2007). In fact we now know that the abnormality is not in the dilated 'megacolon' (which is structurally and functionally normal, but obstructed), but in the distal narrow rectum, which lacks parasympathetic ganglion cells in the myenteric plexus. These cells are derived from neural crest cells and migrate along the bowel in a caudal direction from the oesophagus from the 5^{th} to 12^{th} week of gestation. In patients with Hirschsprung's disease this migration is arrested at varying points, resulting in a variable length of distal bowel which is abnormally innervated and cannot function normally. In around 75% of cases the transition from normal bowel to aganglionic bowel is in the rectosigmoid region, but in around 5 to 10% of cases the entire colon may be aganglionic, along with a variable length of small bowel (Suita et al. 2005).

Infants with Hirschsprung's disease classically present with features of distal intestinal obstruction: failure to pass meconium in the first 48 hours life, abdominal distension, and vomiting which turns bilious. In the majority of infants this can be managed initially with rectal washouts followed by a suction rectal biopsy to confirm the diagnosis followed by planned definitive surgery. In such cases, PN is not usually needed. However, in a minority of patients, an emergency operation may be needed, for example due to intestinal perforation, or enterocolitis which does not respond to conservative treatment with bowel rest, intravenous antibiotics and rectal washouts. Hirschsprung's disease is associated with severe, potentially life-threatening infections: Hirschsprung's disease-associated enterocolitis. This can occur before or after definitive surgery, and some infants are prone to recurrent bouts of infection (Gosain and Brinkman 2015; Vieten and Spicer 2004). At emergency surgery for intestinal perforation or enterocolitis not responding to treatment, a stoma will often need to be formed and levelling biopsies may be taken. Such patients are typically physiologically unwell at the time of surgery and are likely to experience a degree of postoperative ileus and may therefore receive some postoperative PN.

1.2.2 Abdominal Wall Defects

The two main congenital abdominal wall defects are gastroschisis (see Figure 1.1 and section 1.1) and exomphalos (also termed omphalocele). In gastroschisis, the bowel herniates through a small defect in the abdominal wall which is almost always just to the right of the umbilicus, and the bowel has no covering over it. In exomphalos, the bowel (often accompanied by the liver) herniates through the umbilicus itself (in the midline, in the root of the umbilical cord) and is covered by a sac (onto which the umbilical cord

inserts). Exomphalos importantly differs from gastroschisis in that it is often associated with other anomalies, including cardiac and chromosomal anomalies (Kong et al. 2016). These associated anomalies are often the major factors in determining the prognosis of an infant with exomphalos. As the bowel is covered in exomphalos, surgical closure is less urgent. In very large exomphalos defects, surgical closure may not be possible, and this may be managed conservatively, with or without application of disinfectant, awaiting formation of an eschar. In gastroschisis, the bowel is exposed and so there is more urgency for surgical intervention to either close the defect primarily or apply a silo (a synthetic bag, which may be preformed or custom-made) and close the defect in stages. Even after closure of the gastroschisis defect, the bowel remains dysmotile and unable to absorb nutrients for a variable period of time Lap et al. report that in a study of 204 children with gastroschisis the median time to full enteral feeding was 26 days (range 6-515) (Lap et al. 2016). Thus while some neonates may be feeding within a week, other children may be discharged from hospital on home PN after several months. The role of PN is therefore paramount in the management of this condition. Gastroschisis can be complicated by intestinal atresia, perforation, and, rarely, loss of bowel due to antenatal closure of the defect resulting in a very short length of residual bowel.

1.2.3 Necrotising Enterocolitis

Necrotising enterocolitis (NEC) is a serious illness which particularly affects premature infants, characterised by gut necrosis and multisystem organ failure. It can rarely occur in term infants, in whom it may be associated with conditions predisposing to reduced intestinal blood perfusion, such a cardiac anomalies, sepsis, or hypotension. In a UK survey (Rees et al. 2010), infants with NEC accounted for 2% of all neonatal intensive

care admissions, and 14.4% of admissions of infants with birth weight less than 1000 g. Infants are generally initially managed conservatively with bowel rest, intravenous antibiotics and intensive supportive care (often requiring mechanical ventilation, and sometimes inotropic support). Surgery is required if there is intestinal perforation, or if infants fail to respond to medical management. Around one third of infants diagnosed with NEC undergo surgery (Duro et al. 2010; Rees et al. 2010). Around one third of infants who undergo surgery subsequently die (Rees et al. 2010; Thyoka et al. 2012). Overall mortality from NEC was found to be 13%, accounting for 10% of all deaths in neonatal intensive care units (Rees et al. 2010).

A multicentre prospective cohort study in the United States found that 42% of infants undergoing surgery for NEC subsequently developed intestinal failure, which in that study was defined as receiving PN for 90 days or more (Duro et al. 2010). They found that the proportion of the bowel resected or distal to a stoma was a significant predictor of risk of intestinal failure (OR 1.71 per ten percentage point increase in resection, $p = 0.008$). Other significant independent risk factors for development of intestinal failure included birth weight <750 g, and use of ventilator at time of diagnosis.

1.2.4 Miscellaneous Other Conditions

Aside from the three main groups outlined above, any other infants undergoing surgery who are expected to take a significant length of time to tolerate full enteral feeds may benefit from parenteral nutrition. This may include some infants with CDH who experience an unusually prolonged postoperative ileus, infants with intestinal perforation without obstruction from various causes (or occasionally unknown cause), and some

infants with rare conditions such as enteric duplication cysts, pyloric atresia, or even conjoined twins.

1.3 Routes of Infection during Parenteral Nutrition

Perhaps the most obvious route for infective micro-organisms to enter the bloodstream of an infant receiving parenteral nutrition is via the central venous catheter. It is therefore important that appropriate aseptic precautions are strictly observed when such catheters are inserted and accessed. As we shall see in chapter 2, interventions to improve central venous catheter antisepsis have been found to reduce the rate of infections in these patients. However, there is also the possibility that infections can be caused by migration of micro-organisms from the lumen of the intestine into the bloodstream: a phenomenon known as microbial translocation.

1.3.1 Central Venous Catheter-related Infections

A central venous catheter (CVC) refers to an intravascular catheter whose tip lies in the heart or in a 'central vein' – a large vein relatively close to the heart. This is different to a short peripheral cannula in a peripheral vein, which typically cannot be used for more than a few days before becoming occluded, and is generally not suitable for giving parenteral nutrition as the high concentration of glucose in the PN leads to a high risk of thrombophlebitis and extravasation injuries. PN is therefore typically (and more safely) given via a CVC. There are a variety of different types of CVC. In newborns receiving PN, a peripherally inserted central catheter, or PICC line, is often inserted. These are often inserted via basilic, cephalic or brachial veins, aiming to leave the tip in the superior vena cava or brachiocephalic veins, or alternatively via a long saphenous vein aiming to

leave the tip in the inferior vena cava. They can also be inserted via a scalp vein (such as a superficial temporal vein) again aiming to leave the tip in the superior vena cava or brachiocephalic veins. PICC lines can be used for administration of PN in babies for a period of weeks or months.

For longer term access, or when insertion of a PICC line is no longer feasible, a tunnelled CVC can be inserted, usually via the internal jugular vein aiming to leave the tip either in the right atrium or the superior vena cava. Tunnelled CVCs can also be inserted via subclavian or femoral veins. Tunnelled CVCs often have a cuff which becomes integrated into the subcutaneous tissues to prevent migration/dislodgement. These devices were first described in the 1970s (Broviac et al. 1973). Cuffed tunnelled CVCs require a minor surgical procedure for the line to be removed after the cuff has integrated, which is often performed under general anaesthetic in children.

PN can also be given via non-tunnelled central lines which can be inserted into femoral, jugular or subclavian veins, or via umbilical venous catheters in newborns. However, these have a higher risk of infection than PICC lines or tunnelled CVCs and should not be used for more than 7-14 days (Loveday et al. 2014; O'Grady et al. 2011), and should be removed immediately if the patient becomes febrile, whereas with tunnelled CVCs and PICC lines, infections are often treated successfully with intravenous antibiotics without removal of the CVC (Loveday et al. 2014; O'Grady et al. 2011).

All intravascular catheters have risks of thrombosis, blockage, breakage, dislodgement, and insertion carries risks of mechanical complications which may include pneumothorax, haemothorax, air embolism, or cardiac dysrhythmia. There is also a risk

of venous occlusion following insertion of CVCs, which is reduced by use of a percutaneous ultrasound-guided insertion technique for tunnelled CVCs (Wragg et al. 2014); this is an important consideration in children who are likely to require long term venous access.

The Health Protection Agency in England co-ordinates regular national surveys on behalf of the Department of Health to determine the prevalence of healthcare-associated infections in acute hospitals. The most recently published survey (Health Protection Agency 2012) found that CVCs were present in 5.9% of all patients, and 59% of those in Intensive Care Units (ICUs). The overall point prevalence of healthcare associated infections (HCAI) was 6.4% (3360 of 52443 patients surveyed); the prevalence in ICUs was 23%. Bloodstream infections (BSI) accounted for 7.3% of all HCAI (255 patients). 25% of BSI were recorded as attributable to CVCs. In the paediatric population, BSI accounted for 15% of HCAI; 21% of these were CVC-related. Neonates receiving PN are at particularly high risk of infections, due to their special combination of intrinsic vulnerability and exposure to complex intensive care. A study of 1367 infants admitted to a single neonatal unit found that PN significantly and independently increased the risk of bloodstream infection, with an incidence rate ratio of 14.2(Holmes et al. 2008) compared with those not receiving PN. Furthermore, among the population of infants receiving PN, those with a surgical diagnosis have been shown to be at the highest risk, with a reported incidence rate of 12.6 episodes of septicaemia per 1000 PN days in these patients (Donnell et al. 2002). In this study 15% of surgical infants on PN experienced at least one episode of septicaemia; the median duration of PN was 13 days (range 2 to 512 days). In a study of infants with intestinal failure (i.e. those receiving PN for at least 28 days),

those with a surgical diagnosis were found to be at the highest risk of bloodstream infections, with 56% of infants experiencing at least one bloodstream infection; in that study the mean duration of PN treatment was 79 days (range 28–247 days) for all infants (Pichler et al. 2010).

CVC-related infections occur when micro-organisms adhere to the CVC within the bloodstream. Blood vessels are normally free of viable micro-organisms. Micro-organisms may adhere to the intravascular portion of a CVC either during catheter insertion, or they may subsequently migrate down the catheter from the skin (which is known to be colonised with micro-organisms) at the entry point, or they may be introduced by contamination when CVCs are accessed to administer infusate (such as PN) or to take blood samples. Rarely the infusate itself or administration equipment, such as syringes or administration tubing (giving sets) may be contaminated. Aseptic technique is strictly observed during insertion of CVCs, and PN is formulated in aseptic units, stored in refrigerators, and given within a specified time-frame to minimise the risk of contamination. Many interventions to reduce the risk of CVC-related infections have focussed on reducing contamination at the time of accessing CVCs for administration of infusate or blood sampling. Measures which have been shown to be successful in reducing rates of CVC-related infections in both adults and neonates include educating healthcare staff on infection prevention, effective hand hygiene measures, aseptic technique for use of CVCs, and disinfection of catheter hubs when using CVCs (Loveday et al. 2014; O'Grady et al. 2011; Taylor et al. 2015). Some have shown a reduction in rates of BSI by the introduction of dedicated CVC care teams (Taylor et al. 2015; Wilder et al. 2016). There is insufficient evidence to recommend the routine use of systemic

antibiotics before insertion or during use of CVCs (Loveday et al. 2014; O'Grady et al. 2011; Taylor et al. 2015). A recent multicentre randomised trial in paediatric intensive care units (Gilbert et al. 2016) has shown a 75% reduction in catheter-related bloodstream infections with the use of antibiotic-impregnated CVCs compared to standard CVCs (hazard ratio 0.25, 95% CI 0.07 to 0.90). Wide-ranging evidence-based guidelines on the prevention of CVC-related infections have been formulated by working groups both in North America (O'Grady et al. 2011) and in England (Loveday et al. 2014).

1.3.2 Microbial Translocation

The theory that bacteria can cross from the intestine (which is known to be full of micro-organisms) into the bloodstream or peritoneal cavity (which are normally sterile) and cause infection, has been around for over a hundred years (Flexner 1895). Fine and colleagues demonstrated transmural migration of intestinal bacteria in a canine model of haemorrhagic shock shortly after the Second World War (Schweinburg et al. 1950). Schatten et al. subsequently showed in humans that bacteria migrate from the gastrointestinal tract into the portal venous circulation ‘in the absence of infectious processes’ (Schatten et al. 1955). Berg and Garlington coined the phrase ‘bacterial translocation’ in 1979, and defined it as “the passage of viable bacteria from the gastrointestinal tract through the epithelial mucosa into the lamina propria and then to the mesenteric lymph nodes and possibly other organs” (Berg and Garlington 1979). It has been shown that as well as bacteria, other microbes, such as fungi (Krause et al. 1969), and toxic microbial products, such as endotoxin (Alexander et al. 1990; Caridis et al. 1972; Ravin et al. 1960), can also pass through the intestinal barrier. These phenomena can collectively be termed ‘microbial translocation’. A study of 927 adult surgical

patients undergoing laparotomy looked for evidence of microbial translocation by taking samples of mesenteric lymph nodes for culture (Macfie et al. 2006). Evidence of bacterial translocation was found in 14% of patients. Furthermore, there was a correlation with clinical outcome: septic complications were more common in patients with evidence of bacterial translocation (42% vs 20% in those with no organisms cultured; $p < 0.001$). Preoperative total parenteral nutrition was found to be associated with bacterial translocation ($p = 0.015$).

1.4 Micro-organisms Commonly causing Infection in Surgical Infants

The most commonly cultured micro-organisms from blood cultures taken from surgical infants on PN are coagulase-negative staphylococci (CNS) (Donnell et al. 2002), such as *Staphylococcus epidermidis*. These are Gram-positiive cocci which are normally found on the skin and have in the past been dismissed as not pathogenic. However, they have been demonstrated to be an important cause of clinically significant infections (Damjanovic and van Saene 1995; Huebner and Goldmann 1999), particularly in patients who are immunologically less competent, such as neonates, or in the presence of indwelling devices such as central venous catheters (CVCs). There has been some evidence that infants receiving PN show impaired bactericidal activity against CNS (Okada et al. 2000). Furthermore, CNS have also been found to colonise the throat and bowel of neonates, as well as their skin (Damjanovic et al. 1993; Eastick et al. 1996).

Another important Gram-positive coccus is *Staphlococcus aureus*. This is (usually) coagulase positive, and is frequently found in the nose and respiratory tract as well as on the skin. It is considered more potentially pathogenic than CNS: it frequently causes respiratory infections, and skin infections, including abscesses.

Enterococci (especially *Enterococcus faecalis*) are another group of Gram-postive cocci which may cause bacteraemia in this group of infants. They are facultative anaerobes, and are common bowel commensal organisms.

The Enterobacteriaceae are an important family of Gram-negative bacilli (rod-shaped bacteria) which are commonly found in the gastrointestinal tract, and which include a number of important pathogens which can cause septicaemia. These include *Escherichia coli*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Proteus*, and *Serratia*. These Gram-negative organisms contain endotoxins in their cell walls (see section 1.5.2 below) which can trigger a profound inflammatory response.

1.5 Markers of Microbial Translocation

A number of different techniques can be used to detect evidence of microbial translocation. In animal models, direct evidence has been sought using techniques such radio-labelled bacteria (Diniz et al. 1999; Schweinburg et al. 1950), but clinical studies need to rely on less direct evidence.

1.5.1 Blood Cultures

Conventional blood cultures can be used to detect microbes in the bloodstream. Blood samples are taken using aseptic technique in bottles containing special culture medium. The bottles are then placed in a machine which incubates them to 37°C and agitates them (usually for up to five days). The machine automatically detects growth of micro-organisms using colorimetric detection of carbon dioxide (Thorpe et al. 1990). The bottles have a CO_2 sensor bonded to the bottom, which is separated from the culture medium by a membrane which is impermeable to hydrogen ions but permeable to CO_2.

As the bacteria grow, they produce carbon dioxide which diffuses across the membrane and dissolves in water, generating hydrogen ions. As the concentration of hydrogen ions increases, the colour of the sensor changes from blue/dark green to lighter green then yellow. Red light is emitted from a light-emitting diode which is reflected off the sensor onto a red-light absorbing photodiode, which produces a voltage signal proportional to the intensity of the light reflected. As the concentration of CO_2 in the bottle increases, the sensor turns yellow, resulting in increased reflection of red light and hence an increased voltage signal.

However, blood cultures are not 100% sensitive (especially in patients treated with antibiotics), are time-consuming, and do not distinguish for certain between microbes that have translocated from the intestine and those that may have entered the bloodstream via venous catheters or other routes.

1.5.2 Endotoxin Assays

The terms ‘lipopolysaccharide’ (LPS) and ‘endotoxin’ are often used interchangeably to refer to molecules in the cell wall of Gram-negative bacteria which have been found to provoke a marked inflammatory response in many animals. Lipopolysaccharides are large, heat-stable molecules consisting of a polysaccharide antigen (which varies between different bacterial strains), a carbohydrate core, and a lipid A moiety (Hurley 1995). The lipid A moiety seems to be the region that is reactive with most bioassays, and triggers the majority of pathophysiological effects *in vivo*. These effects include fever, leukocytosis, coagulopathy, platelet aggregation and thrombocytopenia. Endotoxin has

been shown to stimulate the complement, coagulation, fibrinolytic, and kinin pathways, and to cause release of cytokines from monocytes and macrophages.

Endotoxin assays can therefore be used to look for evidence of translocation of Gram-negative organisms, or translocation of endotoxin alone. A meta-analysis of clinical studies has found that endotoxaemia is detectable in 53% of cases of Gram-negative bacteraemia, though this proportion was lower when the bacterium was a member of the *Enterobacteriaceae* (Hurley 2009).

There are various assays available for detection of endotoxin, including the rabbit pyrogen test, and immunoassays, but the most widely used by far is the *Limulus* amebocyte lysate (LAL) assay. This has the advantages of being sensitive and quantitative.

Bang observed in 1956 that endotoxin caused fatal intravascular coagulation in the horseshoe crab (*Limulus polyphemus:* Figure 1.9) (Bang 1956). Bang, Levin and co-workers subsequently elucidated the mechanism of this reaction, and recognised its diagnostic potential (Levin and Bang 1964a; Levin and Bang 1964b; Levin and Bang 1968). They found that by osmotic lysis of the crab's blood cells (amebocytes) they could make a reagent (LAL) which could detect the presence of endotoxin (or Gram-negative bacteria) in blood (Levin et al. 1970). By adding a chromogenic substrate (containing a chromophore) to the lysate, the assay is made quantitative and objective, as well as more rapid and sensitive (Nakamura et al. 1977).

However, problems remain with the use of the LAL assay for detection of endotoxin in human blood. Levin noted that there was some interference with the assay due to

Figure 1.9. Horseshoe crabs

Photograph showing two horseshoe crabs (*Limulus polyphemus*), taken by the author.

reversible binding between endotoxin and serum protein(s)' (Levin et al. 1970) – a phenomenon known as inhibition. This can be partly overcome by dilution of plasma and heating (Hurley 1995). Several studies have shown that more endotoxin can be detected in plasma than in serum (Hurley 1995). Collection of plasma in endotoxin-free EDTA-containing Vacutainers has been successfully used by previous investigators (Obayashi et al. 1982).

1.5.3 Lipopolysaccharide Binding Protein

Lipopolysaccharide binding protein (LBP) is synthesised by hepatocytes as part of the acute phase response, and binds endotoxin (lipopolysaccaride), triggering an immune response by presenting the endotoxin to cell of the immune system via membrane-bound receptor molecules including toll-like receptors and CD14. It has been shown to be a sensitive marker of bacterial infection in both neonates (Berner et al. 2002; Orlikowsky et

al. 2006; Pavcnik-Arnol et al. 2004) and older children (Ubenauf et al. 2007), with raised levels detected in infection with both Gram-positive and Gram-negative bacteria (Froon et al. 1995; Ubenauf et al. 2007).

1.5.4 Polymerase Chain Reaction for Bacterial DNA

Polymerase chain reaction (PCR) allows the exponential amplification of selected sections of nucleic acids. Developed by Mullis (Mullis 1990a; Mullis 1990b; Mullis and Faloona 1987), the key components of the reaction include primers (short fragments of DNA complementary to the target DNA), and a heat-stable DNA polymerase – usually Taq polymerase, an enzyme derived from the thermophilic bacterium *Thermus aquaticus* (originally discovered in volcanic geysers at Yellowstone National Park). The reaction relies on thermal cycling i.e. alternate heating and cooling phases: heating to denature the DNA, separating the two complementary strands, followed by cooling to allow annealing (bonding of the primers to the target). The polymerase then binds to the primer-target complex and begins synthesis of a complementary strand. The process is repeated for a number of cycles (e.g. 40). As the amount of product is doubled with every cycle, only a tiny amount (perhaps a single copy) of the target DNA is needed to obtain a positive result.

This gives potential advantages over conventional blood culture in the detection of bacterial translocation: firstly sensitivity may be improved (it may be possible to detect just a small fragment of bacterial DNA); secondly it may be possible to detect non-viable bacteria (e.g. bacteria that have translocated from the bowel and then been killed by antibiotics) or fastidious bacteria, which would not grow in conventional culture media,

or may be obscured by the presence of more readily cultured organisms; thirdly the assay can be quantitative (using real-time PCR, if more of the target DNA is present, the threshold will be reached after fewer cycles); and fourthly, the assay takes less time than conventional blood culture.

However, most bacterial PCR assays are designed specifically to detect a single organism. More recently, broad-range assays have been developed where the targets are nucleotide sequences which are shared by all bacteria, such as ribosomal genes (rDNA), particularly the highly conserved gene coding for the 16S ribosomal subunit (Harris and Hartley 2003). These provide an assay which allows you to search for any bacteria, rather than a particular organism. If the assay is positive, the PCR product can be sequenced, and the organism identified. Clinically this can be useful in detecting unexpected pathogens not grown by culture (Harris et al. 2002), and in a research context it may allow us to look for evidence of bacterial translocation, without looking for a specific organism. Results from 382 clinical samples show that PCR targeting the 16S rDNA gene was able to identify the agent of infection in 71 culture-negative specimens and, equally importantly, did not detect insignificant DNA in other samples (Harris & Hartley 2003). There were 24 false negative PCR results. There has been some evidence in adult surgical patients that PCR (both single-target and broad-range) are more sensitive than conventional blood cultures in detecting evidence of bacteraemia (Kane et al. 1998; Qiao et al. 2009).

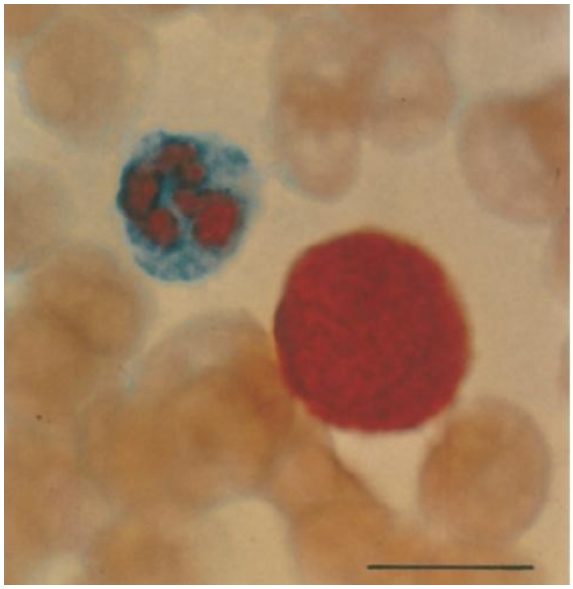
1.5.5 Soluble CD14

CD14 (cluster of differentiation 14) is a protein which plays an important role in immune activation, particularly in response to endotoxin. It exists in two forms. The first is membrane-bound CD14 which is mainly found on monocytes, macrophages and dendritic cells, and binds complexes of endotoxin and LBP, resulting in secretion of inflammatory cytokines (Wright et al. 1990). The second is soluble CD14 (sCD14), which is secreted by monocytes and macrophages in response to endotoxin and binds endotoxin (Kitchens and Thompson 2005). Soluble CD14 is an established marker of monocyte response to endotoxin that has been shown to predict mortality in HIV-infected subjects (Brenchley and Douek 2008; Sandler et al. 2011).

1.6 Marker of Immune Function: Monocyte HLA-DR Expression

Monocytes are white blood cells that play a pivotal role in immune responses to potential pathogens (Figure 1.10). They are derived from precursors in the bone marrow called monoblasts, and can migrate into tissues and differentiate into either macrophages (which can phagocytose foreign material or pathogens, as well as being professional antigen-presenting cells) or dendritic cells (professional antigen-presenting cells). They are important in both innate (non-specific) and adaptive (specific) immunity. The process of antigen presentation is vital in the adaptive immune system, as T cells are not able to recognise most bacterial (non-self) antigens directly, but only when they have been taken up by antigen-presenting cells and are presented on the surface of these cells bound to the antigen-presenting molecules known as the Major Histocompatibility Complex (MHC) proteins. MHC Class I molecules are found on all nucleated cells and present antigens to cytotoxic T lymphocytes, whereas MHC Class II molecules are found only on

Figure 1.10. A monocyte and a neutrophil



A monocyte and a neutrophil stained with combined iron nitrilotriacetate-acid ferrocyanide/α-naphthyl butyrate esterase technique. Scale bar=10 μm. Reproduced with permission from (Barton et al. 1988).

professional antigen-presenting cells and present antigens to helper T lymphocytes.

The level of expression of MHC Class II in the form of Human Leucocyte Antigen – DR isotype (HLA-DR) on circulating monocytes has been found to be variable, and the level of monocyte HLA-DR expression has been shown to be reduced following trauma (Hershman et al. 1990), with the trend in levels of monocyte HLA-DR expression shown to correlate with the risk of sepsis or death following trauma in adult patients (Hershman et al. 1990), and with the risk of infection after surgery in adults (Cheadle et al. 1991). Furthermore, monocyte HLA-DR expression has been shown to be lower in neonates

than in adults, and to be further decreased in neonates with sepsis (Kanakoudi-Tsakalidou et al. 2001).

Low monocyte HLA-DR expression was also shown to be a predictor of increased risk of sepsis/systemic inflammatory response syndrome among infants and children undergoing elective cardiac surgery (Allen et al. 2002). Low monocyte HLA-DR expression has subsequently been shown to be a predictor of increased risk of infection in very low birth weight infants (Palojarvi et al. 2013), and in adult intensive care patients (Lukaszewicz et al. 2009), and a predictor of mortality in neonates with late onset neonatal sepsis (Genel et al. 2010) and in children with severe sepsis in a paediatric intensive care unit (Manzoli et al. 2016). In adult septic shock patients, low monocyte HLA-DR expression was shown to be predictive of both secondary infection and mortality (Manzoli et al. 2016).

1.7 Glutamine, Microbial Translocation and Immune Function

Glutamine is the most abundant free amino acid in the human body (Roth 2008; Vinnars et al. 1975). It is synthesised (mainly in skeletal muscle) by glutamate-ammonia ligase from glutamate and glutamic acid. It is therefore traditionally classified as a nonessential amino acid, and is not included in standard PN formulations. However, there is some evidence that glutamine may be “conditionally essential” i.e. endogenous supply may not be sufficient to meet demand under certain conditions: it has long been established that glutamine levels in skeletal muscle are reduced post-operatively (Vinnars et al. 1975), and that significantly reduced glutamine levels are also found in sepsis, and are a poor prognostic indicator (Roth et al. 1982).

Glutamine has been shown to be the preferred fuel for enterocytes in both rats (Windmueller and Spaeth 1978) and humans (Ashy et al. 1988). Clinical studies have shown that patients receiving standard glutamine-free PN show significantly reduced duodenal villus height and increased intestinal permeability compared to patients receiving glutamine-supplemented PN (van der Hulst et al. 1993).

Glutamine is also a potent stimulator to many components of the immune system, and reduction of glutamine levels *in vitro* has been shown to result in various types of immune dysfunction (Roth 2008), including a significant reduction in monocyte HLA-DR expression (Spittler et al. 1995). Postoperative intravenous glutamine supplementation has been shown to partially prevent the postoperative decrease in monocyte HLA-DR expression (Spittler et al. 2001).

The evidence of glutamine's importance to both the intestine and the immune system supports the hypothesis that glutamine supplementation may prevent bacterial translocation in patients who are glutamine-depleted.

1.7.1 Clinical Trials of Glutamine Supplementation

Review of the literature on randomised clinical trials of therapeutic glutamine reveals conflicting findings. In adults, a number of meta-analyses of randomised controlled trials have reported significant benefits. One such systematic review found that parenteral glutamine supplementation for critically ill patients significantly reduced mortality (risk ratio 0.71 (95% confidence interval 0.55 to 0.92)), infectious complications (risk ratio 0.76 (0.62 to 0.93)), and length of hospital stay (weighted mean difference -3.14 days (-6.03 to -0.24)) (Heyland et al. 2010). However, two subsequent large multicenter

randomised controlled trials in this population showed no benefit (Andrews et al. 2011; Heyland et al. 2013), with one of the studies (which analysed 1216 patients) unexpectedly finding that high dose parenteral and enteral glutamine supplementation in critically ill adults was associated with an increase in mortality – odd ratio 1.28 (95% confidence interval 1.00 to 1.64, p = 0.05) (Heyland et al. 2013). A Cochrane systematic review and meta-analysis has since been performed (Tao et al. 2014). This included 53 studies which enrolled 4671 patients in randomised controlled trials of glutamine supplementation in adults who were critically ill or undergoing major elective surgery. This found that glutamine supplementation had no significant effect on mortality. There was a significant reduction in infectious complications with glutamine supplementation (RR 0.79, 95% CI 0.71 to 0.87, p = 0.00001, I^2 = 8%, moderate quality evidence). Among the subgroup of studies which analysed 456 patients undergoing elective surgery the size of the beneficial effect of glutamine on infectious complications was slightly larger (pooled RR was 0.59 (95% CI 0.41 to 0.86, p = 0.005, I^2 = 0%). In the whole group, glutamine supplementation was associated with a slightly shorter duration of mechanical ventilation (MD - 0.69 days, 95% CI -1.37 to -0.02, p = 0.04, I^2 = 18%, moderate quality evidence) and a shorter length of hospital stay (MD -3.46 days, 95% CI -4.61 to -2.32, p < 0.0001, I^2 = 63%, low quality evidence). The authors caution that some of these results may be influenced by publication bias. The I^2 statistic used above is a measure of the effect of heterogeneity of the various studies (Higgins et al. 2003). The quantity describes the percentage of variation across studies that can be ascribed to heterogeneity rather than chance. A value of 0% indicates no observed heterogeneity, while increasing values

indicate increasing heterogeneity. A value of 50% or more indicates a significant level of inconsistency (Higgins and Green 2011).

Overall, while large studies of unselected adult critical care patients may not show benefit, leading some to question whether there is any role for glutamine supplementation in these patients (Smedberg and Wernerman 2016), it seems there are some subgroups such as elective surgical patients (Tao et al. 2014; Wang et al. 2010) and burns patients (van Zanten et al. 2015) who do benefit from glutamine supplementation.

There is much less evidence available on glutamine supplementation for young infants undergoing gastrointestinal surgery. Glutamine may be expected to be important in young infants as it is found in large quantities in human breast milk (Agostoni et al. 2000; Jochum et al. 2006).

Our unit has reported a prospective double-blind multi-centre randomised controlled trial (Ong et al. 2012) in surgical neonates less than 3 months old who required surgery and PN because of gastro-intestinal dysfunction. Infants received either 0.4 g/kg/day glutamine (treatment group) or isonitrogenous isocaloric PN (placebo group) until full enteral feeding was achieved. 174 patients were randomised of which 164 completed the trial and were analysed (glutamine n=82, placebo n=82). The incidence of clinically evident sepsis (43-51%) and proven septicaemia (22-33%) were both very high in this trial. Glutamine had no effect on overall incidence of clinically evident sepsis (51% glutamine vs. 43% control ($p = 0.27$)) or septicaemia (32% vs. 22% ($p = 0.16$)). In fact, the planned number of patients for this study was 250 patients based on the original power calculation. When the anticipated (and funded) recruitment period had elapsed, the

data monitoring and ethics committee (DMEC) convened to assess whether the study should be continued. They recommended that the trial should halt on the basis that there was less than 5 per cent chance of the specified differences in the primary outcome measure (duration of parenteral nutrition) being observed, even if the trial continued to the target number.

However, *post hoc* analysis showed that during total PN, i.e. before the first enteral feed was introduced, glutamine significantly reduced the risk of developing clinically evident sepsis by 67% (hazard ratio 0.33 [95% confidence interval 0.15-0.72] p = 0.005) but did not appear to have any effect on the risk of having a positive blood culture. In addition, in those patients who did have clinical evidence of sepsis, the time to the first episode of sepsis was significantly longer (12 days) in the glutamine group compared to 4 days in the placebo group (p <0.0001). In this trial glutamine was not supplemented enterally and there was no investigation of whether the glutamine effect on sepsis during total PN was related to increased microbial invasion. There were no adverse effects of glutamine supplementation.

A Cochrane review of glutamine supplementation for young infants with severe gastrointestinal disease has since been published (Brown et al. 2014), which found only three eligible trials (including the study from our unit described above), with a total of 274 infants. There was no significant effect of glutamine demonstrated on mortality, nor on the rate of invasive infection, but the authors note that these studies may be too small to detect some clinically significant effects, and that further studies are required. However, the study by Ong et al. was large enough to show the significant effects described above of glutamine reducing sepsis during the period of total parenteral

nutrition and delaying the first episode of sepsis. Moreover, given that this was a multicentre study conducted over 30 months across 14 UK paediatric surgical centres, it would be challenging to complete a larger scale study than this.

1.8 Aims and Objectives

The aim of this thesis is to investigate the causes of complications in surgical infants, focussing particularly on infections and liver disease in surgical infants receiving parenteral nutrition in the postoperative period, and intraoperative complications of hypercapnia and acidosis in surgery for congenital diaphragmatic hernia and oesophageal atresia with tracheo-oesophageal fistula. I investigated how and why surgical infants on PN get infections, and whether these mechanisms can be modulated by nutritional supplementation with glutamine.

To address these study questions, I have undertaken a series of retrospective clinical studies of surgical infants receiving parenteral nutrition, each of which has formed the basis of a peer-reviewed publication (Bishay et al. 2011b; Bishay et al. 2012a; Bishay et al. 2012b; Bishay et al. 2013b), and performed two prospective randomised controlled trials.

In Chapter 2, I aim to determine whether the introduction of the use of chlorhexidine antisepsis to clean central venous catheter connectors led to a reduction in the rate of septicaemia among infants receiving parenteral nutrition after gastrointestinal surgery. This gives some idea of the rate of CVC-related infections in these infants.

In Chapter 3, I address the question of whether, in surgical infants receiving PN, there is any difference in timing of septicaemia due to gut flora compared to septicaemia due to

coagulase-negative staphylococci. Septicaemia due to gut flora may be more likely to represent bacterial translocation from the gastrointestinal tract.

In Chapter 4, I examine a condition (congenital duodenal atresia/stenosis) which can be treated with or without the use of parenteral nutrition, evaluating the outcomes of the different approaches. I aimed to determine whether growth and infection rate were different in those children who received PN early in the postoperative period, compared to those who did not.

In Chapter 5, I investigate the development of intestinal failure-associated liver disease (IFALD) in surgical infants. My aim was to determine incidence, severity and outcome of IFALD in surgical infants receiving long-term PN. I also aimed to determine whether the development of IFALD was associated with septicaemia, prematurity, or any particular underlying diagnoses or other predisposing factors in this group of infants.

In Chapter 6, I report the prospective randomised controlled trial (the MIGS Trial), which I conducted to investigate whether parenteral and enteral glutamine supplementation affects the incidence of microbial invasion in surgical infants receiving PN. I used a range of techniques to look for evidence of microbial invasion, including conventional blood cultures, endotoxin, LBP and soluble CD14 assays, and broad range and single target PCR. I also investigated the effect of glutamine on immune function as measured by monocyte HLA-DR expression.

In Chapter 7, I report the pilot randomised controlled trial (the CO_2 Trial) I conducted to determine whether thoracoscopic repair of congenital diaphragmatic hernia and oesophageal atresia with tracheo-oesophageal fistula is associated with hypercapnia and

acidosis, compared to open surgery. This has also formed the basis of a publication (Bishay et al. 2013a). Copies of the relevant publications are provided in the appendices.

1.9 Study Selection

The main focus of my research work was the randomised controlled trial to investigate whether parenteral and enteral glutamine supplementation affects the incidence of microbial invasion in surgical infants receiving PN, which is described in Chapter 6. As described in section 1.7.1 and section 6.2, this work followed on from the previous work performed by our unit and others to investigate the role of glutamine supplementation in surgical infants receiving PN. The new trial (the MIGS trial) was set up to investigate whether there was any benefit in enteral as well as parenteral supplementation, and to apply a range of different laboratory techniques to assess whether glutamine supplementation has any effect on microbial invasion and immune function.

Alongside running this trial and performing the laboratory analyses, I undertook a series of retrospective clinical studies looking at surgical infants receiving parenteral nutrition, particularly looking at complications of infections, liver disease and poor growth. These are reported in Chapters 2, 3, 4, and 5. As described in section 2.2, the introduction of chlorhexidine antisepsis for CVC access was associated with a significant decrease in rates of septicaemia among patients on a bone marrow transplant ward. This practice had also been introduced among surgical infants receiving PN, giving me the opportunity to examine whether this led to a similar reduction in the rate of septicaemia in this population, by performing the study described in Chapter 2. As explained in section 3.2, I then examined whether, in surgical infants receiving PN, there is any difference in timing of septicaemia due to gut flora compared to septicaemia due to coagulase-negative

staphylococci – a subject of some controversy in the previous literature. Septicaemia due to gut flora may be more likely to represent bacterial translocation from the gastrointestinal tract. The study in Chapter 4 arose due to a difference in practice among surgeons in our unit as to whether to routinely commence parenteral nutrition following surgery for congenital duodenal obstruction – this afforded an opportunity to assess the outcomes of these different management approaches. I was then interested to investigate the development of intestinal failure-associated liver disease (IFALD) in surgical infants, as surgical infants are noted to have a higher incidence of IFALD than other hospitalised children (Btaiche and Khalidi 2002; Buchman 2002; Koglmeier et al. 2008; Pichler et al. 2012) and sepsis was previously reported to be an important risk factor for the development of IFALD in surgical infants (Beath et al. 1996). I therefore aimed to investigate the incidence, severity and outcome of IFALD in surgical infants receiving long-term PN, as well as investigating whether the development of IFALD was associated with septicaemia or other predisposing factors, as reported in Chapter 5.

Concurrently with all these studies, an opportunity arose for me to take on the running of the pilot randomised controlled trial described in Chapter 7 to investigate whether thoracoscopic repair of congenital diaphragmatic hernia and oesophageal atresia with tracheo-oesophageal fistula is associated with hypercapnia and acidosis, compared to open surgery. This study came about due to concerns raised during the adoption of this recent innovation in surgical technique. While this is not linked to the themes of parenteral nutrition and infection which feature in the other chapters, it is linked to the themes of complications of neonatal surgery (this time focussing on intraoperative rather than postoperative complications) and of improving the evidence base for care of infants

requiring surgery. This is a field in which there is so far a paucity of evidence from randomised trials to inform surgical practice - randomised controlled trials represent less than 0.05% of all publications involving paediatric surgery (Ostlie and St Peter 2010), and the conduct of randomised trials in emergency neonatal surgery poses particular challenges. I felt it was important to embrace these challenges and take this opportunity to perform this study alongside the other strands of investigation.

Chapter 2. Effect of Chlorhexidine Antisepsis on Infection in Surgical Infants Receiving Parenteral Nutrition

2.1 Summary of Chapter 2

2.1.1 Aim

Following a change in national policy, central venous catheter (CVC) antisepsis with chlorhexidine was introduced in our hospital. My aim was to evaluate whether this change was associated with a reduction in the rate of infection seen during parenteral nutrition (PN) in infants requiring gastro-intestinal surgery.

2.1.2 Methods

Two groups of consecutive infants were compared in this single centre retrospective cohort study. *Control*: 98 infants who had CVC antisepsis with 70% isopropanol alone. *Chlorhexidine*: 112 infants who had CVC antisepsis with 2% chlorhexidine in 70% isopropanol. Incidence rates of sepsis (blood cultures taken) and septicaemia (blood cultures positive) were compared by Poisson regression analysis.

2.1.3 Results

71% of infants experienced clinically suspected sepsis; the incidence of septicaemia was 32%. The incidence rate ratio for sepsis was 0.72 (95% CI 0.61 - 0.84) for the chlorhexidine group versus control ($p < 0.0005$). The incidence rate ratio for septicaemia was 0.49 (95% CI 0.36 - 0.67) ($p < 0.0005$), i.e. over a given period of PN, patients had half the rate of positive blood cultures after the introduction of chlorhexidine antisepsis compared to before.

2.1.4 Conclusions

The incidence of sepsis and septicaemia among surgical infants on PN for gastrointestinal anomalies is high.

The introduction of chlorhexidine antisepsis was associated with significantly decreased infection rates in surgical infants, including a halving in the rate of septicaemia, and this effect appears to be largely due to a decrease in coagulase-negative staphylococcal septicaemia.

2.2 Introduction

Infants requiring gastrointestinal surgery and receiving parenteral nutrition (PN) are at high risk of infection: Donnell et al. in a prospective cohort study of such infants found a 15% rate of septicaemia with an incidence rate of 12.6 episodes of septicaemia per 1000 PN days (Donnell et al. 2002). Pichler et al. studied infants with intestinal failure (those receiving PN for at least 28 days) and found that the subgroup at highest risk of bloodstream infections were, by a significant margin, the surgical infants, 56% of whom experienced a bloodstream infection (Pichler et al. 2010). As outlined in Chapter 1, these infants receive PN via a central venous catheter (CVC), which is a potential source of bloodstream infections.

The use of chlorhexidine to clean CVC connectors before and after use is now recommended in guidelines to prevent infection formulated by working groups both in North America (O'Grady et al. 2011) and in England (Loveday et al. 2014). However, these recommendations are based on expert consensus and refer to *in vitro* work on disinfection of catheter hubs (Salzman et al. 1993) and studies of microbial colonisation (Casey et al. 2003) rather than on any clinical evidence of actual reduction in infections associated with the use of chlorhexidine disinfection of CVC connectors.

The use of chlorhexidine wipes to clean CVC connectors was introduced in our hospital in 2007. This was shown to be associated with a dramatic reduction (75%) in the incidence of catheter-related septicaemia in children on the bone marrow transplant ward, in an observational before/after study which used a Poisson model to assess significance (Soothill et al. 2009). The aim of this study was to compare the rates of sepsis and septicaemia in infants receiving PN and requiring gastrointestinal surgery before and after

introduction of chlorhexidine and establish whether the introduction of chlorhexidine was associated with a similar reduction in septicaemia among these surgical infants to that reported among the bone marrow transplant patients.

2.3 Methods

This was a single centre retrospective comparative cohort study. I studied 210 surgical infants (corrected gestational age up to 3 months at start of PN) receiving PN for at least 5 days via CVC for congenital or acquired intestinal anomalies, admitted under the care of general paediatric surgeons. Audit approval for this study was obtained.

Data collected included diagnosis, duration of PN, episodes of clinically suspected sepsis (defined as blood cultures taken), episodes of septicaemia (defined as growth of micro-organisms from blood culture), and organisms cultured. Information on duration of PN was obtained from the pharmacy database, while data on blood cultures taken and results of culture were obtained from the pathology/microbiology database. Blood cultures taken less than 24 hours after starting PN were not counted as episodes of sepsis/septicaemia during PN, and consecutive blood cultures taken within 24 hours were counted as a single episode of sepsis/septicaemia. Blood cultures were read automatically using the BioMérieux BacT/ALERT system (BioMérieux , Marcy L'Étoile, France). Diagnosis and other clinical details were obtained from the hospital administrative database and electronic surgical handover archive.

Two groups were compared: *Control* (Jan 2005 - Dec 2006): 98 consecutive infants who had CVC antisepsis with 70% isopropanol alone (Iso-sachets from Griffiths and Nielsen Ltd, Billingshurst, UK) prior to the introduction of chlorhexidine antisepsis.

Chlorhexidine (Jul 2007 – Jun 2009) 112 consecutive infants treated after CVC antisepsis was changed to 2% chlorhexidine in 70% isopropanol (Clinell wipes from Gama Healthcare, London, UK). The six-month gap between the two periods was to allow full implementation of the policy change across all wards and units in our hospital. In both groups the recommended technique for cleaning connectors before and after use was to swab for 30 seconds then leave to dry completely.

Proportions were compared using Fisher's exact test and continuous variables compared using Mann-Whitney test, as the data were not normally distributed. Kaplan-Meier curves and log-rank test were performed censoring for patients finishing parenteral nutrition, and an event occurring as an episode of septicaemia. Incidence rates were compared by Poisson regression analysis (Stata Intercooled version 10.0, College Station, TX, USA) adjusting for diagnosis, with length of time on PN as the exposure variable. Data are presented as median (range).

2.4 Results

The underlying diagnoses of patients in both groups are given in Table 2.1; the distribution of diagnoses was similar between the two time periods. Overall 45% of patients had necrotising enterocolitis; 30% had intestinal obstruction (including intestinal atresias, meconium ileus, malrotation and volvulus, anorectal malformations, Hirschsprung's disease, and milk curd obstruction); 17% had abdominal wall defects (gastroschisis and exomphalos), and 7% had miscellaneous other causes of intestinal dysfunction requiring surgery (including congenital diaphragmatic hernia, conjoined twins, and oesophageal perforation). There was no difference in pre-operative requirement for intensive care, and no difference in outcomes (achieving full enteral feeds and death) between the two groups. The duration of PN given in the two groups was not significantly different (13 [5-260] days in the control group vs. 13 [5-200] days in the chlorhexidine group; $p = 0.92$).

Overall, 71% of infants experienced at least one episode of clinically suspected sepsis, and the incidence of septicaemia was 32%. In the control group, 70 infants (71%) experienced a total of 300 episodes of sepsis and 36 infants (37%) experienced 104 episodes of septicaemia. In the chlorhexidine group, 80 infants (72%) experienced 297 episodes of sepsis and 31 infants (28%) experienced 64 episodes of septicaemia. The distribution of number of episodes of sepsis is shown in Figure 2.1.

Table 2.1 Patient diagnosis, level of care and outcomes

	Control (n = 98)	Chlorhexidine (n = 112)	P-value
Necrotising enterocolitis	49 (50)	46 (41)	0.22
Intestinal obstruction	29 (30)	35 (31)	0.88
Abdominal wall defect	15 (15)	21 (19)	0.58
Miscellaneous intestinal dysfunction requiring surgery	5 (5)	10 (9)	0.42
Pre-operative intensive care	73 (74)	74 (66)	0.23
Achieved full enteral feeds	49 (50)	50 (45)	0.49
Died	9 (9)	8 (7)	0.62

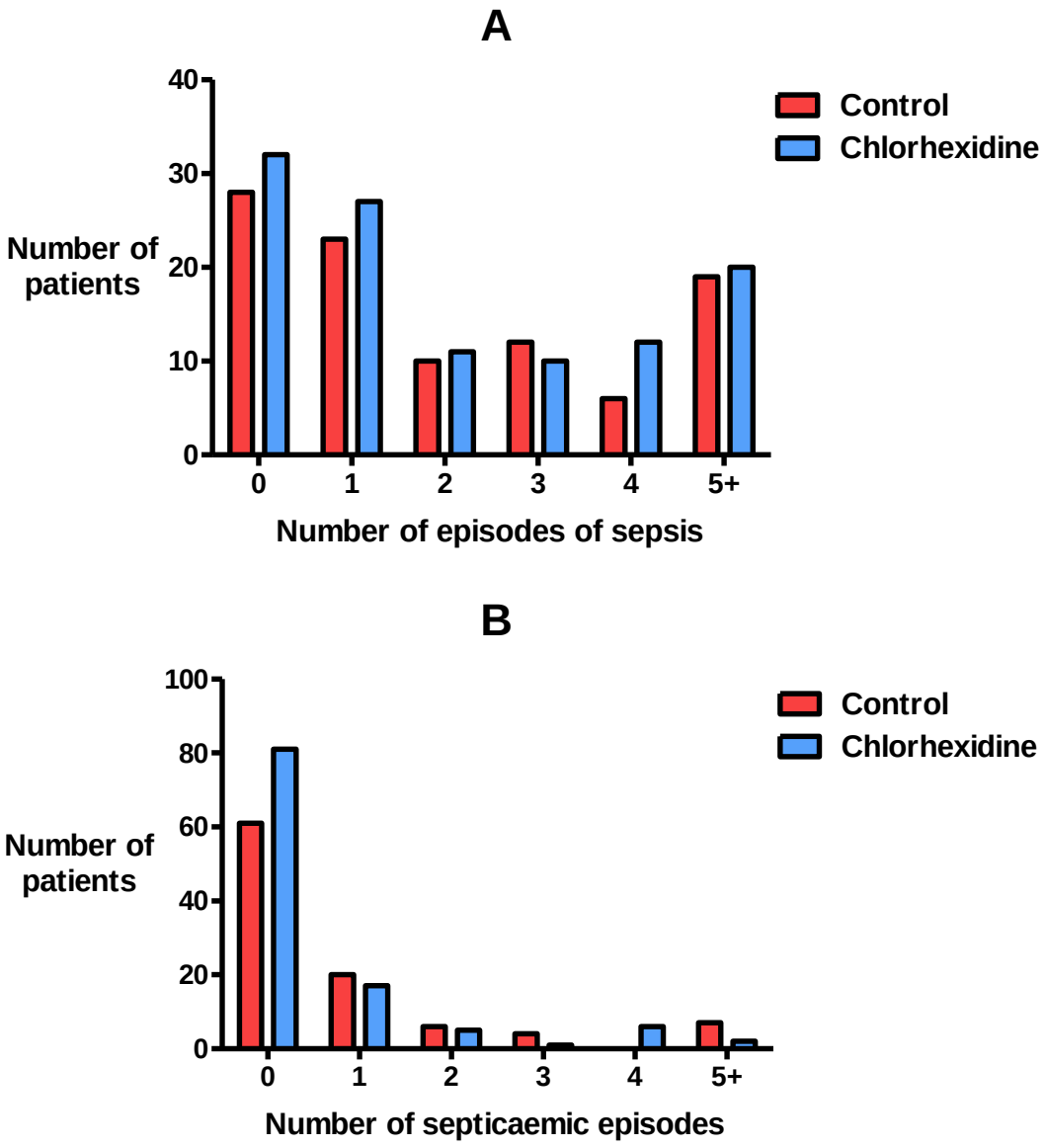
Data presented as number (percentage) and compared using Fisher's exact test.

Table 2.2 Incidence of sepsis and septicaemia

	Control (n = 98)	Chlorhexidine (n = 112)	Incidence rate ratio (95% CI)	P-value
Sepsis per 100 days of PN	10 (0,42)	10 (0,44)	0.72 (0.61, 0.84)	<0.0005
Septicaemia per 100 days of PN	0 (0,17)	0 (0,31)	0.49 (0.36, 0.67)	<0.0005

Rates of sepsis and septicaemia in Control and Chlorhexidine groups presented as median (range) and compared using Poisson regression analysis. CI = confidence interval

Figure 2.1 Frequency of sepsis and septicaemia

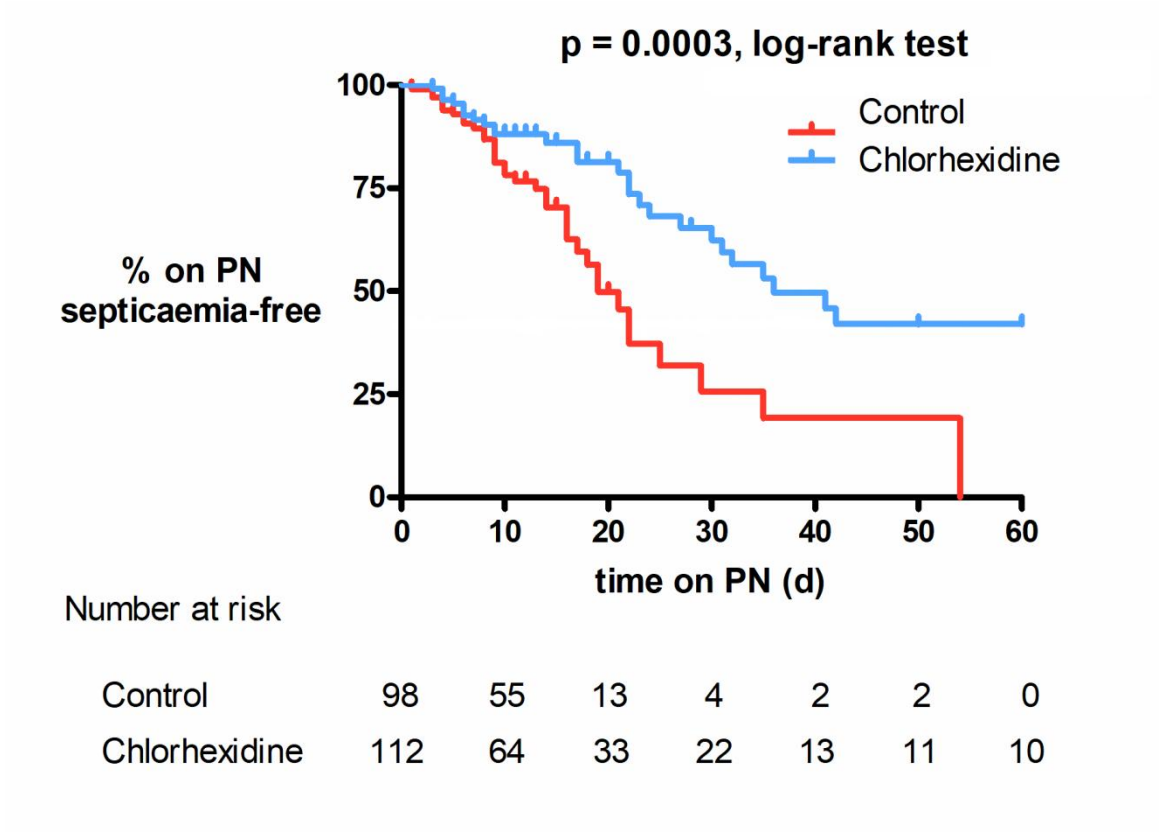


Frequency of [A] clinical suspicion of sepsis and [B] septicaemia (sepsis with positive blood culture) before (control) and after introduction of chlorhexidine antisepsis of central venous catheter connectors.

To account for differing durations of parenteral nutrition, the number of episodes of sepsis/septicaemia per 100 days of PN were calculated (Table 2.2 above). In order to adjust for length of time on PN, diagnosis and number of patients, the rates of sepsis and septicaemia were compared by Poisson regression analysis. The incidence rate ratio for sepsis was 0.72 (95% CI 0.61 - 0.84) for the chlorhexidine group compared with controls ($p < 0.0005$), i.e. there was a 28% decrease in the rate of sepsis after introduction of chlorhexidine. The incidence rate ratio for septicaemia was 0.49 (95% CI 0.36 - 0.67) for the chlorhexidine group compared with controls ($p < 0.0005$), i.e. over a given period of PN, patients had less than half the rate of positive blood cultures after the introduction of chlorhexidine antisepsis compared to before.

There was no significant effect of chlorhexidine on timing of the first sample sent for blood culture (log-rank test $p = 0.131$). However, chlorhexidine significantly delayed the occurrence of the first positive blood culture, as shown in Figure 2.2 ($p = 0.0003$), and this effect appeared to be present from relatively early during the course of PN. The median septicaemia-free survival time was 19 days in the control group, compared to 36 days in the chlorhexidine group.

Figure 2.2 Kaplan-Meier curves showing septicaemia-free time on parenteral nutrition for the Control and Chlorhexidine groups



Chlorhexidine was associated with significant delay in the onset of septicaemia compared to Control group (p = 0.0003, log-rank test).

The most commonly cultured organisms were coagulase-negative staphylococci, which were grown from 70% of positive cultures overall (Table 2.3). The distribution of pathogens cultured was broadly similar in both groups (Table 2.4). Positive blood cultures of coagulase-negative staphylococci and Gram-negative bacilli were significantly reduced in the chlorhexidine group (in keeping with the overall reduction in septicaemia). However, I did not find a reduction in cultures of enterococci, which therefore accounted for a larger proportion of positive cultures in the chlorhexidine group (25%, compared to 12% in the control group).

Table 2.3 Pathogens cultured

Micro-organisms	No of episodes	%*
Coagulase-negative staphylococci	119	70
Enterococci	29	17
Gram-negative bacilli	25	15
Enterobacteriaceae (including Enterobacter, Citrobacter & Proteus spp)	10	6
Escherichia coli	8	5
Klebsiella spp	6	4
Pseudomonas aeruginosa	1	1
Staphylococcus aureus	6	4
Candida spp	2	1
Group B beta-hemolytic streptococci	1	1

*numbers add up to more than 100% as some samples grew more than one organism.

Table 2.4 Distribution of pathogens

Micro-organisms	Control (n)	% of positive cultures*	Chlorhexidine (n)	% of positive cultures*	Incidence rate ratio (95% CI)	P-value
CNS	74	72	45	69	0.48 (0.34, 0.71)	<0.0005
GNB	19	18	7	11	0.28 (0.11, 0.67)	0.004
Enterococci	12	12	16	25	0.87 (0.42, 1.81)	0.711
Others	5	5	5	8	0.74 (0.21, 2.55)	0.631

*numbers add up to more than 100% as some samples grew more than one pathogen.
CNS = Coagulase-negative staphylococci. GNB = Gram-negative bacilli

2.5 Discussion

In this large retrospective study, I showed that the incidence of sepsis and septicaemia among surgical infants on PN for gastrointestinal anomalies is high, and that chlorhexidine CVC antisepsis was associated with a significant reduction in this incidence.

The main limitation of this study is that it is retrospective and therefore demonstrates an association rather than causation. As historical controls are used it is possible that other changes in infection control over the time period, such as audits of handwashing and reinforcement of staff training in aseptic non-touch technique when accessing CVCs, may have been confounding factors which also influenced infection rates. Like the use of chlorhexidine, I would expect such measures to primarily impact on CVC-related infections rather than endogenous routes of infection such as translocation from the gut. Another important limitation related to the retrospective nature of the study is the use of non-standard definitions of sepsis and septicaemia: it was not possible to reliably include clinical criteria in the definition of sepsis. Clearly different clinicians may choose to take blood cultures (or not) in various different situations. However, I can see no reason to think there would be any systematic bias which would undermine the comparison of the two groups within this study. In children, an international panel of experts have published definitions of systemic inflammatory response syndrome (SIRS), infection, sepsis, severe sepsis, and septic shock based on physiological and laboratory variables – see Figure 2.3 for full definitions (Goldstein et al. 2005). These were modified from previous published definitions for adults (Bone et al. 1992; Levy et al. 2003).

Figure 2.3 International consensus definitions for paediatric sepsis and organ dysfunction

SIRS[a]

The presence of at least two of the following four criteria, **one of which must be abnormal temperature or leukocyte count:**

- Core[b] temperature of >38.5°C or <36°C.
- Tachycardia, defined as a mean heart rate >2 SD above normal for age in the absence of external stimulus, chronic drugs, or painful stimuli; or otherwise unexplained persistent elevation over a 0.5- to 4-hr time period **OR for children <1 yr old: bradycardia, defined as a mean heart rate <10th percentile for age in the absence of external vagal stimulus, β-blocker drugs, or congenital heart disease; or otherwise unexplained persistent depression over a 0.5-hr time period.**
- Mean respiratory rate >2 SD above normal for age or mechanical ventilation for an acute process not related to underlying neuromuscular disease or the receipt of general anesthesia.
- Leukocyte count elevated or depressed for age (not secondary to chemotherapy-induced leukopenia) or >10% immature neutrophils.

Infection

A suspected or proven (by positive culture, tissue stain, or polymerase chain reaction test) infection caused by any pathogen OR a clinical syndrome associated with a high probability of infection. Evidence of infection includes positive findings on clinical exam, imaging, or laboratory tests (e.g., white blood cells in a normally sterile body fluid, perforated viscus, chest radiograph consistent with pneumonia, petechial or purpuric rash, or purpura fulminans)

Sepsis

SIRS in the presence of or as a result of suspected or proven infection.

Severe sepsis

Sepsis plus one of the following: cardiovascular organ dysfunction OR acute respiratory distress syndrome OR two or more other organ dysfunctions. Organ dysfunctions are defined in Table 4.

Septic shock

Sepsis and cardiovascular organ dysfunction as defined in Table 4.

Modifications from the adult definitions are highlighted in boldface.

[a]See Table 3 for age-specific ranges for physiologic and laboratory variables; [b]core temperature must be measured by rectal, bladder, oral, or central catheter probe.

International consensus definitions of systemic inflammatory response syndrome (SIRS), infection, sepsis, severe sepsis, and septic shock in paediatrics. Reproduced with permission from (Goldstein et al. 2005).

Figure 2.4 The SOFA (Sepsis-related Organ Failure Assessment) score

SOFA score	1	2	3	4
Respiration PaO_2/FiO_2, mmHg	<400	<300	<200	<100
			—— with respiratory support ——	
Coagulation Platelets $\times 10^3/mm^3$	<150	<100	<50	<20
Liver Bilirubin, mg/dl (μmol/l)	1.2 – 1.9 (20 – 32)	2.0 – 5.9 (33 – 101)	6.0 – 11.9 (102 – 204)	>12.0 (<204)
Cardiovascular Hypotension	MAP < 70 mmHg	Dopamine ≤5 or dobutamine (any dose)[a]	Dopamine >5 or epinephrine ≤0.1 or norepinephrine ≤0.1	Dopamine >15 or epinephrine >0.1 or norepinephrine >0.1
Central nervous system Glasgow Coma Score	13 – 14	10 – 12	6 – 9	<6
Renal Creatinine, mg/dl (μmol/l) or urine output	1.2 – 1.9 (110 – 170)	2.0 – 3.4 (171 – 299)	3.5 – 4.9 (300 – 440) or <500 ml/day	>5.0 (>440) or <200 ml/day

[a] Adrenergic agents administered for at least 1 h (doses given are in μg/kg·min)

The SOFA (Sepsis-related Organ Failure Assessment) score. Reproduced with permission from (Vincent et al. 1996).

These adult international consensus definitions for sepsis and septic shock have recently been updated (Singer et al. 2016). The task force concluded that the term severe sepsis was redundant and that sepsis should be defined as life-threatening organ dysfunction caused by dysregulated host response to infection. For clinical practice, organ dysfunction is represented by an increase in the Sequential [Sepsis-related] Organ Failure Assessment (SOFA) score (see Figure 2.4 above) of 2 points or more, which in adults is associated with greater than 10% in-hospital mortality. Septic shock is defined as a subset of sepsis in which underlying circulatory and cellular/metabolic abnormalities are profound enough to substantially increase mortality. Clinically these patients are identified as those with sepsis and persisting hypotension requiring vasopressors to maintain mean arterial pressure of 65 mmHg or greater and serum lactate level greater than 2 mmol/L despite adequate volume resuscitation. With these criteria, hospital mortality is in excess of 40%. Outside of the critical care setting, adult patients with suspected infection can be rapidly identified as being more likely to have poor outcomes typical of sepsis if they have at least 2 of the following clinical criteria: respiratory rate of 22/min or greater, altered mentation, or systolic blood pressure of 100 mmHg or less. The non-standard definitions used in this retrospective study make it impossible to directly compare the reported rates with those from other studies. The internal comparison between the two groups within the study (*Control* and *Chlorhexidine*) remains valid as the definitions are applied consistently.

The retrospective nature of the study meant it was not possible to accurately document the influence of other possible confounding factors including bowel length, proportion of

enteral feeds, and timing of line insertion in relation to surgery, which could potentially influence the incidence of infections. Nonetheless, it is reassuring that the groups were similar in terms of the baseline characteristics available, including diagnostic groups and also in the duration of PN overall. It is remarkable that the Poisson regression analysis adjusting for diagnosis and length of time on PN was able to reveal important clinically and statistically significant associations which were not evident from simple summary measures due to the skewed non parametric distribution of the data. A further limitation of this group is the heterogeneity in terms of underlying diagnoses. These are uncommon conditions and it would be difficult to draw any conclusions from examining diagnostic subgroups individually. The statistical methods used here have allowed me to adjust for diagnostic group and demonstrate highly significant associations.

Most information on the incidence of blood stream infections (BSIs) in children comes from data from intensive care units (ICUs). In the US, the latest data from the National Healthcare Safety Network (NHSN) show a pooled mean of 3.0 BSIs per 1000 catheter-days across 129 paediatric medical/surgical centres (Edwards et al. 2009). Estimates of the costs of BSIs in children in US ICUs range from US$36 000 to US$50 000 (Dominguez et al. 2001; Elward et al. 2005; Slonim and Singh 2001). Neonates are at particularly high risk, due to their special combination of intrinsic vulnerability and exposure to complex intensive care. A study of 1367 infants admitted to a neonatal unit found that PN significantly increased the risk of bloodstream infection, with an incidence rate ratio of 14.2 (Holmes et al. 2008) compared with those not receiving PN. Any intervention shown to significantly reduce this high rate of infection among these susceptible patients is therefore worthwhile.

Chlorhexidine has a broad spectrum microbicidal activity which is thought to be mediated by disruption of microbial cell membranes (Kuyyakanond and Quesnel 1992). It is widely used in topical skin cleansing preparations (including for pre-operative skin preparation), and in oral rinses and other products on sale to the public. The use of alcoholic chlorhexidine for cleaning CVC catheters before and after access is now recommended by the Department of Health in England as part of national guidelines for preventing healthcare-associated infections (Loveday et al. 2014; Pratt et al. 2007), as well as in similar North American guidelines (O'Grady et al. 2011). Use of chlorhexidine skin disinfection is recommended by the US 2002 Centers for Disease Control and Prevention guidelines for the prevention of intravascular catheter-related infections, but not for infants under 2 years of age (Garland et al. 2002). One US children's hospital reported a 75% reduction in the relative risk of infection with CVC over an 8 year period, following the stepwise introduction of a series of infection control interventions including the use of 2% chlorhexidine for skin disinfection in all age groups (Bhutta et al. 2007). Chlorhexidine solutions leave a residue that may provide a longer lasting microbicidal activity than isopropanol alone. Chlorhexidine patch dressings have been shown to decrease CVC colonisation in a randomised controlled trial in neonates, but there was no decrease in the incidence of infection (Garland et al. 2001).

In view of these previous studies, and the application of the chlorhexidine wipes to catheter hubs, it seems reasonable to ascribe the observed effects of chlorhexidine to prevention of infection via the CVC route, improving sterility when the line is accessed to draw blood or administer PN. These findings underline the importance of strict hygiene when accessing CVC connectors. However it is noticeable that despite this reduction, the

rates of sepsis and septicaemia among these patients remained remarkably high. Moreover the effect seen among these patients was not as dramatic as that reported on the haemopoietic stem cell transplant ward in the same hospital (where the incidence rate fell by 75%; (Soothill et al. 2009). Thus, while I have observed a significantly reduced rate of infection in surgical infants on PN following the introduction of chlorhexidine wipes for CVC antisepsis, we are far from eradicating infections in this patient group.

It is interesting to note that chlorhexidine was associated with delay in the onset of septicaemia. A previous prospective study of infection rates among surgical infants receiving PN (Donnell et al. 2002) found evidence that infections with coagulase-negative staphylococci (which may be more likely to be involved in CVC-related infections) occurred significantly earlier in the course of PN than infections with enterococci and potentially pathogenic micro-organisms (which are more likely to originate from the gastrointestinal tract). The authors hypothesised that infants might be more vulnerable to translocation from the gut later in the course of PN due to immunosuppression and liver dysfunction. In support of this, they found that serum bilirubin was significantly higher at the time of infection with enterococci and potentially pathogenic micro-organisms, compared to infection with coagulase-negative staphylococci. If chlorhexidine does reduce CVC-related infections, and staphylococcal infections, we may therefore expect its effects to become evident early in the course of PN. This hypothesis appears to be true, as judged from the Kaplan-Meier curves (Figure 2.2), which can be seen to diverge relatively early.

The persistently high rate of infections and the failure to match the rate of infection reduction seen in other patients with CVCs suggest that routes of infection other than

CVC-related are important in this group of patients. This is borne out to some extent by the persistent level of enteroccocal infections after the introduction of chlorhexidine; while there was a significant reduction in overall septicaemia, and in coagulase-negative staphylococcal septicaemia, I found there was no corresponding decrease in the rate of enterococcal infection, which may be more likely to originate from the gastrointestinal tract. It therefore seems plausible that endogenous routes, such as translocation from the gastro-intestinal tract, play a significant role in causing infection in these infants with gastrointestinal anomalies, as well as the exogenous route of infection via CVCs.

2.6 Conclusions

In conclusion, in this study I have shown that the incidence of sepsis and septicaemia among surgical infants on PN for gastrointestinal anomalies is high. I have also shown that the introduction of chlorhexidine antisepsis was associated with significantly decreased infection rates in surgical infants, including a halving in the rate of septicaemia, and this effect appears to be largely due to a decrease in coagulase-negative staphylococcal septicaemia.

Chapter 3. Timing of Septicaemia with Different Organisms in Surgical Infants receiving Parenteral Nutrition

3.1 Summary of Chapter 3

3.1.1 Aim

The purpose of this study was to determine whether, in surgical infants requiring parenteral nutrition (PN), septicaemia due to enterococci or Gram-negative bacilli occurs later than septicaemia due to coagulase-negative staphylococci (CNS).

3.1.2 Methods

I retrospectively studied 112 consecutive surgical infants (corrected gestational age up to 3 months) receiving PN for at least five days for congenital or acquired intestinal anomalies over a two-year period (July 2007 – June 2009). Data collected included diagnosis, duration of PN, episodes of septicaemia (defined as growth of bacteria from blood culture), and organisms cultured. I compared the time to first occurrence of septicaemia due to CNS, with the times to first occurrence of septicaemia due to enterococci, Gram-negative bacilli, or other micro-organisms, using Kruskal-Wallis nonparametric ANOVA test and Dunn's Multiple Comparisons test. Data are given as median (range).

3.1.3 Results

31 patients (28%) had a total of 65 episodes of septicaemia. Septicaemia due to CNS was most common, occurring in 22% of patients, after 17 days (1-239) of PN. Septicaemia due to enteric organisms was less common and occurred significantly later: at 59 (24-103) days for enterococci ($p<0.01$), and 55 (30-106) days for Gram-negative bacilli ($p<0.05$).

3.1.4 Conclusions

Septicaemia due to enterococci or Gram-negative bacilli occurs later in the course of PN than septicaemia due to CNS, in surgical infants. This may suggest that these infants become more vulnerable to translocation of enteric micro-organisms after a longer period of parenteral nutrition.

3.2 Introduction

As I and others have shown, infants requiring gastrointestinal surgery and receiving PN are at high risk of infection (Donnell et al. 2002; Pierro et al. 1996; Pierro et al. 1998). Pierro et al. have shown that in surgical neonates and infants who are receiving PN, septicaemia may be a gut-related phenomenon and this may be due to microbial translocation (Pierro et al. 1996). In addition these authors (Pierro et al. 1998) have shown that approximately half of this patient population develop abnormal gut flora which predisposes to septicaemia. There has previously been some controversy in the literature as to whether infections in these infants are due to different causative organisms after different durations of parenteral nutrition (Donnell et al. 2002; Donnell et al. 2004; Holden et al. 2003; Van Camp et al. 1994). Donnell et al. prospectively studied a cohort of 208 surgical infants receiving PN at a UK centre (Donnell et al. 2002). They found that septicaemia due to enterococci and 'potentially pathogenic micro-organisms' occurred significantly later than septicaemia due to coagulase-negative staphylococci (CNS). Holden et al. responded to this study providing data regarding septicaemia in infants receiving PN in a different UK centre (Holden et al. 2003). These data apparently relate to all infants receiving PN (not only those with surgical pathology) and showed no clear temporal association with different micro-organisms.

The purpose of this study was to determine whether, in surgical infants receiving PN, septicaemia due to enterococci or Gram-negative bacilli occurs later than septicaemia due to CNS.

3.3 Patients and Methods

This was a single centre retrospective comparative cohort study. I studied 112 consecutive surgical infants (corrected gestational age up to 3 months at start of PN) receiving PN for at least 5 days via a CVC for congenital or acquired intestinal anomalies, admitted under the care of general paediatric surgeons over a two-year period (July 2007 – June 2009). Audit approval for this study was obtained.

Data collected included diagnosis, duration of PN, episodes of clinically suspected sepsis (defined as blood cultures taken), episodes of septicaemia (defined as growth of micro-organisms from blood culture), and organisms cultured. Information on duration of PN was obtained from the pharmacy database, while data on blood cultures taken and results of culture were obtained from the pathology/microbiology database. Blood cultures taken less than 24 hours after starting PN were not counted as episodes of sepsis/septicaemia during PN, and consecutive blood cultures taken within 24 hours were counted as a single episode of sepsis/septicaemia. Blood cultures were read automatically using the BioMérieux BactAlert system (BioMérieux, Marcy L'Étoile, France). Diagnosis and other clinical details were obtained from the hospital administrative database and electronic surgical handover archive.

I compared the time to first occurrence of septicaemia due to CNS, with the times to first occurrence of septicaemia due to enterococci, Gram-negative bacilli, or other micro-organisms, using Kruskal-Wallis nonparametric ANOVA test and Dunn's Multiple Comparisons test. Data are given as median (range), or mean ± standard deviation.

3.4 Results

The underlying diagnoses are summarised in Table 3.1. The diagnosis was necrotising enterocolitis in 41% of infants; 31% had intestinal obstruction (including intestinal atresias, meconium ileus, malrotation and volvulus, anorectal malformations, Hirschsprung's disease, and milk curd obstruction); 19% had abdominal wall defects (gastroschisis and exomphalos), and 9% had miscellaneous other causes of intestinal dysfunction requiring surgery (including congenital diaphragmatic hernia, conjoined twins, and oesophageal perforation). Data on clinical progress are given in Table 3.2.

The rates of sepsis and septicaemia are illustrated in Figure 3.1: 80 infants (72%) experienced 297 episodes of clinically suspected sepsis and 31 infants (28%) experienced 65 episodes of septicaemia. The rate of episodes of sepsis was 11.0 ± 10.3 per 100 days of PN, with 2.1 ± 4.8 episodes of septicaemia per 100 days of PN. The most commonly cultured organisms were coagulase-negative staphylococci (Table 3.3), which were grown from 69% of positive cultures. Enterococci were grown from 25% of positive cultures, and Gram-negative bacilli (*Enterobacter cloacae*, *Proteus mirabilis*, *Escherichia coli* and *Citrobacter freundii)* from 11%. 9 samples grew more than one organism.

Table 3.1 Patient diagnoses

Necrotising enterocolitis	46 (41)
Intestinal obstruction	35 (31)
Abdominal wall defect	21 (19)
Miscellaneous intestinal dysfunction requiring surgery	10 (9)

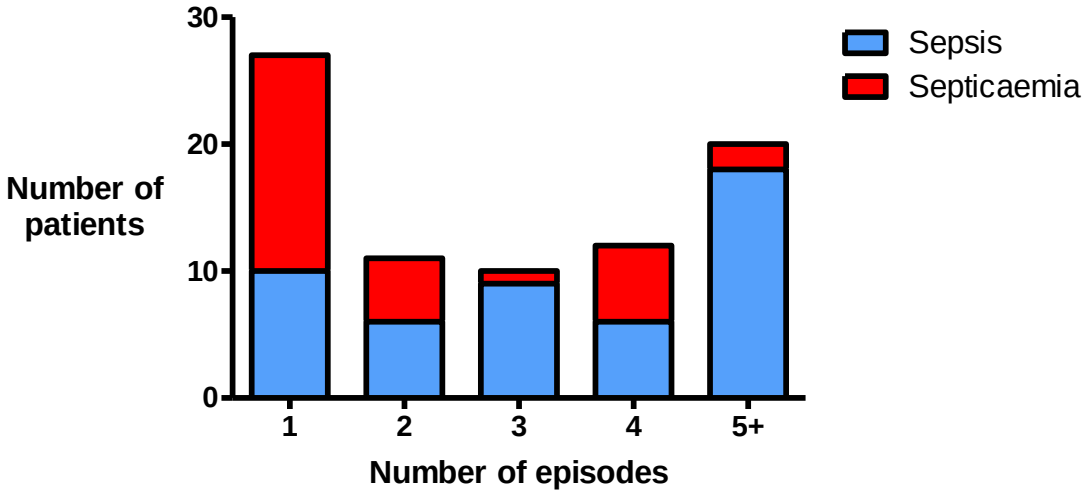
Data presented as number (percentage)

Table 3.2 Clinical Progress

Duration of parenteral nutrition	13 (5-200)
Pre-operative intensive care	74 (66)
Achieved full enteral feeds	50 (45)
Died	8 (7)

Data presented as median (range) or number (percentage)

Figure 3.1 Frequency of sepsis and septicaemia



Frequency of clinical suspicion of sepsis and septicaemia among surgical infants receiving parenteral nutrition. The blue bars represent episodes with negative blood culture, while the red bars represent episodes with positive blood culture.

Table 3.3 Pathogens cultured

Micro-organisms	No of episodes	%*
Coagulase-negative staphylococci	45	69
Enterococci	16	25
Gram-negative bacilli	7	11
Enterobacter cloacae	3	5
Citrobacter freundii	1	2
Proteus mirabilis	2	3
Escherichia coli	1	2
Others	6	9
Staphylococcus aureus	3	5
Candida parapsilosis	1	2
Group B beta-hemolytic streptococci	1	2
Gram-positive bacilli	1	2

*numbers add up to more than 100% as some samples grew more than one pathogen.

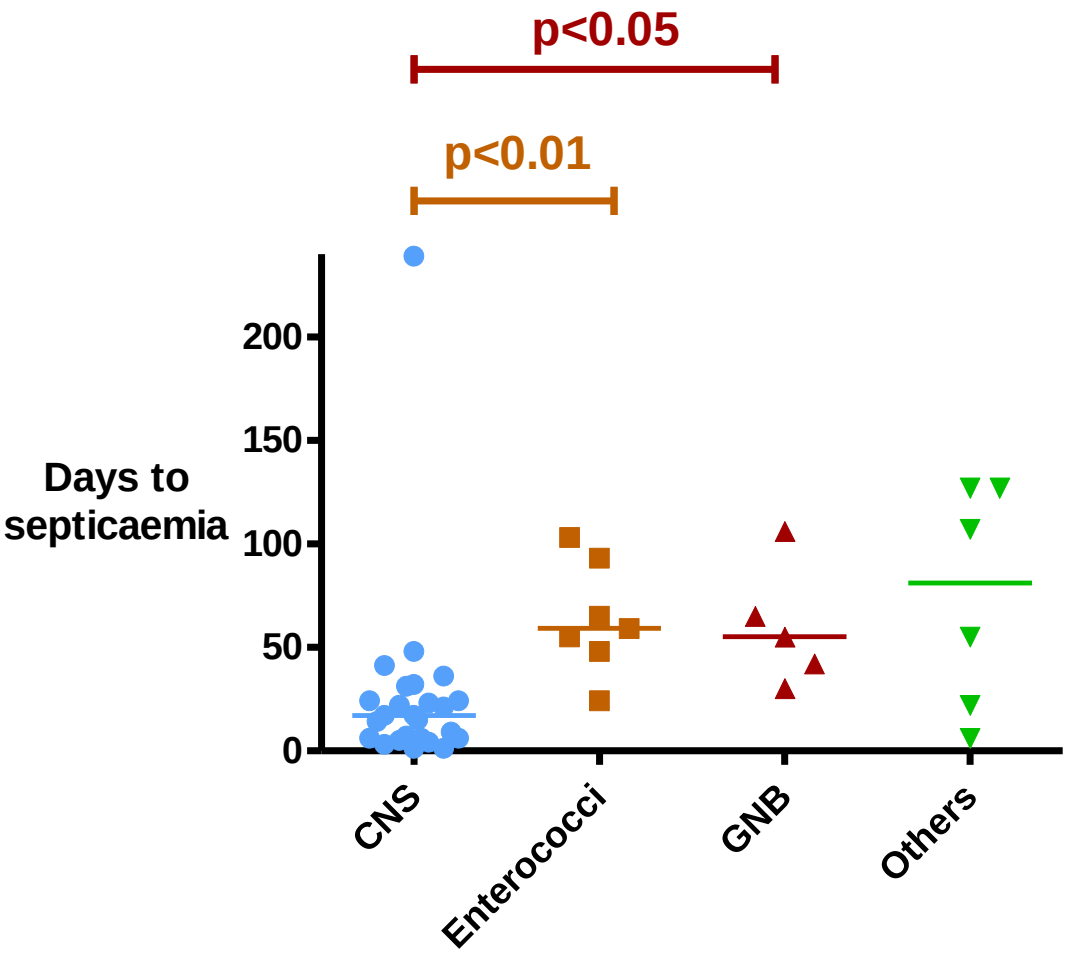
The data on timing of septicaemia due to different micro-organisms are shown in Table 3.4 and Figures 3.2 and 3.3. Septicaemia due to enteric organisms occurred significantly later than septicaemia due to CNS. No infections due to enteric organisms occurred within the first three weeks of PN. Figure 3.4 shows the organisms causing a first episode of septicaemia, compared to organisms causing subsequent episodes of septicaemia. Enterococci accounted for 14% of primary episodes, and 29% of recurrent episodes.

Table 3.4 Timing of septicaemia due to different pathogens

Micro-organism	Number of patients (%)	Days of PN to first septicaemia with relevant micro-organism	P-value (Compared to CNS)
CNS	25 (22)	17 (1-239)	N/A
Enterococci	7 (6)	59 (24-103)	<0.01
GNB	5 (4)	55 (30-106)	<0.05
Others	6 (5)	81 (6-127)	NS

CNS = Coagulase-negative staphylococci. GNB = Gram-negative bacilli
Data given as number (percent) or median (range) and compared using Kruskal-Wallis nonparametric ANOVA test and Dunn's multiple comparisons test.

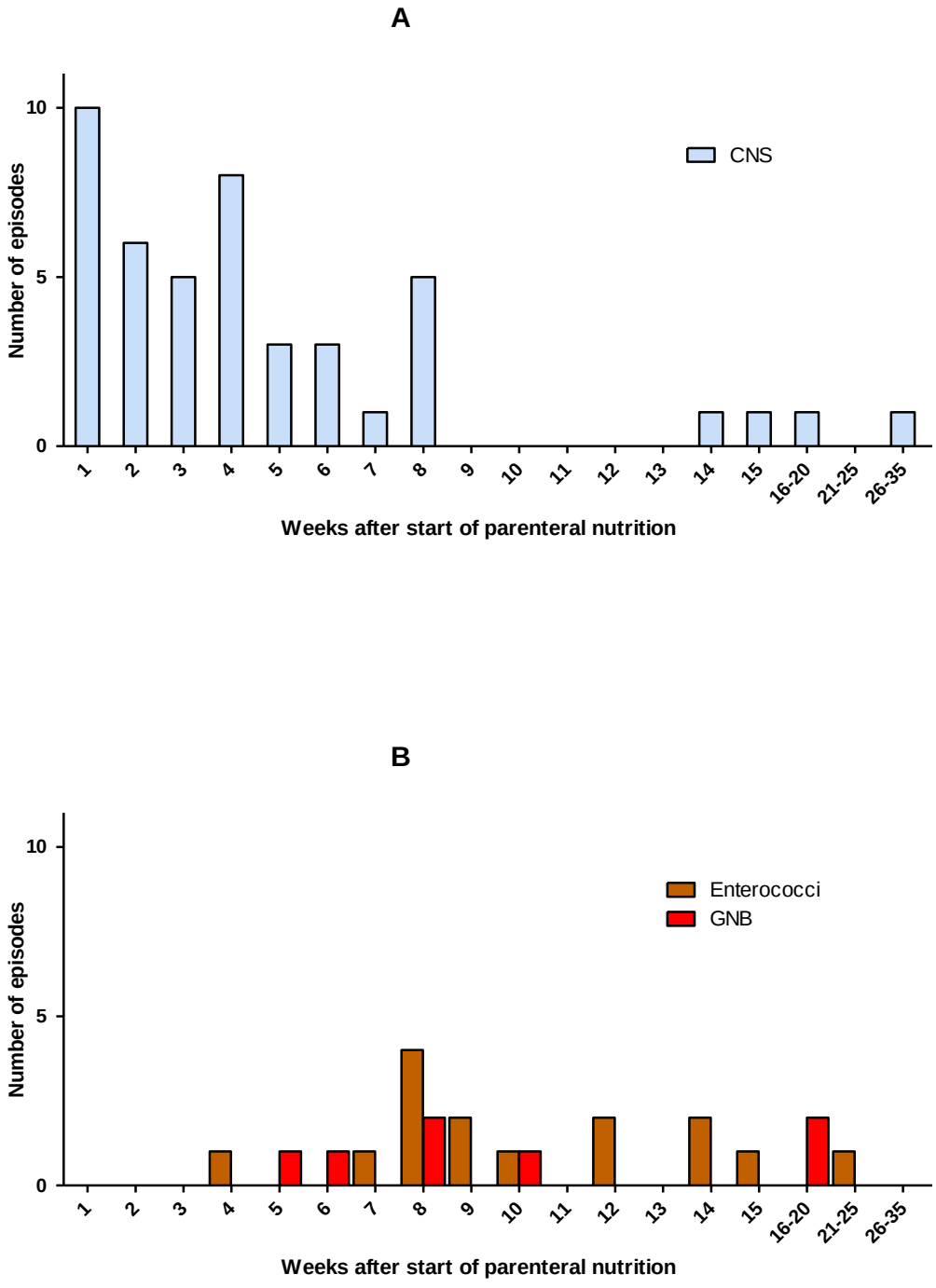
Figure 3.2 Timing of first septicaemia due to different micro-organisms



CNS = coagulase-negative staphylococci. GNB = Gram-negative bacilli.

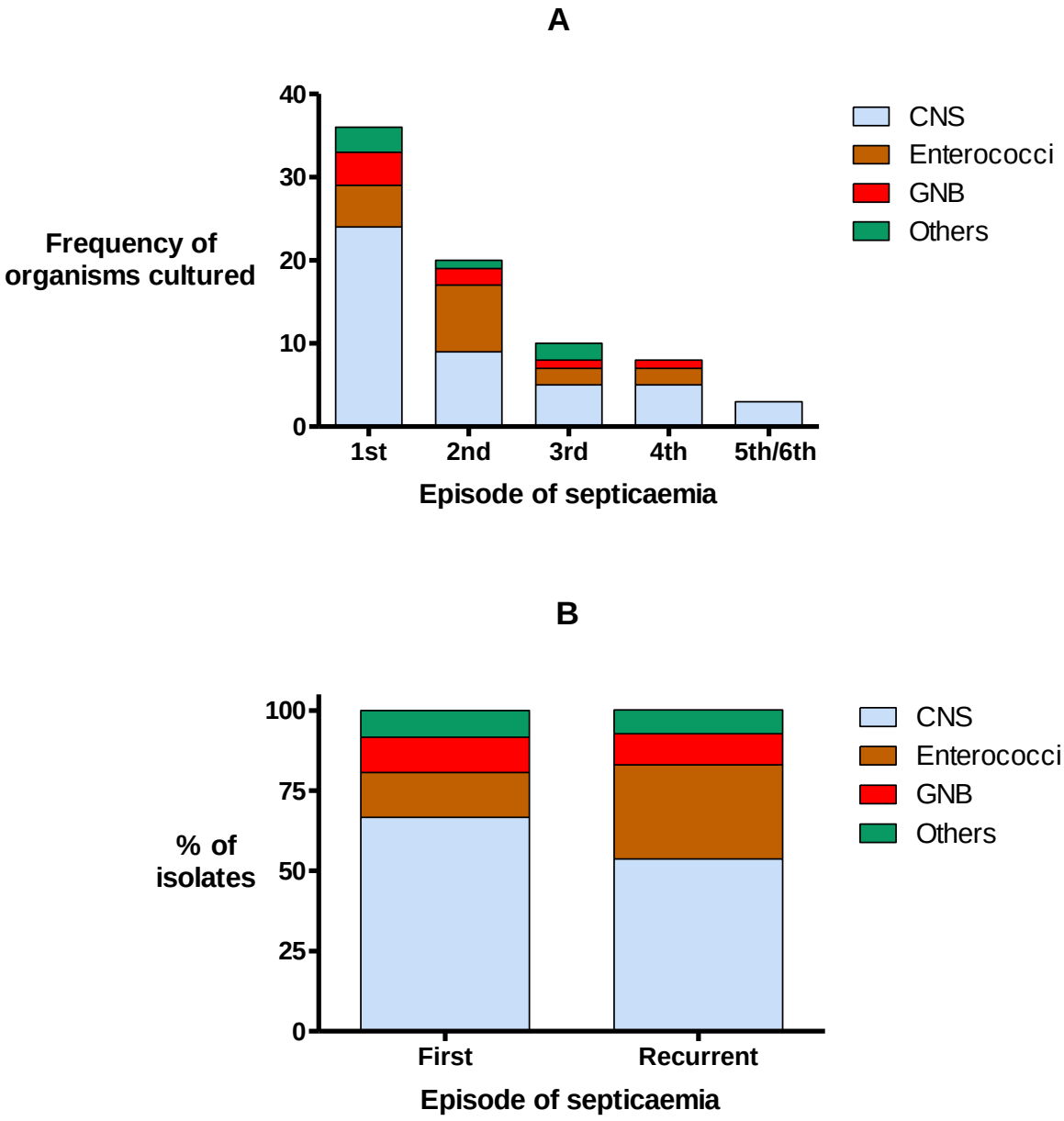
I compared the time to first occurrence of septicaemia due to CNS, with the times to first occurrence of septicaemia due to enterococci, Gram-negative bacilli, or other micro-organisms, using Kruskal-Wallis nonparametric ANOVA test and Dunn's multiple comparisons test. Among these surgical infants receiving parenteral nutrition, septicaemia due to enteric organisms occurred significantly later than septicaemia due to CNS.

Figure 3.3 Timing of septicaemia due to different micro-organisms



Timing of septicaemia due to [A] coagulase-negative staphylococci (CNS) and [B] enterococci and Gram-negative bacilli (GNB).

Figure 3.4 Frequency of organisms cultured at first and subsequent episodes of septicaemia



CNS = coagulase-negative staphylococci. GNB = Gram-negative bacilli.

Among these surgical infants receiving parenteral nutrition, enterococci accounted for 14% of first episodes of septicaemia, and 29% of recurrent episodes.

3.5 Discussion

In this large retrospective study, I showed that the incidence of sepsis and septicaemia among surgical infants on PN for gastrointestinal anomalies is high, and that septicaemia due to enterococci or Gram-negative bacilli occurs later in the course of PN than septicaemia due to CNS.

As with Chapter 2, the main limitations of this study are related to the heterogeneity of the patients and the retrospective nature of the study, and the same issues apply with the same non-standard definition of sepsis which precludes direct comparison with other studies.

Pierro *et al*, on the basis of prospective observational studies on surgical infants receiving long-term PN, have indicated the importance of gut flora and microbial translocation for the development of septicaemia (Pierro et al. 1996; Pierro et al. 1998). A further similar prospective study found evidence that infections with coagulase-negative staphylococci (which may be more likely to be involved in CVC-related infections) occurred significantly earlier in the course of PN than infections with enterococci and potentially pathogenic micro-organisms (which are more likely to originate from the gastrointestinal tract) (Donnell et al. 2002).

It has been hypothesised that infants might be more vulnerable to translocation from the gut later in the course of PN due to immunosuppression and liver dysfunction (Donnell et al. 2002; Pierro et al. 1996; Pierro et al. 1998). In support of this, Donnell et al. found that serum bilirubin was significantly higher at the time of infection with enterococci and potentially pathogenic micro-organisms, compared to infection with coagulase-negative

staphylococci (Donnell et al. 2002). My findings are also in keeping with another prospective study in similar infants which found that in infants receiving PN for more than 2 months, Gram-negative organisms caused nearly three times as many positive isolates as CNS (Page et al. 2000). It has previously been shown that in surgical infants, immune function (including phagocytosis and killing of CNS) is impaired after long-term PN (Okada et al. 1999a; Okada et al. 1999b; Okada et al. 2000).

However, in a response to the study by Donnell *et al*, another UK centre reported that they found no temporal association in septicaemia with different causative organisms in infants on parenteral nutrition (Holden et al. 2003). Significantly, this report did not specify whether the infants had had gastrointestinal surgery. The fact that I have confirmed this finding in a group of surgical infants, and that this temporal association was not seen in a broader group of infants receiving parenteral nutrition, suggests that these infants with structural gastrointestinal anomalies may be particularly prone to bacterial translocation from the gastro-intestinal tract after prolonged periods of parenteral nutrition. This is in addition to the risk of exogenous infection via CVCs, which is common to all infants receiving parenteral nutrition, and is reduced by infection control interventions to improve CVC care (Bishay et al. 2011b).

As surgical infants receiving PN for more than three weeks appear to be at increased risk of infection from gut-derived organisms, there may be a role for specific therapies to minimise bacterial overgrowth and microbial translocation. Selective digestive contamination has been shown to decrease infectious complications in adult intensive care units (de Smet et al. 2011; Liberati et al. 2009) and in adult patients undergoing gastrointestinal surgery (Roos et al. 2011). Although it has been advocated in surgical

infants (Donnell et al. 1998), there is little evidence for or against routine use in this population. Probiotics alter the intestinal microflora, and have also been advocated in preterm infants (Deshpande et al. 2010; Sawh et al. 2016), but there is currently a lack of evidence supporting their use in surgical infants. Other potential strategies, such as use of epidermal growth factor, aim to increase gut barrier function, but also probably increase enteral adaptation and absorption, so may shorten time to full enteral feeds and prevent bacterial translocation (Lamb-Rosteski et al. 2008; Rowland et al. 2013).

3.6 Conclusions

In this study I have shown that septicaemia due to enterococci or Gram-negative bacilli occurs later in the course of PN than septicaemia due to CNS, in surgical infants. This may suggest that these infants become more vulnerable to translocation of enteric micro-organisms after a longer period of parenteral nutrition.

Chapter 4. The Role of Parenteral Nutrition Following Surgery for Duodenal Atresia or Stenosis

4.1 Summary of Chapter 4

4.1.1 Aim

In our institution, some children routinely receive parenteral nutrition (PN) following surgery for duodenal atresia/stenosis, while others do not. My aim was to compare growth and infection rate between these two treatment strategies.

4.1.2 Methods

This was a retrospective study of all children undergoing surgery for duodenal atresia/stenosis over seven years.

4.1.3 Results

Of the 54 children, 19 commenced PN soon after surgery (the *'Initial PN'* group). Of the remaining 35 children (the *'No Initial PN'* group), 13 (37%) subsequently required PN (the *'Delayed PN'* group). The remaining 22 never received PN (the *'Never PN'* group). The proportion of patients experiencing clinically suspected sepsis was higher in those receiving PN *('Initial'* plus *'Delayed'*; 41%) compared with those who never received PN (14%; $p = 0.04$). The *'Initial PN'* and *'Never PN'* groups did not show a significant change in weight Z-score over time. However, the *'Delayed PN'* group showed a significant decrease in weight Z-scores from the time of operation to the time of achieving full enteral feeds, and failed to catch up by the time of last follow up.

4.1.4 Conclusions

Children with duodenal atresia/stenosis can be managed without PN. However, a third of these children subsequently require PN, lose weight centiles, and have a high rate of sepsis.

4.2 Introduction

The role of supportive nutrition is pivotal in the management of congenital duodenal obstruction. While surgical repair of duodenal atresia was first successfully performed in 1916 by Ernst (Ernst 1916; Madsen 1977), mortality remained very high for several decades – Ehrenpreis in 1949 reported 9 survivors out of 20 children undergoing surgery for congenital duodenal obstruction (Ehrenpreis and Sandblom 1949). Survival was improved somewhat, from 39% to 75% with the introduction of the transanastomotic feeding tube (Nixon and Tawes 1971). Following the widespread use of parenteral nutrition since the mid-1970s, survival has improved further, and postoperative PN has become the standard of care according to several paediatric surgical texts (Applebaum et al. 2006; Dagli 2005; Davenport 2009). This nutritional support allows the neonate to grow and develop, while the dilated and dysmotile proximal duodenum begins to function satisfactorily.

The duration of the period of postoperative ileus following surgery for duodenal atresia/stenosis is highly variable. Different methods of postoperative nutrition are used by different surgeons. Some surgeons commence all infants on parenteral nutrition (PN) soon after surgery, whereas other surgeons do not routinely commence infants on PN with the anticipation that there will only be a short period before enteral feeds can be tolerated.

Some surgeons pass a transanastomotic tube at the time of surgery, in the hope that feeding distal to the anastomosis will be better tolerated than gastric or oral feeding. This technique was originally advocated prior to the introduction of PN in surgical infants, and is favoured by some surgeons in the modern era to try to avoid/minimise the use of PN

(Hall et al. 2011; Nixon & Tawes 1971). There is conflicting evidence as to whether this technique results in a shorter time to full enteral feeds: some studies report a benefit (Arnbjornsson et al. 2002; Hall et al. 2011), while others report the opposite (Mooney et al. 1987; Upadhyay et al. 1996).

In our institution, some children have routinely received PN, while others have not, and were maintained on peripheral intravenous fluids while enteral nutrition is commenced via nasogastric tube soon after surgery and increased as tolerated. This variation was due to different practice being favoured by different consultant surgeons, not related to clinical features of the individual patient, and therefore afforded an opportunity for systematic analysis. I hypothesised that growth and infection rate might differ depending on the use and timing of PN in these infants i.e. there may be a difference in outcomes including growth (assessed by serial comparison of weight Z-scores), and in rates of sepsis and septicaemia between the two groups: those who commenced on PN initially after surgery and the others who did not.

4.3 Methods

This was an institutionally approved single centre retrospective comparative cohort study (audit approval was obtained). I studied 54 consecutive children undergoing surgery for duodenal atresia/stenosis between January 2005 and January 2012.

Data collected included diagnosis, duration of PN, episodes of clinically suspected sepsis (defined as blood cultures taken), episodes of septicaemia (defined as growth of micro-organisms from blood culture), and serial weights. Information on duration of PN was obtained from the pharmacy database, while data on blood cultures taken and results of culture were obtained from the pathology/microbiology database. Blood cultures taken less than 24 hours after surgery were not counted as episodes of postoperative sepsis/septicaemia, and consecutive blood cultures taken within 24 hours were counted as a single episode of sepsis/septicaemia. Blood cultures were read automatically using the BioMérieux BactAlert system (BioMérieux , Marcy L'Étoile, France). Diagnosis, weight measurements, and other clinical details were obtained from clinical records.

Routine practice following operation for duodenal obstruction is to leave a nasogastric tube on free drainage initially, and commence enteral feeds via the tube when aspirates start to decrease in volume. Only one patient in the study period had a transanastomotic feeding tube. The decision of whether and when to commence parenteral nutrition was made by the consultant surgeon in each case. PN was administered centrally in almost all cases via central venous catheters such as tunnelled devices (with or without a cuff) or peripherally inserted neonatal long lines. Occasionally PN was administered peripherally on the intensive care unit.

Proportions were compared using Fisher's exact test (when comparing two groups) or χ^2 test for independence (when comparing three groups). Weight measurements (at time of operation, at time of full enteral feeds and at time of last follow-up) were converted into Z-scores based on British 1990 reference data (Freeman et al. 1995). These three time points were then compared using repeated measures ANOVA with Tukey's multiple comparisons test. Other continuous variables were compared using Mann-Whitney test (when comparing two groups) or Kruskal-Wallis test with Dunn's multiple comparisons test (when comparing three groups). Data are presented as median (range).

4.4 Results

During the study period, 54 children underwent surgery for duodenal atresia or stenosis. One further patient with duodenal stenosis and congenital acute myeloid leukaemia died without undergoing surgery. 50 of the patients underwent surgery during the neonatal period. At operation, 35 patients were found to have duodenal atresia (complete duodenal obstruction), while 19 had duodenal stenosis (partial obstruction). In 34 patients the obstruction was described as being in the second or third part of the duodenum, with only two proximal to this (in the first part) and one distal (near the duodenojejunal flexure; in the remaining cases the site of duodenal obstruction was not further specified). One child was found to have a double duodenal atresia with two obstructing septa. In twelve cases a laparoscopic approach was used; four of these required conversion to open surgery. A duodenoduodenostomy was performed in 47 cases, duodenojejunostomy in 4 cases, and duodenoplasty in 3 cases.

The demographics of the whole group are shown in Table 4.1. 51 out of the 54 children were less than 28 days old at the time of operation. As well as the high rates of prematurity (59%) and cardiac anomalies (44%), 31% of children had trisomy 21, and a wide range of other comorbidities were observed, including craniofacial, musculoskeletal, renal and genitourinary anomalies, and other chromosomal abnormalities. Coexisting gastrointestinal anomalies included intestinal malrotation, anorectal malformations, oesophageal atresia and tracheo-oesophageal fistula, congenital diaphragmatic hernia, and Hirschsprung's disease. Two patients developed necrotising enterocolitis following surgery. Only eight patients were not noted to have any significant comorbidity.

Of the 54 children, 19 commenced PN within 3 days of surgery (the '*Initial PN*' group). The remaining 35 who did not commence PN within 3 days, I have designated the '*No Initial PN*' group. There was no significant difference between the two groups in age, sex, gestational age, prematurity, baseline weight Z-score, trisomy 21, cardiac anomalies or antenatal diagnosis (Table 4.1). The '*Initial PN*' group included two children born extremely prematurely (at 26 and 28 weeks), who were receiving PN prior to surgery. Cases completed laparoscopically were significantly less likely to be commenced on PN initially ($p = 0.04$), perhaps reflecting an assumption that the less invasive approach would result in quicker progress to full enteral feeds. However five out of the eight did subsequently require PN

Of the 35 infants in the '*No Initial PN*' group, 13 children (37%) subsequently required PN (the '*Delayed PN*' subgroup), which was commenced 7 (4 – 30) days after surgery. The remaining 22 never received PN (the '*Never PN*' subgroup).

Table 4.1 Patient characteristics

	All patients (n=54)	Initial PN (n=19)	No Initial PN (n=35)	Never PN (n=22)	Delayed PN (n=13)
Age at operation (days)	2 (0, 408)	3 (0, 408)	2 (0, 251)	1 (0, 251)	2 (1, 58)
Females	35 (65%)	11 (58%)	24 (69%)	14 (64%)	10 (77%)
GA at birth (weeks)	36 (26, 41)	35 (26, 41)	36 (30, 40)	36 (30, 41)	37 (33, 40)
Prematurity (birth GA <37 weeks)	32 (59%)	13 (68%)	19 (54%)	12 (55%)	7 (54%)
Baseline weight Z-score	-0.9 (-6.0, 1.5)	-0.9 (-6.0, 1.5)	-1.0 (-5.4, 1.1)	-1.0 (-5.4, 1.1)	-0.9 (-2.3, 0.7)
Trisomy 21	17 (31%)	6 (32%)	11 (31%)	7 (32%)	4 (31%)
Cardiac anomaly	24 (44%)	10 (53%)	14 (40%)	10 (45%)	4 (31%)
Antenatally diagnosed	25 (46%)	6 (32%)	19 (54%)	13 (59%)	6 (46%)
Laparoscopically repaired	8 (15%)	0 (0%)	8 (23%)	3 (14%)	5 (38%)

GA = Gestational age

Table 4.2 summarises the nutritional management and outcomes. Comparing the '*Initial PN*' group with the '*No Initial PN*' group showed no difference in time to reach full feeds or hospital stay (using Mann-Whitney test; Figure 4.1A). However, considered separately, the '*Never PN*' subgroup had a significantly shorter time to reach full feeds, and a significantly shorter hospital stay than the other two groups (Figure 4.1B). With regard to time to commence feeds, there is evidence of a trend towards shorter time to commence feeding in the '*Never PN*' group compared to the '*Delayed PN*' group.

The proportion of patients experiencing clinically suspected episodes of sepsis or sepitcaemia was not significantly different in the '*Initial PN*' group compared to the '*No Initial PN*' group. However, the proportion of patients experiencing clinically suspected episodes of sepsis was higher in those receiving PN ('*Initial PN*' plus '*Delayed PN*'; 41%) compared with those who never received PN (14%; $p = 0.04$). Four patients had positive blood cultures. The organisms isolated were coagulase-negative staphylococci (in two cases), *Staphylococcus aureus*, *Streptococcus mitis*, and *Candida parapsilosis*. One of the children with septicaemia due to coagulase-negative staphylococci required readmission to the intensive care unit for three days due to septic shock. She was in the '*Delayed PN*' group, and was on PN at the time. She had previously suffered an extravasation injury while on peripheral intravenous fluids (10% glucose) prior to starting PN. Two other children in the '*Never PN*' group also suffered extravasation injuries while on peripheral intravenous fluids (10% glucose), although one of these was prior to surgery. One girl in the '*Initial PN*' group suffered an extravasation injury while on peripheral PN in the intensive care unit (she subsequently had a tunnelled central venous catheter inserted).

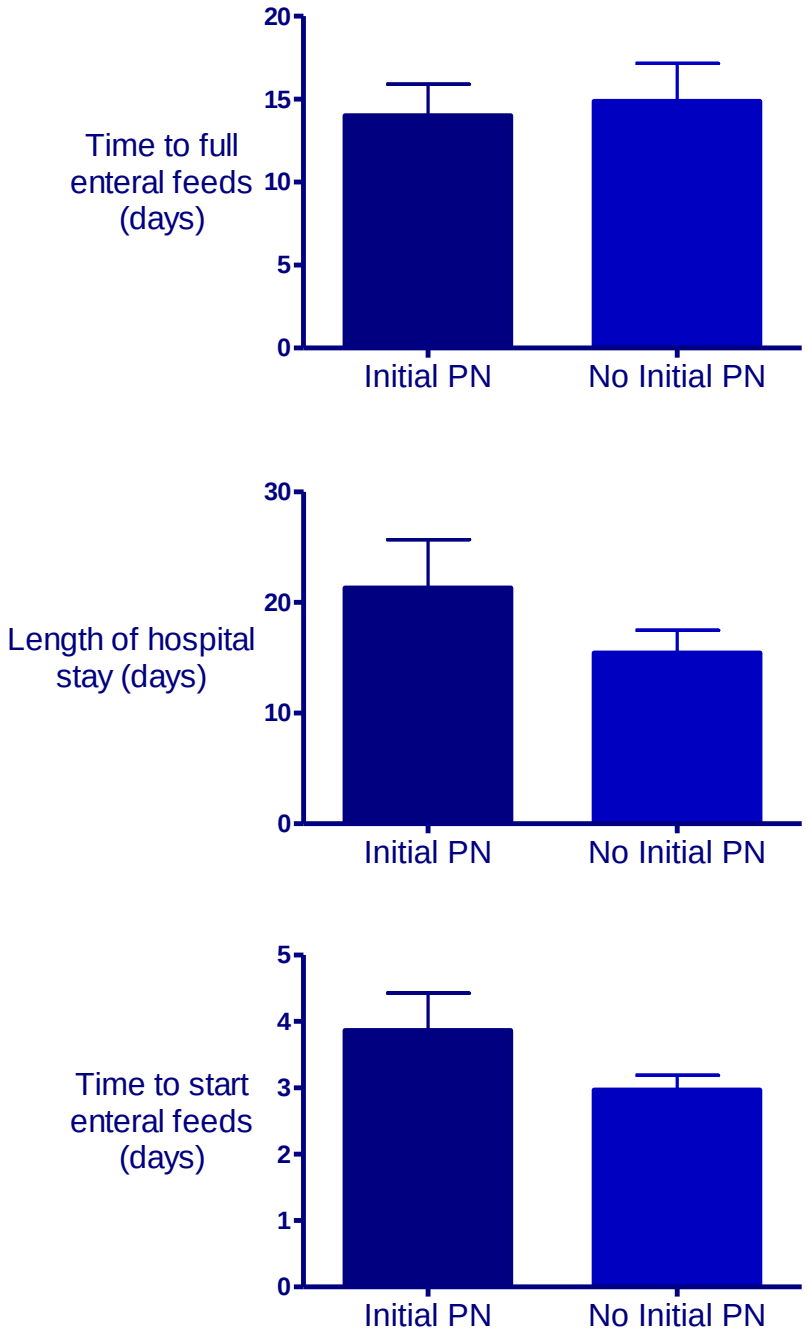
Table 4.2 Nutritional management and outcomes

	All patients (n=54)	Initial PN (n=19)	No Initial PN (n=35)	Never PN (n=22)	Delayed PN (n=13)
Time to start feeds (days)	3 (1, 9)	3 (2, 9)	3 (1, 6)	2 (1, 4)	4 (2, 6)
Time to full feeds (days)	11 (3, 48)	12 (5, 31)	10 (3, 48)	7 (3, 12)	17 (9, 48)
Time to start of PN	3 (0, 30) [a]	1 (0, 3)	7 (4, 30) [a]	N/A	7 (4, 30)
Duration of PN (days)	9 (5, 45) [a]	9 (5, 45)	9 (5, 21) [a]	N/A	9 (5,21)
Sepsis	16 (30%)	7 (37%)	9 (26%)	3 (14%)	6 (46%)
Septicaemia	4 (7%)	2 (11%)	2 (6%)	0 (0%)	2 (15%)
Length of stay (days)	14 (7, 68)	14 (7, 68)	12 (7, 60)	8 (7, 30)	25 (13, 60)
Follow up (months)	17 (3, 68)	14 (3, 39)	10 (3, 68)	10 (3, 68)	19 (3, 55)

'*Sepsis*' = children experiencing at least one episode of clinically suspected sepsis. '*Septicaemia*' = children experiencing at least one episode of septicaemia.

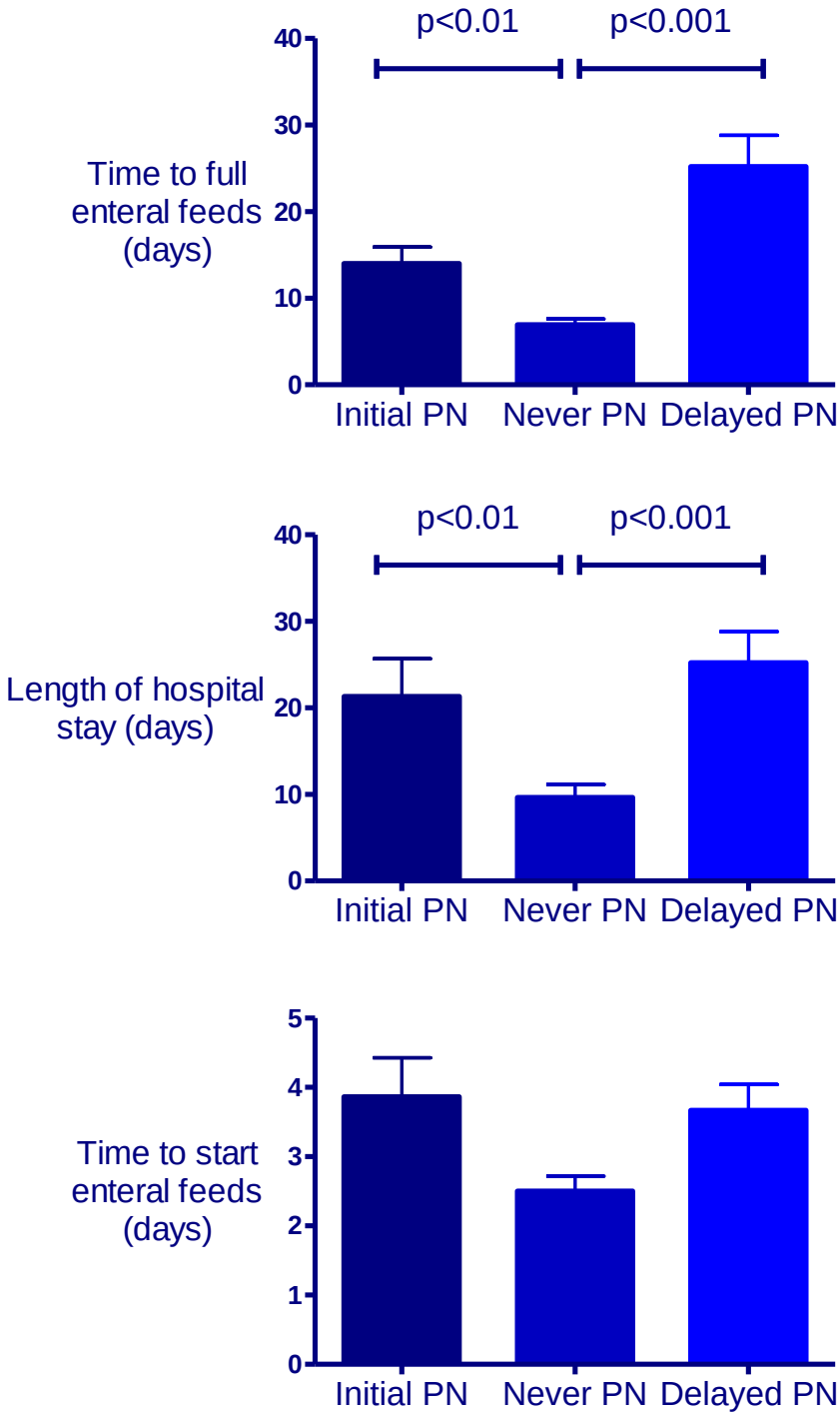
[a] For patients who received parenteral nutrition (PN).

Figure 4.1A Timing of enteral feeds and length of hospital stay



Comparing the '*Initial PN*' group with the '*No Initial PN*' group showed no difference in time to reach full feeds, length of hospital stay or time to start enteral feeds (groups compared using Mann-Whitney test).

Figure 4.1B Timing of enteral feeds and length of hospital stay

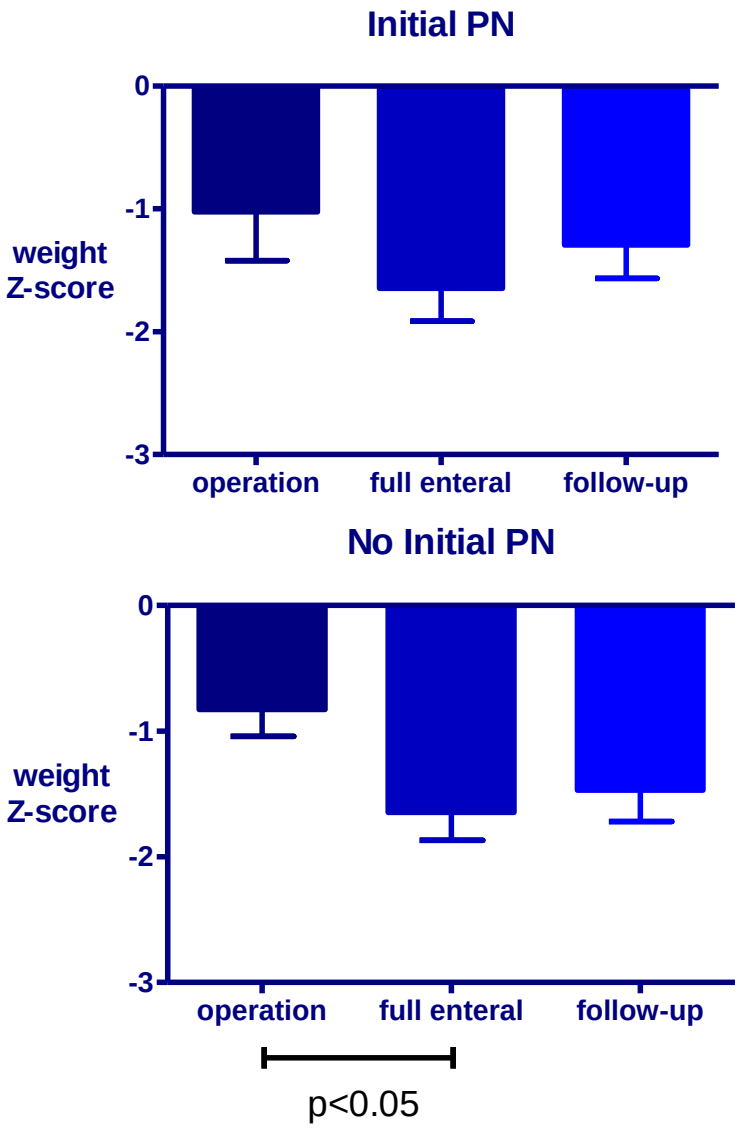


The '*Never PN*' subgroup had a significantly shorter time to reach full feeds, and a significantly shorter hospital stay than the other two groups (groups compared using Kruskal-Wallis test with Dunn's multiple comparisons).

The '*Initial PN*' group did not show a significant change in weight Z-score over time (Figure 4.2A), while the '*No Initial PN*' group did show a significant decrease in weight Z-scores from the time of operation to the time of achieving full enteral feeds. Analysing the subgroups, the '*Never PN*' group showed no significant change in weight Z-score over time. However, the '*Delayed PN*' group showed a significant decrease in weight Z-scores from the time of operation to the time of achieving full enteral feeds, and failed to catch up by the time of last follow up (Figure 4.2B). Patients were followed up for 17 (3, 68) months. There was no difference in the length of follow-up between the three groups.

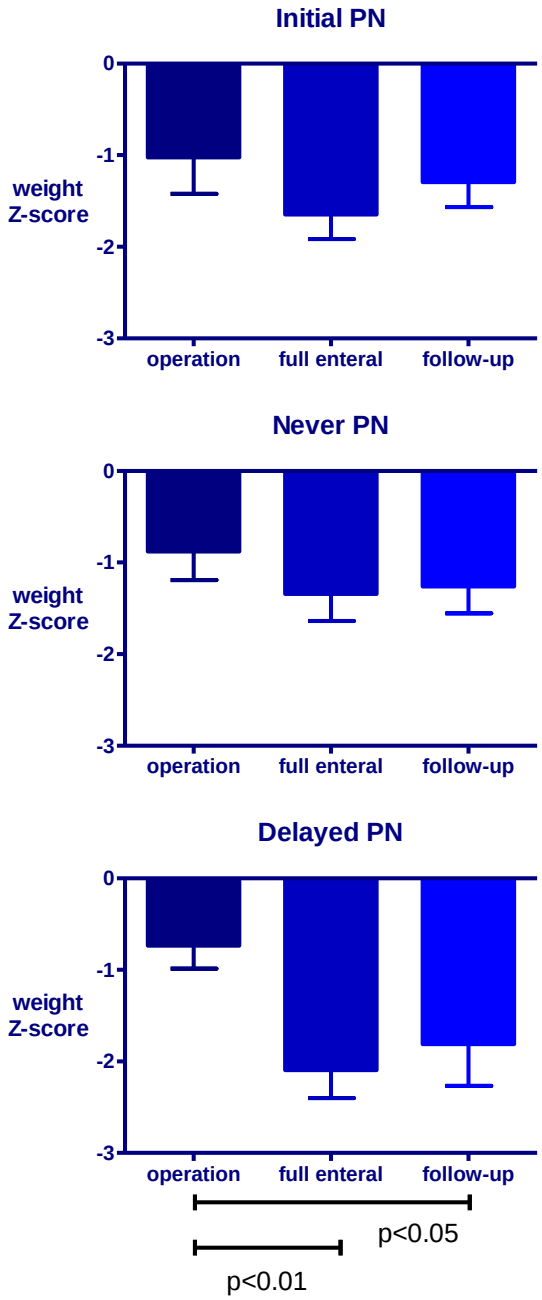
Complications related to surgery were recorded in ten cases, including one anastomotic leak requiring re-operation, one duodenal ulcer with perforation, one anastomotic stricture treated by balloon dilatation under fluoroscopic guidance, three children with recurrent or persisting duodenal obstruction requiring re-operation (one following initial laparoscopic repair, one following initial duodenoplasty for partial obstruction; a duodenojejunostomy was performed at re-operation in all three cases), three superficial wound separations, and a port site hernia. Three children died without reaching full enteral feeds; all had major congenital cardiac anomalies. Two were considered too small for cardiac surgery, while the third suddenly deteriorated and died two days after undergoing cardiac surgery.

Figure 4.2A Change in weight Z-score following surgery for congenital duodenal obstruction



The '*Initial PN*' group did not show a significant change in weight Z-score over time, while the '*No Initial PN*' group did show a significant decrease in weight Z-scores from the time of operation to the time of achieving full enteral feeds. The weight Z-scores at the three time points were compared using repeated measures ANOVA with Tukey's multiple comparisons test.

Figure 4.2B Change in weight Z-score following surgery for congenital duodenal obstruction



The '*Never PN*' subgroup showed no significant change in weight Z-score over time. However, the '*Delayed PN*' subgroup showed a significant decrease in weight Z-scores from the time of operation to the time of achieving full enteral feeds, and failed to catch up by the time of last follow up (repeated measures ANOVA with Tukey's multiple comparisons test).

4.5 Discussion

There is considerable variability in the amount of time taken for adaptation to occur following surgery for congenital duodenal obstruction, and for enteral feeds to be tolerated satisfactorily. The fact that some children can tolerate full feeds within a few days (the shortest time to full enteral feeds in the current series was just three days after operation) leads some surgeons to withhold PN in the initial postoperative period.

This large retrospective comparative study shows that some children with duodenal atresia/stenosis can be managed without PN. This policy was successful in the majority of cases, and was associated with a quicker time to full enteral feeds and shorter hospital stay in those cases, with no loss of weight centiles, as well as avoiding the complications associated with the use of PN and central venous access. However, over a third of children initially managed without PN did go on to subsequently require PN. These children had a high rate of sepsis and prolonged hospital stay (similar to the '*Initial PN*' group), and (unlike the other two groups) lost weight centiles during their hospital stay. They had not regained their baseline centiles by the time of latest follow-up (median 19 months). It is not known whether this poor growth in early life has any long term effects particularly on neurodevelopmental outcome.

Regarding time to commence feeds, the trend towards shorter time to commence feeds in the '*Never PN*' group compared to the '*Delayed PN*' group is of interest. It may be that the patients in the '*Never PN*' group showed less dysmotility and lower volume of aspirates and were therefore commenced on enteral feeds sooner. Alternatively, it may be that commencing feeds earlier predisposes to improved tolerance of feeds.

While transanastomotic tubes were originally introduced before the use of PN became widespread, some surgeons advocate their use in the modern era, based on the rationale that the dysmotility is principally due to the dilated segment proximal to the anastomosis so that feeding distally will be tolerated sooner (Hall et al. 2011; Nixon & Tawes 1971). Some studies have claimed that this can avoid the need for PN and central venous access (with their attendant complications), and reduce the time to establish full enteral feeds (Arnbjornsson et al. 2002; Hall et al. 2011) (though it may not reduce the time to full oral feeds and therefore does not appear to reduce hospital stay). However, these findings are in conflict with those reported by others who found that the use of a transanastomotic tube was associated with longer time to full enteral feeds and/or longer hospital stay (Mooney et al. 1987; Upadhyay et al. 1996). Furthermore, complications associated with the use of such tubes have been reported, including anastomotic leak and intestinal perforation (Hall et al. 2011; Millar et al. 2000; Upadhyay et al. 1996). Tube malfunction (including occlusion, migration into the stomach, or dislodgement) is commonly reported (Hall et al. 2011; Millar et al. 2000). For example, in the retrospective study by Hall et al. four out of seventeen patients with transanastomotic tubes experienced tube displacement and one had an anastomotic leak with jejunal perforation (Hall et al. 2011). On the other hand, 15 of the 17 avoided the need for CVC and PN, compared to 10 of 38 patients with no transanastomotic tube. Six of the infants managed without a transanastomotic tube experienced CVC-related complications (5 infections, 1 PN extravasation). The use of a transanastomotic tube is therefore a potentially useful option in the management of these infants.

Minimally invasive surgery has been used for congenital duodenal obstruction: eight patients in the current series underwent laparoscopic repair, and there are numerous reported series in the literature (Bax et al. 2001; Hill et al. 2011; Kay et al. 2009; Kozlov et al. 2011; Parmentier et al. 2015; Rothenberg 2002; Son et al. 2015; van der Zee 2011). Laparoscopic repair of congenital duodenal obstruction is technically very challenging, and one centre which reported initial promising results subsequently abandoned the technique for three years due to a high rate of surgical complications (anastomotic leaks), before resuming with some technical adjustments and additional experience in intracorporeal suturing (van der Zee 2011). So far laparoscopic repair of duodenal obstruction has not been shown to result in any reduction in time to full enteral feeding, compared to open surgery (Hill et al. 2011). In the present study 8 patients underwent laparoscopic surgery. None were initially started on PN, but the majority (five of the eight) subsequently required PN.

The timing of parenteral nutrition in critically ill adults has been studied: a systematic review in 2014 (Bost et al. 2014) found four randomised controlled trials and two prospective observational studies comparing early supplementary parenteral nutrition (commenced within 48 hours of admission to intensive care) with late PN (postponing PN until day 8 after admission). This review was undertaken due to conflicting guidance available at the time: the 2009 European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines (Singer et al. 2009) recommend using supplementary PN within 24 to 48 hours in patients who are expected to be intolerant to enteral nutrition within 72 hours of admission, while the 2009 American Society for Parenteral and Enteral Nutrition (ASPEN) guidelines (McClave et al. 2009) recommend postponing the initiation of PN

until day 8 after ICU admission. The six studies included in the review analysed a total of 10,060 patients. Overall there was no benefit from early parenteral nutrition with respect to mortality or morbidity including infection rates or length of hospital stay. In critically ill children, a multicentre randomised controlled trial involving 1440 children (Fivez et al. 2016) compared early PN (commenced within 24 hours of admission to intensive care) with late PN (PN not provided until the 8^{th} day after admission). Late PN was found to be associated with lower infection rates, shorter duration of mechanical ventilation, lower proportion of patients requiring renal replacement therapy, and shorter hospital stay, compared to early PN. 653 patients (45%) were under one year old. Post hoc subgroup analysis of 209 term neonates found that the benefits of late PN in this subgroup were similar to or greater than those seen in older children. However, episodes of hypoglycaemia were seen in 9% of all patients in the late PN group, compared to 5% of those in the early PN group ($p = 0.001$). Based on these findings, the latest consensus guidelines (Mehta et al. 2017) suggest withholding PN for a week in critically ill children with normal baseline nutrition state and low risk of nutrition deterioration (excluding neonates). Based on expert consensus, PN supplementation is suggested for children who are unable to receive any EN during the first week in intensive care. For patients who are severely malnourished or at risk of nutrition deterioration, it is suggested that PN may be supplemented in the first week if they are unable to advance past low volumes of EN. There is thus very limited evidence to guide management this group of infants with congenital duodenal obstruction. This study is therefore important in showing that for infants with congenital duodenal obstruction, infants not commenced on PN initially

show a decrease in weight Z-scores compared to those who are commenced on PN initially.

The present study has important limitations, mainly related to its retrospective nature. With this design there is a risk of selection bias; however the results show similar baseline characteristics between the groups, supporting the premise that group allocation was largely unrelated to clinical features but was rather a reflection of the practice of the individual surgeon. Techniques of propensity score matching have been developed to overcome selection bias in observational studies (Austin 2011); such techniques are useful when the groups compared in an observational study are dissimilar due to confounding covariates which influence both group allocation and outcome. This was not the case in the present study, where the groups had similar baseline characteristics, and the only confounding covariate which was found to significantly influence group allocation was whether surgery was completed laparoscopically, which was only the case for 8 patients, and therefore could not be meaningfully used in propensity score matching. Logistic regression analysis to identify confounding covariates may not be reliable with such a limited sample size.

Regarding growth data, unfortunately data regarding head circumference growth were not available retrospectively. Condition-specific growth reference data were not used for patients with trisomy 21. This is of limited significance as the main aim was to compare serial weight Z-scores for individual patients. Furthermore condition-specific growth reference data are not available for the other comorbidities encountered such as congenital cardiac anomalies. This study again uses the same non-standard definitions of

sepsis and septicaemia as in the preceding chapters which limit the ability to compare findings with other studies.

4.6 Conclusions

Despite these limitations, this study provides important information: it demonstrates that patients with congenital duodenal obstruction can often be managed with gastric feeds without PN or a transanastomotic tube (an approach which is not widely reported in the modern literature). However, while avoidance of initial PN is successful for almost two thirds of these cases, the remaining third will go on to require PN, and this group show poorer growth than children who commenced PN soon after surgery; a fact which must be balanced against the advantages conferred upon the majority.

Chapter 5. Intestinal Failure-Associated Liver Disease in Surgical Infants

5.1 Summary of Chapter 5

5.1.1 Aims

My aim was to determine the incidence, severity and outcome, as well as predisposing factors and underlying diagnoses, of intestinal failure-associated liver disease (IFALD) in surgical infants requiring long-term parenteral nutrition (PN).

5.1.2 Methods

I retrospectively studied surgical infants receiving PN for at least 28 days for congenital or acquired intestinal anomalies over a five-year period (January 2006–December 2010). Type 1, early IFALD was defined as a persistent elevation of alkaline phosphatase (ALP) for at least 6 weeks. Type 2, established IFALD was defined as type 1, but in addition with an elevated total bilirubin. Type 3, late IFALD, was defined as type 2, but with clinical signs of end-stage liver disease. Ordinal regression analysis was used to determine risk factors for IFALD.

5.1.3 Results

Eighty seven infants required PN for at least 28 days. IFALD occurred in 29 infants (33%). IFALD was managed medically in all but two patients who underwent intestinal elongation. None were referred for intestinal or liver transplant. IFALD has been reversed in 17 (59%) of cases to date. Sixty one patients receiving long-term PN (70%) have achieved enteral autonomy, while 12 (14%) require home PN. Severity of IFALD was significantly associated with duration of PN, and female gender. Development of IFALD was not associated with septicaemia.

5.1.4 Conclusions

IFALD remains a fairly common, but rarely life-threatening, complication of intestinal failure in surgical infants. IFALD can be reversed in over half of these children and enteral autonomy achieved in over two thirds, even with minimal use of intestinal elongation. This is the first study to demonstrate an association between the severity of IFALD in surgical infants and female gender. Septicaemia was not found to be a risk factor for the development of IFALD.

5.2 Introduction

Parenteral nutrition (PN) has dramatically improved outcomes for surgical infants unable to be fed enterally (Fillingham & Rankin 2008; Puntis 2001; Schwaitzberg et al. 1982) and it plays a key role in the management of gastroschisis, necrotising enterocolitis (NEC), and congenital intestinal obstruction due to various causes. However, PN is associated with a number of potentially life-threatening complications, including infections and liver disease (Bishay et al. 2011b; Donnell et al. 2002; Koglmeier et al. 2008; Pichler et al. 2010; Pierro et al. 1998; Puntis 2001). Intestinal failure-associated liver disease (IFALD) has been reported in 40% to 60% of children requiring long-term PN (Btaiche & Khalidi 2002; Buchman 2002; Koglmeier et al. 2008).

Hepatobiliary complications associated with the use of PN range from mild derangement of liver function tests through steatosis, cholestasis, biliary sludge and cholelithiasis to fibrosis, cirrhosis, and established life-threatening liver failure requiring transplantation. The aetiology of IFALD is multifactorial. Factors reported to be associated with the development of IFALD included longer duration of PN, less enteral feeding, prematurity, sepsis, early exposure to PN, and excess lipid calories (Beath et al. 1996; Beath on behalf of the BSPGHAN Nutrition Working Group 2010; Kelly 2006). Strategies to prevent IFALD thus include increasing enteral feeding, prevention and prompt management of sepsis, cyclic infusion of PN/lipids, and restriction of lipid calories or use of alternative sources of lipids (Abu-Wasel and Molinari 2014; Beath on behalf of the BSPGHAN Nutrition Working Group 2010; Kelly 2010; Nandivada et al. 2013; Nandivada et al. 2016). The standard initial source of lipids in PN has been soybean-based emulsions, but there have been concerns that these contain phytosterols (plant-derived cholesterol-like

compounds), which have been shown to reduce bile flow in animal models (Clayton et al. 1998) and have been associated with the development of cholestatic liver disease in children receiving PN (Kelly 2010; Nandivada et al. 2016). Other components of intravenous lipid emulsions have also been implicated in the development of IFALD (Raphael and Duggan 2012).

Among hospitalised children with intestinal failure, intestinal surgery has been found to be the greatest independently significant risk factor for development of IFALD (Pichler et al. 2012).

My aim was therefore to determine the incidence, severity and outcome of IFALD in infants requiring long-term PN following gastrointestinal surgery. I also aimed to test the hypotheses that the development of IFALD was associated with septicaemia, prematurity, or diagnostic group (congenital bowel obstruction, necrotising enterocolitis, or abdominal wall defects) or other predisposing factors (such as sex or age at starting PN) in this group of infants.

5.3 Methods

This was a single centre retrospective cohort study. With institutional audit approval I retrospectively studied surgical infants receiving PN for at least 28 days for congenital or acquired intestinal anomalies over a five-year period (January 2006–December 2010). IFALD was identified and graded according to BSPGHAN (British Society of Paediatric Gastroenterology, Hepatology and Nutrition) guidelines (British Society for Paediatric Gastroenterology Hepatology And Nutrition (BSPGHAN) Nutrition Working Group 2009). Briefly, type 1, early IFALD was defined as a persistent elevation of alkaline phosphatase (ALP) 1.5 times the upper limit of reference range for at least 6 weeks. Type 2, established IFALD was defined as type 1, but in addition with an elevated total bilirubin (>50 μmol/L), with a conjugated fraction 50% or more. Type 3, late IFALD, was defined as type 2, but with clinical signs of end-stage liver disease.

Management of PN was as described previously in a paper from our group describing IFALD in children of all ages from 2006-2009 (Pichler et al. 2012) (this general review of 279 children with IFALD resulting from both medical and surgical conditions in our institution includes some data on seventy of the infants described in the current series). Briefly, PN was prescribed according to the Guidelines on Paediatric Parenteral Nutrition of the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the European Society for Clinical Nutrition and Metabolism (ESPEN) (Koletzko et al. 2005). The formulation was usually ‘tailor made’ for the individual infant. The initial standard intravenous lipid emulsion used during the time period studied was a soya-based lipid (Intralipid 20%®). The lipid was changed when ALP, alanine aminotransferase (ALT), or γ-glutamyl transferase (γ-GT) were elevated to 1.5 times the

upper limit of the reference range for more than two weeks, or conjugated bilirubin was more than 2mg/dl (>50 μmol/L). In premature infants, the lipid was changed within a week of raised enzyme levels. Alternative lipids used were an MCT/LCT mixture (Lipofundin®) or a lipid with LCT, MCT, olive and fish oil (SMOF®). Enteral feed was introduced at the earliest possible opportunity, and increased as tolerated.

Data collected included diagnosis, duration of PN, episodes of septicaemia (defined as growth of micro-organisms from blood culture), and organisms cultured. Information on duration of PN was obtained from the pharmacy database, while data on number of blood cultures taken and results of culture were obtained from the pathology/microbiology database. Data are presented as median (range). Data were compared by Fisher's exact test, Mann-Whitney test, chi-squared test for trend, or ordinal regression analysis, which was used to determine risk factors for IFALD. Ordinal regression analysis was performed using severity of IFALD grade as the outcome and sex, gestational age, diagnostic group, age at start of PN, duration of PN and septicaemia as co-variates. Kaplan-Meier curves were constructed for duration of parenteral nutrition, and compared using log-rank test; patients were censored at the last available follow-up. A P-value of <0.05 was considered significant.

5.4 Results

Eighty seven infants required PN for at least 28 days, of whom 50 were male (57%). Age at start of PN was 27 (1-347) days, and the duration of PN given was 62 (28-310) days. 49 (56%) were born prematurely. Fifty-three infants (61%) experienced at least one episode of septicaemia. The underlying diagnoses (see Table 5.1) were necrotising enterocolitis in 37 (43%), abdominal wall defects in 21 (24%), and congenital bowel obstruction in 25 (29%).

IFALD occurred in 29 infants (33%). Seven of these were graded as early (type 1), 13 as established (type 2), and 9 as late (type 3). As expected, IFALD was associated with longer PN duration (Table 5.1; see also Figure 5.1). However, unexpectedly, IFALD was also associated with female sex (Table 5.1). Further analysis showed that female sex was associated with increasing severity of IFALD ($p = 0.006$, using chi squared for trend; Figure 5.2), and this association remains even after adjusting for gestational age, diagnostic group, age at start of PN, duration of PN and presence of septicaemia ($p = 0.017$ by ordinal regression analysis). Although the majority of infants with intestinal failure were male, the majority of those with grade 2 or 3 IFALD were female. Unexpectedly, infants who experienced at least one episode of septicaemia (with positive blood culture) were no more likely to develop IFALD than those who did not.

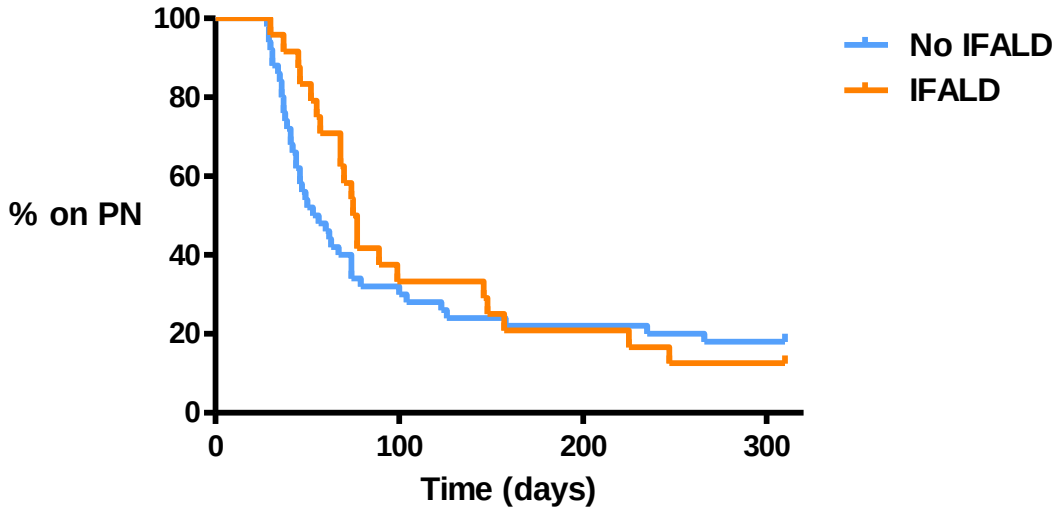
As shown in Table 5.1, there was no association between prematurity or underlying diagnoses and development of IFALD.

Table 5.1 Patient characteristics and diagnoses

	Non-IFALD (n=58)	IFALD (n=29)	P-value
Males	38 (66)	12 (41)	0.04
Age at start of PN (days)	19 (1-347)	45 (4-270)	0.12
Duration of PN (days)	48 (28-310)	77 (30-276)	0.002
Preterm	32 (55)	17 (59)	0.82
Septicaemia	35 (60)	18 (62)	1.00
NEC	24 (41)	13 (45)	0.82
Congenital Bowel Obstruction	17 (29)	8 (28)	1.00
Abdominal Wall Defect	13 (22)	8 (28)	0.60
Other intestinal failure	4 (7)	0	0.30

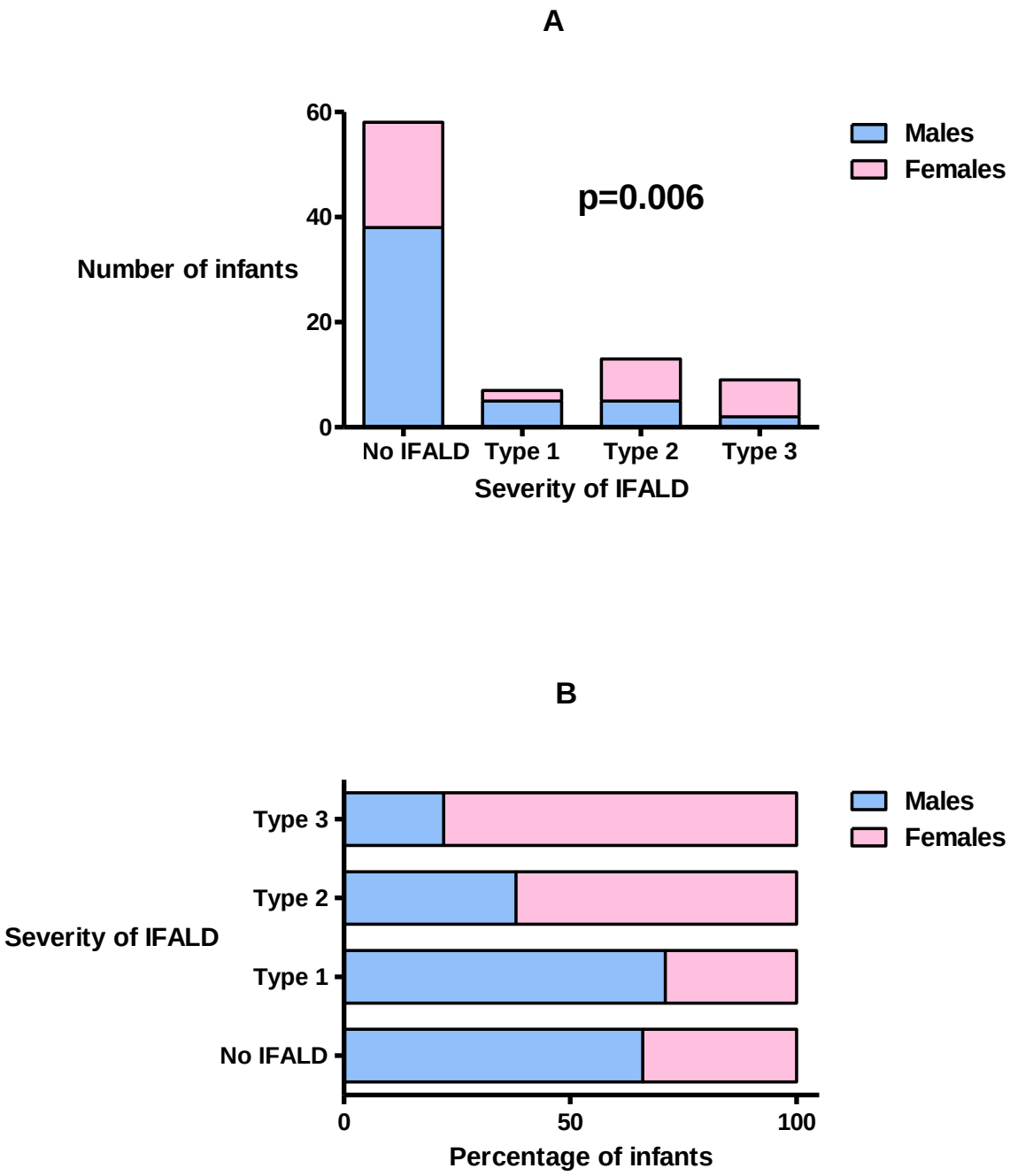
Data presented as number (percentage) or median (range). Continuous variables compared by Mann-Whitney test, categorical variables by Fisher's exact test.

Figure 5.1 Duration of parenteral nutrition and development of intestinal failure-associated liver disease



Kaplan-Meier curves showing duration of parenteral nutrition (PN) in infants who developed intestinal failure-associated liver disease (IFALD) and those who did not. No significant difference between curves by log-rank test (p = 0.5).

Figure 5.2 Sex and severity of intestinal failure-associated liver disease



A. Numbers of male and female infants with different grades of intestinal failure-associated liver disease (IFALD). Chi-squared for trend p = 0.006.

B. Percentage of male and female infants with different grades of IFALD. While the majority of infants with no IFALD or type 1 IFALD were male, the majority of those with more severe IFALD were female.

Clinical outcomes at a follow-up of 24 (5-62) months are summarised in Table 5.2. No patients were referred for intestinal or liver transplant, as they did not meet the relevant criteria. IFALD was managed medically in all, except two patients who underwent STEP (serial transverse enteroplasty) procedures (1 died of sepsis and in 1 IFALD was reversed and the child achieved enteral autonomy after a period of home PN). To date, IFALD has been reversed in 17 (59%) infants. Sixty one children receiving long-term PN (70%) have achieved enteral autonomy, while 12 (14%) require home PN. There were 13 deaths overall (15%); 9 deaths were PN-related (eight due to sepsis and one due to liver failure). Five of the thirteen deaths were in patients who developed IFALD. Eight of the thirteen infants who died were born prematurely. Two of the others had congenital diaphragmatic hernia with severe pulmonary hypertension.

Table 5.2 Patient outcomes

	Total (n=87)	Non-IFALD (n=58)	IFALD (n=29)
Achieved enteral autonomy	61 (70)	40 (69)	21 (72)
On home PN	12 (14)	9 (16)	3 (10)
Died	13 (15)	8 (14)	5 (17)
PN-related deaths	9 (10)	6 (10)	3 (10)
Death due to liver failure	1 (1)	0	1 (3)

Data presented as number (percentage). The follow-up was 24 (5-62) months.

5.5 Discussion

This large retrospective study shows that IFALD remains a fairly common, but rarely life-threatening, complication of intestinal failure and long-term parenteral nutrition in surgical infants. IFALD can be reversed in more than half of these children and enteral autonomy can be achieved in more than two thirds, even with very limited use of intestinal lengthening. Mortality, both overall and PN-related, is similar in those infants with IFALD and those without; only one death was due to liver failure.

The 33% incidence of IFALD in my study is slightly lower than the 40-60% reported in other studies of hospitalised children requiring long term PN, despite the fact that surgical infants are noted to have a higher incidence of IFALD than other hospitalised children (Btaiche & Khalidi 2002; Buchman 2002; Koglmeier et al. 2008; Pichler et al. 2012). The finding that IFALD was not associated with sepsis is in contrast to a previous comparable study (Beath et al. 1996), but is in keeping with findings in the mixed medical/surgical cohort of children with IFALD with which the current group of surgical infants with IFALD overlaps (Pichler et al. 2012). This may reflect improvements in the prevention and treatment of sepsis. In particular, the development of a service for CVC insertion using a percutaneous technique (provided by the interventional radiology department in this centre) may have led to a lower threshold for removing central lines when there is suspicion of infection, as replacement of CVCs is seen as both easier to arrange and less likely to result in venous occlusion (Wragg et al. 2014). Alternatively, this may reflect the difference between using a definition of IFALD which requires a persistent elevation of alkaline phosphatase (ALP), with or without elevated bilirubin, compared to a definition based purely on a single plasma bilirubin level - as used in the study by Beath et al.

(Beath et al. 1996), which may be transiently raised during an episode of sepsis. In contrast to the wider mixed medical/surgical cohort of children with IFALD (Pichler et al. 2012), I found no association of IFALD with prematurity. This difference is explained by the high number of term non-surgical children in the mixed cohort who started PN older than one year of age and are less likely to develop IFALD.

As with the previous chapters, this study is limited by its retrospective nature and heterogeneous patient group. Again septicaemia was defined as positive blood culture, without correlation to clinical status at the time of culture, which may therefore include some contaminants.

Surgical techniques for autologous intestinal reconstruction by intestinal tailoring and lengthening have been used in children with intestinal failure with a short length of dilated bowel (Sommovilla and Warner 2014). In this series, intestinal lengthening procedures have been used in only two patients, precluding any conclusions as to the merits of this intervention. The role of these procedures in management of intestinal failure in infants should be evaluated in prospective studies and trials, as well as using international registry data. There have been reports of improved enteral nutrition and growth following the use of intestinal lengthening surgery (Ching et al. 2009; Jones et al. 2013; Modi et al. 2006), but it is difficult to interpret these in the absence of comparative studies and clear selection criteria. These techniques are used relatively infrequently and different surgical centres vary in the extent to which they have used them (Jones et al. 2013). An international registry was set up to record data from various centres on patients undergoing the STEP procedure. A report from this database on 111 patients found that 47% achieved enteral autonomy, 11% died and 5% progressed to intestinal

transplantation (Jones et al. 2013). The current series shows that even in patients with intestinal failure and liver disease, relatively good results can be obtained with very limited use of such techniques.

The finding that female sex was associated with development of IFALD is also in contrast to previous studies, some of which found no association between sex and development of IFALD (Blau et al. 2007; Duro et al. 2011; Robinson and Ehrenkranz 2008), and two of which found an increased incidence of cholestasis among male infants on PN (Albers et al. 2002; Takemoto et al. 2009). However, one of the latter studies was relatively small, included both surgical and non-surgical infants, and included infants with much shorter duration of PN (some as short as one week). Furthermore, the majority of cases had "mild cholestasis", with only one case of "severe cholestasis" (Takemoto et al. 2009). The other study included surgical patients receiving PN for more than one week, and defined PN-associated cholestasis as PN for at least two weeks with an elevated bilirubin (Albers et al. 2002). These authors do not describe the severity of cholestasis (though it may be inferred that many such children would have a milder degree of cholestasis than children with IFALD as defined by the BSPGHAN criteria, which require persistently deranged liver function tests over a six week period) and did not look for any association of sex with more severe cholestasis. The reason for the discrepancy between studies is not known, but may be in part related to the difference in the length of PN; it is noteworthy that in my study, the majority of patients receiving PN for longer than 28 days were male. There is a body of evidence that various steroid sex hormones influence the function and proliferation of cholangiocytes (Mancinelli et al.

2010). The finding of an association between female sex and severe IFALD in surgical infants warrants further investigation.

Although previous papers have considered outcomes from IFALD, it is difficult to make meaningful comparisons because of the differences in definition of IFALD – a consensus staged definition as used in this paper may provide a useful benchmark for future studies rather than widely varying definitions of cholestasis such as those based on a single plasma bilirubin level.

5.6 Conclusions

In this study, I showed that IFALD remains a fairly common, but rarely life-threatening, complication of intestinal failure and long-term parenteral nutrition in surgical infants. IFALD can be reversed in more than half of these children and enteral autonomy can be achieved in more than two thirds, even with very limited use of intestinal lengthening. In contrast to previous work, I found no association with septicaemia, and also no association with prematurity. I found an unexpected association of female sex with development and severity of IFALD, which warrants further investigation.

Chapter 6. A Randomised Controlled Trial to Assess the Effects of Parenteral and Enteral Glutamine Supplementation on Microbial Invasion in Surgical Infants Receiving Parenteral Nutrition

6.1 Summary of Chapter 6

6.1.1 Aims

To determine whether parenteral plus enteral glutamine supplementation influences microbial invasion and immune function in surgical infants requiring parenteral nutrition.

6.1.2 Methods

This was an ethically-approved prospective double-blind randomised controlled trial studying surgical infants receiving parenteral nutrition for at least five days for congenital or acquired intestinal anomalies (July 2009 – March 2012) with a target of 60 infants. Infants were randomised using minimisation to receive either glutamine supplementation (parenteral plus enteral; total 400mg/kg/day) or isonitrogenous control. The primary endpoint was microbial invasion evaluated after five days of supplementation and defined as either: i) positive conventional blood culture; ii) evidence of microbial DNA in blood (PCR); iii) plasma endotoxin level ≥50 pg/mL; or iv) plasma level of lipopolysaccharide-binding protein (LBP) ≥50 ng/mL. Data are given as median (range) and compared by binary logistic regression. Secondary outcomes included comparison of monocyte HLA-DR expression.

6.1.3 Results

Sixty infants were randomised and reached the primary endpoint. Age at enrolment was 6 days (0-95), gestational age 37 weeks (24-49), and weight 2.3 kg (0.6–4.6). The underlying diagnoses were: 25 patients had congenital/neonatal intestinal obstruction, 19 had anterior abdominal wall defects, 13 had necrotising enterocolitis, and 3 had other

causes of intestinal dysfunction. Thirty six infants showed some evidence of microbial invasion during the study: 17 of these were not detected by conventional blood culture. There was no significant difference between the two groups in the primary outcome: evidence of microbial invasion after five days was found in 9/31 in the control group and 8/29 in the glutamine group: odds ratio 0.83 (0.24 – 2.86; p = 0.77). Monocyte HLA-DR expression was significantly higher among the infants receiving glutamine supplementation, both after five days and at the end of the study. Exploratory analysis showed that in the subgroup of infants with monocyte HLA-DR expression <60% at enrolment, glutamine supplementation reduced the rate of microbial invasion from 7 episodes per 100 days (0 – 33) in the control group to 0 episodes per 100 days (0 – 17) in the glutamine group; p = 0.04 (Mann-Whitney test).

6.1.4 Conclusions

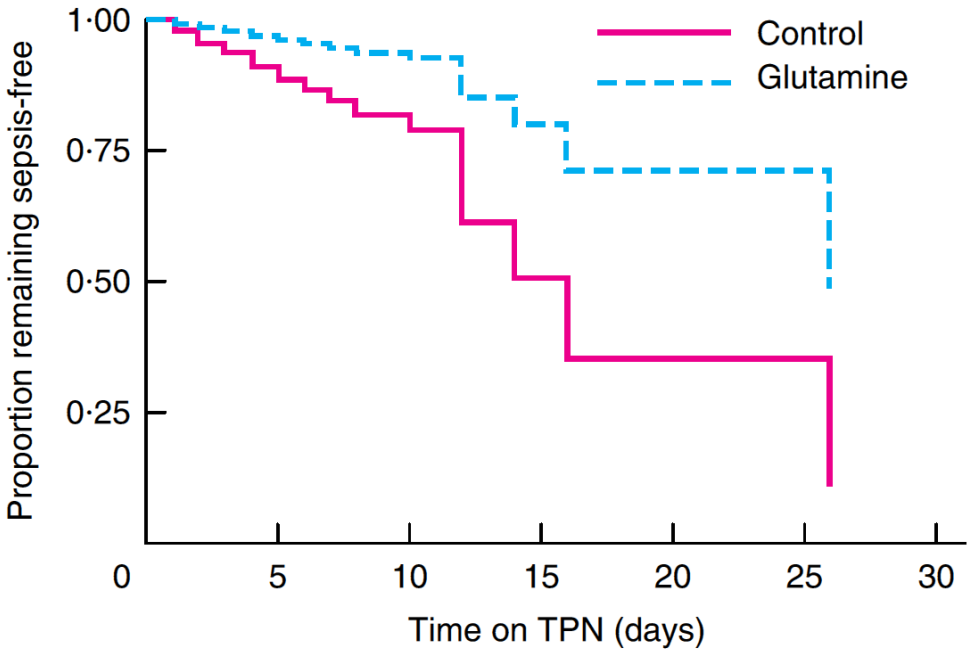
More than half of surgical infants requiring parenteral nutrition showed evidence of microbial invasion. Approximately half of this was not detectable by conventional blood cultures. Parenteral plus enteral glutamine supplementation had no effect on the incidence of microbial invasion in surgical infants. However, glutamine supplementation did assist recovery of post-operative immunoparesis, and glutamine did prevent microbial invasion in those with the most severe immunoparesis, suggesting that a targeted population may benefit from supplementation.

6.2 Introduction

Infants requiring gastrointestinal surgery for conditions including gastroschisis, congenital intestinal obstruction and necrotising enterocolitis are unable to absorb enteral nutrition for a variable period postoperatively and thus depend on parenteral nutrition (PN). However, during PN, these infants are at high risk of infection with bloodstream infections found in 27% of surgical infants receiving PN (Ong et al. 2012), and 61% of surgical infants receiving PN for 28 days or more (Bishay et al. 2012a). These infections may arise from the central venous catheter (Bishay et al. 2011b), or by bacterial translocation i.e. migration of micro-organisms from the intestinal lumen across the gut wall (Pierro et al. 1996). The high rate of these infections may be related to various factors, such as a deficiency of nutritional components which are found in enteral feeds but not supplied in parenteral nutrition, leading to worse immune function or loss of gut mucosal integrity (Rossi et al. 1993).

The amino acid glutamine is not routinely provided in PN, but as it may become conditionally essential, various studies have investigated whether glutamine supplementation is of benefit to patients receiving PN, as discussed in section 1.7.1. Our group has previously shown, in a multicentre randomised controlled trial of intravenous glutamine supplementation in surgical infants (Ong et al. 2012), that the incidence of sepsis was significantly lower in the glutamine group during the period of *total* parenteral nutrition (i.e. before the introduction of enteral feeds) – see Figure 6.1.

Figure 6.1 Parenteral glutamine supplementation and risk of sepsis during total parenteral nutrition



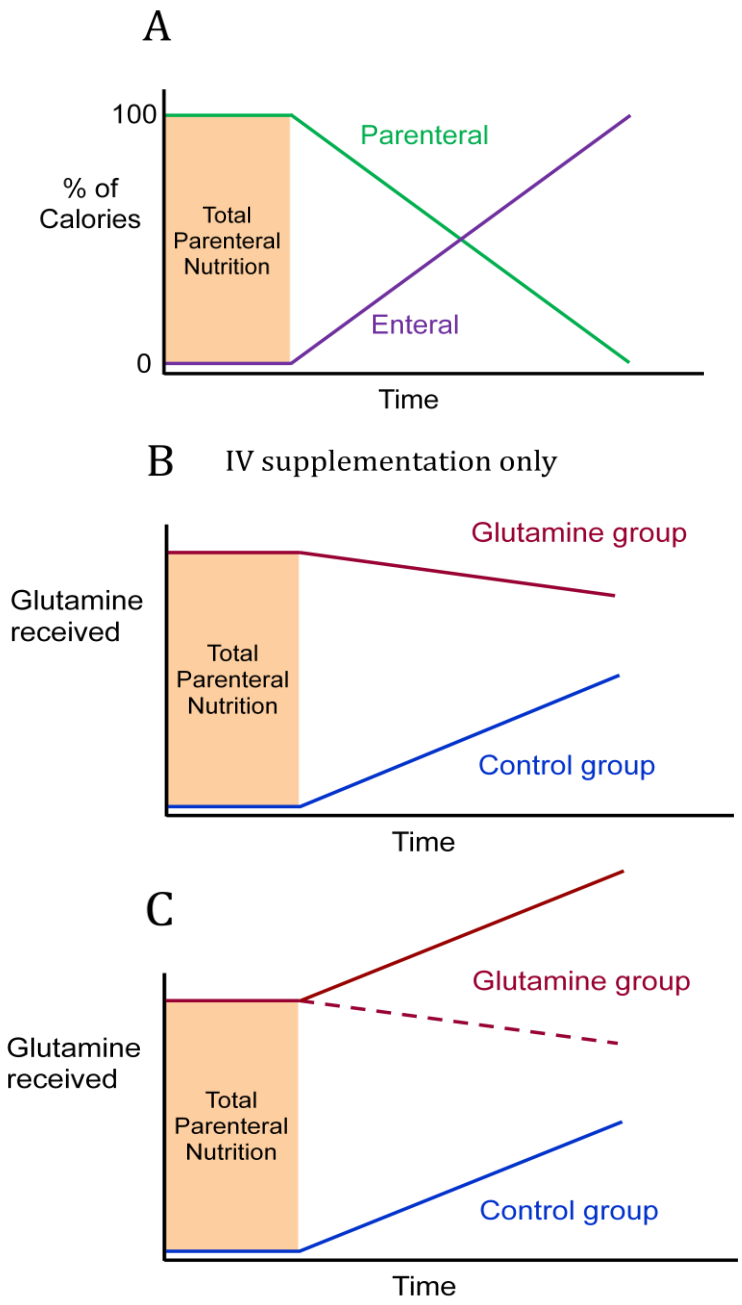
No. at risk						
Control	69	26	9	2	1	
Glutamine	73	36	13	4	3	1

Data from a multicentre randomised controlled trial showed that parenteral glutamine supplementation in surgical infants was associated with a significant decrease in the risk of sepsis during the period of total parenteral nutrition. Hazard ratio 0.33 (95 per cent confidence interval 0.15 to 0.72; p = 0.005), analysed by Cox regression. (Ong et al. 2012).

In that trial, glutamine was only supplemented intravenously, so during the period of mixed enteral/parenteral feeding, the difference in glutamine intake between the two groups was decreasing as enteral feed increased (all enteral feeds contain significant amounts of glutamine).

The aim of this randomised controlled trial was to determine whether glutamine supplementation of both parenteral and enteral nutrition in surgical newborn infants leads to a reduction in microbial invasion. In this trial, I aimed to maintain the difference in glutamine intake between the two groups during the entire study including the period of combined parenteral and enteral feeding (see Figure 6.2). Evidence of microbial invasion was sought using molecular techniques as well as conventional blood cultures.

Figure 6.2. Rationale for study of enteral and parenteral glutamine supplementation



A Schematic representation of transition from total parenteral nutrition to full enteral feeds. B In the previous study with only intravenous glutamine supplementation, the difference in glutamine received between the two groups diminished during the period of transition to enteral feeds. C In the current study (solid line), I aimed to maintain the difference in glutamine received between the two groups during the period of mixed parenteral and enteral feeding by glutamine supplementation of enteral and parenteral nutrition. Dashed line represents previous study, as shown in B.

6.3 Methods

6.3.3 Study Design

The trial was a single centre double blind randomised controlled trial comparing surgical infants receiving glutamine supplementation (study group n=30) with a group receiving isonitrogenous solution (placebo n=30). The supplementation was performed during both total parenteral nutrition and partial parenteral/enteral nutrition at a single UK paediatric surgical centre (Great Ormond Street Hospital) and an associated neonatal unit (University College London Hospital). The full trial protocol is supplied in Appendix 1.

6.3.3.1 Power Calculation

The target recruitment was 60 infants, based on the data from the previous study regarding decreased risk of sepsis during total parenteral nutrition. In our previous trial, 50% of infants had at least one episode of clinical sepsis, and during total PN, glutamine decreased the risk of sepsis by 67% - see Figure 6.1 above; hazard ratio 0.33 (95 per cent confidence interval 0.15 to 0.72; p = 0.005), analysed by Cox regression. Assuming that 50% of surgical infants have evidence of microbial invasion at 5 days, using the primary endpoint described above, and that glutamine decreases this risk by 68%, 30 infants in each arm would be required to detect this difference at 80% power, alpha = 0.05.

6.3.3.2 Inclusion and exclusion criteria

Infants aged less than 3 months (corrected gestational age), admitted under the care of a paediatric surgeon for congenital or acquired gastrointestinal anomalies requiring abdominal surgery and parenteral nutrition, were eligible for the trial. Parenteral nutrition was indicated in all surgical infants who were not expected to tolerate enteral feeds for at

least five days. These would include infants with gastroschisis, meconium ileus, necrotising enterocolitis, bowel atresia or intestinal surgery for other reasons.

Infants who had already been receiving parenteral nutrition for 5 days or more before enrolment were excluded. Patients enrolled in another trial, or suffering from renal failure, inborn errors of metabolism or immune deficiency, were also excluded.

6.3.3.3 Ethics, Funding and Registration

Written informed consent was obtained from parents or guardians of all participants. The trial was registered with Current Controlled Trials (registration no. ISRCTN54742344; http://www.controlled-trials.com). Research ethics committee approval was obtained (Reference 08/H0713/31) – see Appendix 2. A Data Monitoring and Ethics Committee was convened which was independent of both the trial organisers and those providing therapy. Financial support was generously provided by Sparks, who did not have any input into the trial design, running of the trial, analysis of data or reporting of the study.

6.3.3.4 Randomisation

Infants were randomised to glutamine and control groups using balanced minimisation (Wade et al. 2006) with the following criteria: length of functional small bowel (normal small bowel length – no intestinal resection, remaining small bowel length at least 30 cm, remaining small bowel length less than 30 cm); diagnosis (congenital intestinal obstruction, congenital defect of the abdominal wall, necrotising enterocolitis, other); gestational age (derived from last menstrual period) at the time of enrolment in the study (less than 30 weeks, 30–36 weeks, over 36 weeks); ileocaecal valve in continuity without proximal diversion (yes, no); and weight at the time of enrolment in the study (less than 1

kg, 1–2 kg, over 2 kg). In order to prevent the allocation being entirely predictable based on the minimisation criteria, an element of purely random allocation was included as is usual for minimisation (Wade et al. 2006); the probability of being allocated to minimise the difference between groups was 80% and that of random allocation was 20%. All factors were weighted equally. Randomisation was performed in the pharmacy department by the pharmacists using SIMIN software (after participants were recruited by the trial co-ordinator and information relevant to randomisation was collected and passed to the pharmacist).

6.3.3.5 Treatment in intervention and control groups

In the glutamine group, parenteral glutamine was given as a chemically stable dipeptide solution (Dipeptiven®, Fresenius-Kabi, Runcorn, Cheshire, UK; L-alanyl-L-glutamine 200 mg/mL) in an intial dose of 0.4 g/kg/day glutamine equivalent to 0.6 g/kg/day Dipeptiven® which ensures that the nitrogen intake of the intervention and control infants is equal and that no more than 35% of the total nitrogen intake will be provided by Dipeptiven®. The dose is equal to that used in our group's previous randomised controlled trial which demonstrated that there were no negative effects from administration of glutamine and no abnormal levels of serum ammonia, urea nitrogen and glucose (Ong et al. 2012). This level is also based on published research which has confirmed beneficial effects on enteral mucosa at this dose (Allen et al. 1993). All patients were prescribed not less than 1·5 g per kg per day of amino acid while receiving total parenteral nutrition. No more than 35 per cent of total amino acids were replaced to prevent essential amino acid deficiency (A.S.P.E.N.Board of Directors and The Clinical Guidelines Task Force 2002; Dollery 1999; Koletzko et al. 2005b). The control group

received an isonitrogenous, isocaloric parenteral nutrition solution: Vaminolact® (Fresenius-Kabi, Runcorn, Cheshire, UK; this contains no glutamine).

During the period of partial enteral feeding, in which the parenteral intake of glutamine/placebo was reducing, the enteral feeds were supplemented with the balance of glutamine which was no longer being given parenterally to maintain a total dose dose of 0.4 g/kg/day glutamine. This glutamine was given as Adamin-G® (SHS International Ltd, Liverpool, UK). The control group received isonitrogenous supplementation of enteral feeds using Complete Amino Acid Mix (SHS International Ltd, Liverpool, UK; contains 0.7% glutamine).

6.3.3.6 Blinding

Parenteral nutrition prescriptions and the labels used for the parenteral nutrition bags specified the amount of nitrogen and not its source (whether control or glutamine). The total nitrogen content was specified on the bags to maintain clinical standards but, as the two groups received the same amount of nitrogen, this did not unblind care staff. All parents and staff (nurses, physicians, surgeons, etc.) involved in the care of the patients were blinded to allocation group, except for the parenteral nutrition manufacturing pharmacists and the dieticians preparing the enteral supplementation. The trial coordinator, who was responsible for data collection, was similarly blinded throughout and did not have access to the randomisation lists until after data analysis.

6.3.4 Endpoints

The primary end point was evidence of microbial invasion. Microbial invasion was evaluated after five days of supplementation and defined as either i) positive conventional

blood culture; ii) evidence of microbial DNA in blood by polymerase chain reaction (PCR); iii) plasma endotoxin level ≥50 pg/mL; or iv) plasma level of lipopolysaccharide-binding protein (LBP) ≥50 ng/mL. Blood samples were also taken at the beginning of the trial, at the introduction of first enteral feeding and when full enteral feeding is achieved (parenteral feeding was stopped when more than 75% of the patient's full nutritional requirement was tolerated enterally for a minimum of 24 hours). Samples were also taken during clinically suspected episodes of sepsis. Sepsis was defined as the clinical state of generalised inflammation manifested by at least 3 of the following clinical signs: fever, hyperthermia (>38°C) and/or hypothermia (<36°C), lethargy, poor perfusion, age-related tachycardia and tachypnoea, and hypotension (Saez-Llorens and McCracken-GH 1993).

Secondary endpoints are given in full in the study protocol (see Appendix 1) and included clinically suspected episodes of sepsis, time to full feeds, monocyte HLA-DR expression (as a marker of immune function), soluble CD14 (as an additional marker of microbial invasion) and growth.

6.3.4.1 Blood Sampling

Where possible, blood samples were taken from central lines when these are accessed for connection of parenteral nutrition and/or clinically indicated blood sampling. Aseptic non-touch technique was used in accordance with hospital policy. Conventional blood cultures are sent to the clinical microbiology laboratory. A further sample of up to 1.5mL was taken and placed in a BD Vacutainer® EDTA tube (Becton Dickinson, Oxford, UK).

6.3.4.2 Blood Sample Processing and Storage

Samples were processed immediately using endotoxin-free tips. 200-400 microlitres of whole blood was stored at 4°C for DNA extraction for PCR in 0.5mL Elkay polypropylene microtubes (Elkay, Birmingham, UK). 300 microlitres of whole blood was placed in BD Falcon polystyrene round-bottomed tubes for flow cytometry and stained immediately (see below). The rest of the sample (500-800 microlitres) was centrifuged at 1500rpm for 15 minutes. The supernatant plasma was pippetted into 0.5mL Elkay polypropylene microtubes and frozen at -80°C for endotoxin, LBP and soluble CD14 assays.

6.3.4.3 Flow Cytometry for Measurement of Monocyte HLA-DR Expression

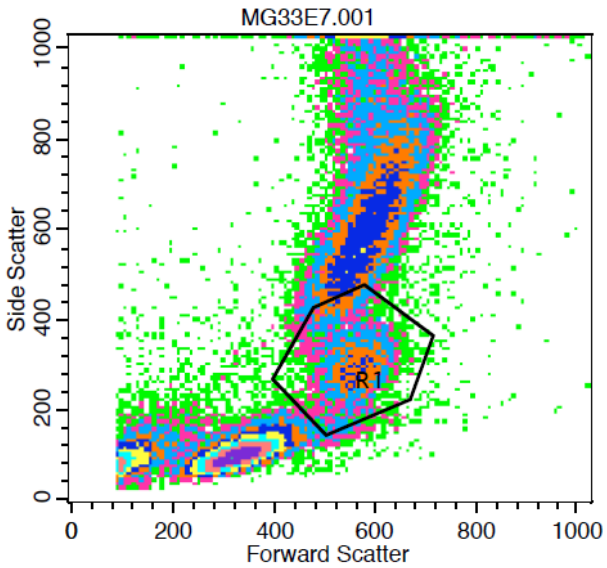
A dual staining technique was used to determine monocyte HLA-DR expression, as described by Allen *et al* (Allen et al. 2002). Monocytes are stained by using an R-phycoerythrin (RPE) conjugated antibody to CD14 (TUK4; DAKO, Ely, UK), and HLA-DR expression is determined with fluorescein isothiocyanate (FITC) conjugated antibody to HLA-DR (CR3/43, DAKO). Fifty microliters of whole blood are incubated with 5µL of each of the antibodies for 10 minutes at room temperature. Red cells are removed by lysis with FACS lysing solution (Becton Dickinson) followed by washing and centrifuging at 1500 rpm with buffer containing 0.1% sodium azide, and the samples are fixed in 1% formaldehyde (CellFIX, Becton Dickinson). The cells are analysed on a FACScan flow cytometer using Cellquest software (Becton Dickinson). Nonspecific staining is determined with appropriate mouse IgG2a monoclonal antibodies labelled with PE and FITC (DAKO). Monocytes were identified by their physical characteristics and positive CD14 staining. The cytometer passes the cells in a stream of fluid through

the beam of a laser. The degree of forward scatter (FSC) detected correlates to the volume of the cells, while the side scatter (SSC) correlates to the granularity. Monocytes can thus be identified as the group of cells with intermediate size and granularity (as opposed to lymphocytes, which are smaller and less granular, or neutrophils, which tend to be more granular; Figure 6.3). The identification of the monocytes can then be confirmed by using the CD14 RPE staining (Figure 6.4).

HLA-DR expression was determined on 5000 gated events (data was acquired until 5000 cells of intermediate size and granularity had been detected) and is expressed as the percentage positive fluorescence (percentage of monocytes staining >99% of that observed with the negative control IgG2a monoclonal antibody) and as median fluorescence intensity (MFI; see Figure 6.4).

Figure 6.3 Example of a flow cytometry plot

A. The monocytes (within the polygonal gate) have intermediate forward scatter (which correlates to size) and side scatter (which correlates to granularity).



B. The identity of the monocyte population is confirmed by gating on the cells with highest expression of CD14.

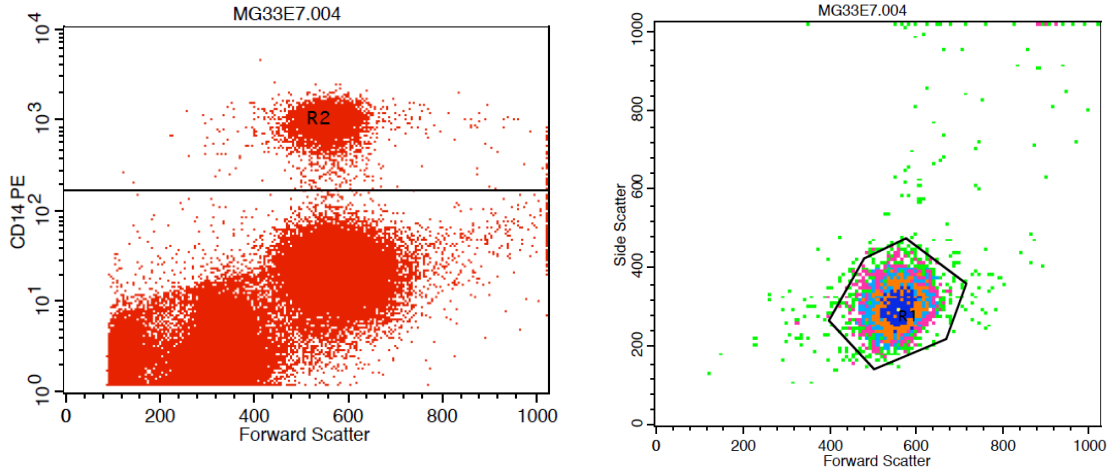
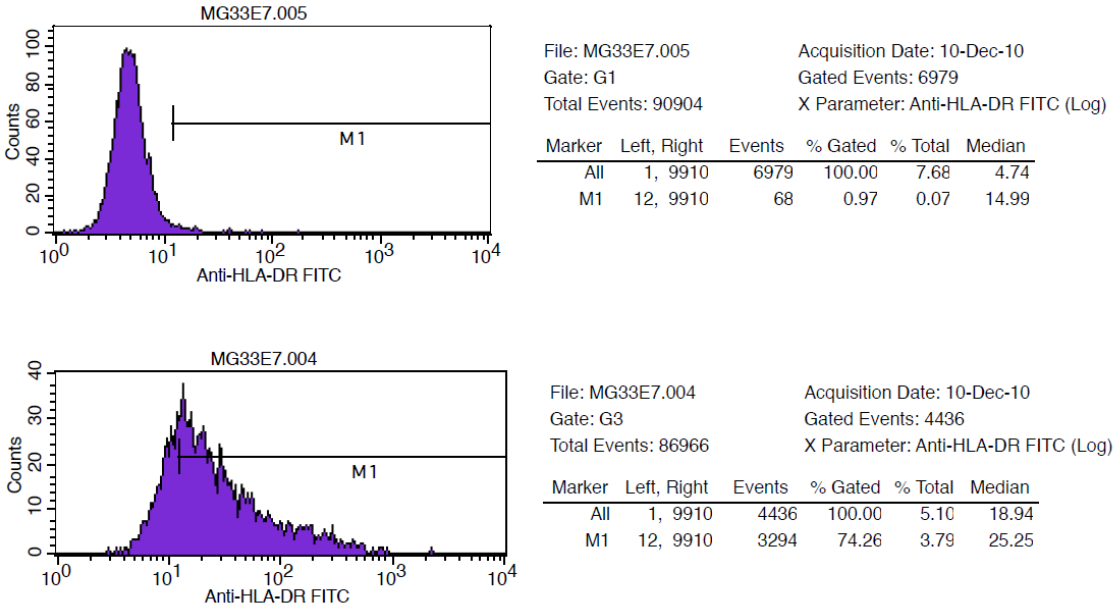


Figure 6.4 Gating to quantify monocyte HLA-DR expression



The upper histogram shows the negative isotype control (labelled with IgG2a FITC). The marker is set so that <1% of the events in the upper histogram are included. The lower histogram shows cells labelled with Anti-CD14 RPE and Anti-HLA-DR FITC within the monocyte gate. In this example 74.26% of monocytes are expressing HLA-DR, and the MFI is 18.94.

6.3.4.4 DNA Extraction and PCR

Methods were those used by the clinical microbiology service, and as described by Harris and Hartley (Harris & Hartley 2003).

6.3.4.4.1 DNA Extraction

DNA was extracted from whole blood, using the QIAmp DNA mini kit (Qiagen), following the manufacturer's protocol. Complete lysis of bacterial cells is ensured by using a Ribolyser cell disrupter (Hybaid) according to the manufacturer's instructions. Briefly, following Proteinase K digestion, Ribolyser resin for bacterial cells is added to the sample and the sample is placed in the Ribolyser and run at maximum speed for 40 s. One negative control (200 µl sterile UV-irradiated water) is included in each extraction run. Epstein Barr virus (EBV) is used as internal positive control (10 µl of Raji cells, infected with ~50 copies EBV per cell).

6.3.4.4.2 16S rDNA broad-range PCR

The PCR reaction mixture is as follows: 5 µl 10X PCR buffer (Molzym), 1·5 mMMgCl2, 0·1 mM dNTPs (Bioline), 1 U Taq polymerase (Molzym), 0·4 µM of each of the following primers: 16SFa: 5′-GCT CAG ATT GAA CGC TGG-3′, 16SFb: 5′-GCT CAG GAT GAA CGC TGG-3′, 16SR: 5′-TAC TGC TGC CTC CCG TA-3′ and sterile UV-irradiated water to give a final volume of 45 µl. Five microlitres of extracted DNA is added to this mixture and the reactions are heated to 94°C for 3 min, followed by 32 cycles of 94°C for 30 s, 63°C for 40 s and 72°C for 90 s. A final extension is carried out at 72°C for 10 min. Each PCR run includes two positive controls (1 µl each of *Escherichia coli* ~100 c.f.u. μl^{-1} and *Staphylococcus aureus* ~100 c.f.u. μl^{-1}). PCR

reactions are electrophoresed through a 2% agarose gel containing 2 µl 500 nM ethidium bromide and bands are visualised by UV transillumination (see Figure 6.6).

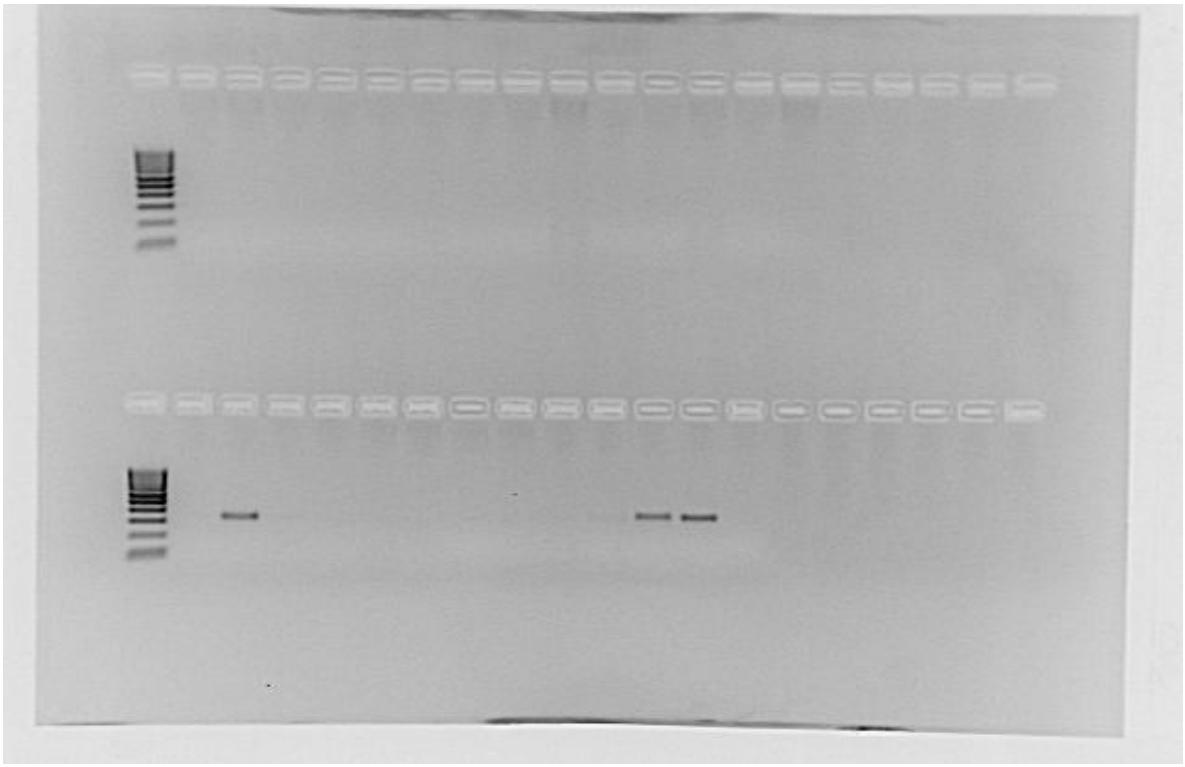
6.3.4.4.3 Staphylococcus aureus (coA) real-time PCR(Sabet et al. 2006)

The PCR reaction mixture is as follows: 14 µl of 1X Quantitec mastermix (Qiagen), 0·7 µl of each of the following primers: Sa-3-F (coA): 5′- GTA GAT TGG GCA ATT ACA TTT TGG AGG -3′, Sa-4-R (coA): 5′-CGC ATC TGC TTT GTT ATC CCA TGT A-3′, Probe coA: 5′(FAM)-CGC TAG GCG CAT TAG CAG TTG CAT C- (TAMRA)3′, 0·3 µl of each of the following primers: EBV-F: 5′-CCG GTG TGT TCG TAT ATG GAG-3′, EBV-R: 5′-GGG AGA CGA CTC AAT GGT GTA-3′, Probe EBV: 5′(JOE)-TGC ACC AGA CCC GGG CTC AGG TAC TCC GA-(TAMRA)3′, and sterile UV-irradiated water to give a final volume of 18 µl. Ten microlitres of extracted DNA is added to this mixture and the reactions are heated to 50°C for 2 min, followed by 94°C for 10 min, followed by 45 cycles of 94°C for 15 s, and 60°C for 60 s. Each PCR run includes a positive control (methicillin-resistant *Staphylococcus aureus* ~1 c.f.u. μl^{-1}). A positive result was any target detected with a cycle-threshold (CT) value <38.

6.3.4.4.4 Staphylococcus aureus (mecA/Sa442) real-time PCR(Sabet et al. 2006)

The PCR reaction mixture is as follows: 14 µl of 1X Quantitec mastermix (Qiagen), 0·3 µl of each of the following primers: Sa442-F: 5′-TGC GTA CAC GAT ATT CTT CAC-3′, Sa442-R: 5′-ACT CTC GTA TGA CCA GCT TC-3′, Probe Sau3A: 5′(JOE)-TAC TGA AAT CTC ATT ACG TTG CAT CGG AAA CA-(TAMRA)3′, 0·7 µl of each of the following primers: Sa-1-F (mecA): 5′-CGG TAA CAT TGA TCG CAA CGT TCA -3′,

Figure 6.5 Agarose gel from 16S rDNA broad-range PCR



Example of agarose gel from 16S rDNA broad-range PCR visualised by ultraviolet irradiation, showing two positive controls (*Escherichia coli* and *Staphylococcus aureus*; lower row, columns 12 and 13) and one band from positive sample (lower row, third column).

Sa-2-R (mecA): 5′-CTT TGG AAC GAT GCC TAA TCT CAT-3′, Probe mecA: 5′(FAM)-TTC CAG GAA TGC AGA AAG ACC AAA GCA- (TAMRA)3′, and sterile UV-irradiated water to give a final volume of 18 µl. Ten microlitres of extracted DNA is added to this mixture and the reactions are heated to 50°C for 2 min, followed by 94°C for 10 min, followed by 45 cycles of 94°C for 15 s, and 60°C for 60 s. Each PCR run includes a positive control (methicillin-resistant *Staphylococcus aureus* ~1 c.f.u. μl^{-1}). A positive result was any target detected with a CT value <38.

6.3.4.4.5 Staphylococcal (tuf) real-time PCR (Tann et al. 2014)

The PCR reaction mixture is as follows: 12 µl of 1X Quantitec mastermix (Qiagen), 0·5 µl of each of the following primers: tuf-F: 5′-CAT TCC AAC TCC AGA ACG TGA YT-3′, tuf-R: 5′- CAC GAC CAG TGA TTG AGA ATA CG-3′, tuf-Probe: 5′(CY5)-TGA YAA ACC ATT CAT GAT GCC AGT TGA GG-(BBQ)3′. Ten microlitres of extracted DNA is added to this mixture and the reactions are heated to 50°C for 2 min, followed by 94°C for 10 min, followed by 45 cycles of 94°C for 15 s, and 60°C for 60 s. Each PCR run includes a positive control (methicillin-resistant *Staphylococcus aureus* ~1 c.f.u. μl^{-1}). A positive result was any target detected with a CT value <38.

6.3.4.4.6 Real time PCR for Enterobacteriaceae

K Harris and co-workers in the microbiology department at Great Ormond Street Hospital have recently developed a novel real time PCR which detects *Enterobacteriaceae* (Tann et al. 2014). It has been shown to detect *Escherichia coli*, *Klebsiella*, and *Enterobacter* at levels much lower than the broader-range 16S rDNA PCR (as low as ~1 c.f.u. per reaction). I used this assay to analyse my samples (prior to its publication), with guidance and permission. The PCR reaction mixture is as follows:

13 µl of 1X QuantiTect mastermix (Qiagen), and 1.0 µl of each of the following primers: Ent-dnaK-F: 5' ACC TGG GTA CWA CCA ACT CTT GTG T-3′, Ent-dnaK-R: 5' GTC ACT GCC TGA CGT TTA GC-3′, Ent-dnaK-probe: 5' (FAM)-AGG ATG GTG AAA CTC TGG TWG GTC AGC C-(BHQ-1)-3′, to give a final volume of 16 µl. Ten microlitres of extracted DNA is added to this mixture and the reactions are heated to 50°C for 2 min, followed by 94°C for 10 min, followed by 45 cycles of 94°C for 15 s, and 60°C for 60 s. Each PCR run includes a positive control (*Escherichia coli* ~1 c.f.u. μl^{-1}). A positive result was any target detected with a CT value <38.

6.3.4.4.7 Sequencing

Enterobacteriaceae and 16S rDNA PCR positives were further identified by amplicon sequencing using Big-Dye 3.1 Cycle-sequencing kit (Life Technologies) and analyzed on a 3130 Genetic Analyse (Life Technologies). Sequences were compared to in-house databases of sequences using MegAlign (Lasergene 10, DNAStar, Madison, WI, USA) or by BLAST searching against the Genbank database (http://www.ncbi.nlm.nih.gov).

6.3.4.5 Blood cultures

Blood cultures were analysed by the clinical microbiology service, and were read automatically using the BioMérieux BactAlert system (BioMérieux, Marcy L'Étoile, France).

6.3.4.6 Endotoxin assays

Plasma samples were diluted to 10% using endotoxin-free water then heated to 80°C for 15 minutes to inactivate plasma proteins. A series of experiments were performed to determine this optimum dilution and heat inactivation. Plasma endotoxin levels were then

measured using a commercially available kinetic chromogenic *Limulus* amebocyte assay, according to the manufacturer's protocol (Lonza, Walkersville, MD). Samples were run in duplicate and the background subtracted.

6.3.4.7 Lipopolysaccharide binding protein assays

LBP was measured using a commercially available enzyme-linked immunosorbent assay according to the manufacturer's protocol (Cell Sciences, Canton, MA) using plasma serially diluted to 1:1000. Plasma level of LBP ≥50 μg/mL was taken as positive evidence of microbial invasion. This was a modification of the original trial protocol, which was agreed by the Trial Steering Committee, while still blinded as to allocation. The original protocol was to take plasma level of LBP ≥13 μg/mL as positive, based on the study by Ubenauf et al (Ubenauf et al. 2007). However, when I analysed my samples, I found that this cut-off would have been around the 25th centile. I discussed this with the Trial Steering Committee, and the decision was made to modify the cut-off value to 50 μg/mL. This was around the 88th centile, and is in keeping with cut-off values determined by receiver operating characteristic (ROC) curve analysis in various studies (Kitanovski et al. 2014; Oude Nijhuis et al. 2003; Pavcnik-Arnol et al. 2007; Tsalkidou et al. 2013).

6.3.4.8 Soluble CD14 assays

Soluble CD14 was measured using a commercially available enzyme-linked immunosorbent assay according to the manufacturer's protocol (R&D Systems, Minneapolis, MN) using plasma diluted to 0.5%.

6.3.5 Statistical Analysis

Data are given as median (range) and groups are compared using Fisher's exact test for categorical variables, unpaired *t* test (with Welch correction where appropriate) for normally distributed continuous variables, and Mann-Whitney test for non-normally distributed continuous variables. Binary logistic regression was performed to analyse the primary outcome, adjusting for the minimisation criteria (SPSS v. 21). Growth Z-scores were analysed by multi-level modelling using MLWin v2.11, taking into account repeated measures on the same patients, and examining the interaction between glutamine administration and time, with diagnosis as a covariate.

6.4 Results

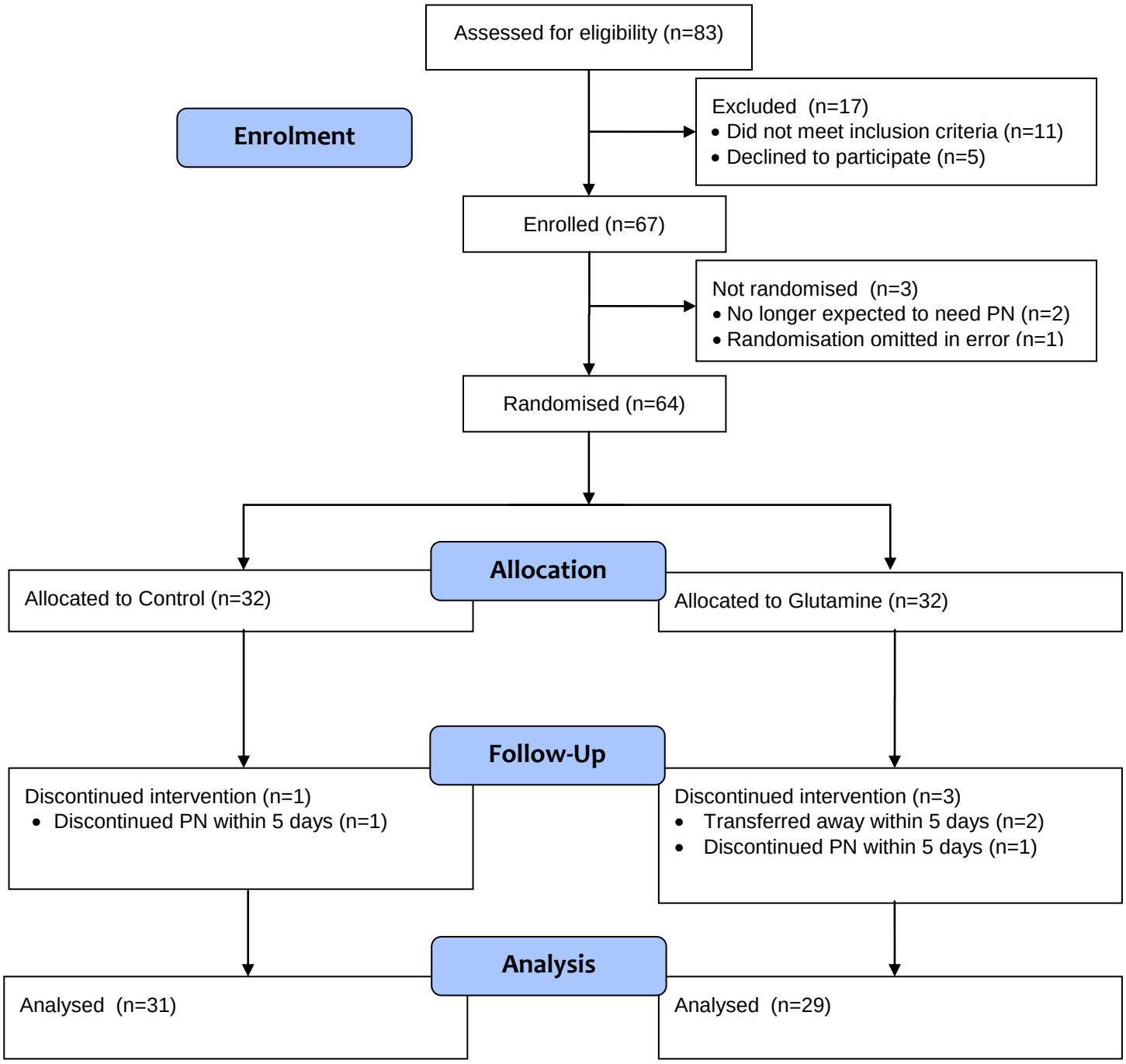
6.4.1 Early Challenges

Ethical amendments were obtained to alter the original protocol in order to correct and clarify various technical details of the study, and also to extend the study to allow continued supplementation and collection of follow-up samples and data at University College London Hospitals NHS Trust if patients are transferred there before enteral feeding is established. These amendments were approved and recruitment began on 22nd July 2009.

6.4.2 Patients

Between July 2009 and December 2011, 83 infants were assessed for eligibility (see Figure 6.6 for CONSORT flow chart). The last blood samples were taken from participants in March 2012. 67 infants were enrolled in the study. Two were withdrawn prior to randomisation as they were no longer expected to require parenteral nutrition. One was not randomised due to an oversight in the pharmacy department. Four infants were randomised but did not receive five days of trial supplementation (and therefore the primary endpoint could not be assessed as they ended their involvement in the study prior to the fifth day and did not have the relevant blood samples taken) due to either achieving full feeds in less than five days (n=2) or being transferred to a non-participating centre (n=2). The protocol did not make clear whether additional infants should be recruited if infants did not reach the primary endpoint (which was assessed after five days of participation in the study); clarification on this matter was therefore sought from the

Figure 6.6 Trial CONSORT flow chart



DMEC, who advised that additional infants should be recruited, and ratified by the trial steering committee (I was still blinded at this point and no patients had had the primary endpoint fully assessed as some of the analyses were performed using frozen plasma after the trial was completed). Additional patients were therefore enrolled to achieve the target number of 60 infants for whon the primary endpoint could be assessed. The full target number of sixty infants were enrolled, randomised, reached the primary endpoint and were analysed.

Age at enrolment was 6 days (0-95), gestational age 37 weeks (24-49), and weight 2.3 kg (0.6–4.6) – see Table 6.1. The underlying diagnoses were: 25 patients had congenital/neonatal intestinal obstruction, 19 had anterior abdominal wall defects, 13 had necrotising enterocolitis, and 3 had other causes of intestinal dysfunction.

Baseline characteristics were similar between the two groups (Table 6.1), except that the glutamine group was found to have slightly lower baseline levels of LBP.

6.4.3 Adverse Events

There were no serious adverse events related to the study, and no incidents where unblinding of either the clinical team or the investigators was felt to be necessary.

Three children died during the study. One was an infant (in the glutamine group) with complex congenital heart disease, which was eventually deemed not amenable to surgical correction, and a decision was made to withdraw care electively thirteen days after laparotomy for duodenal atresia. The second was a premature infant (in the control group), born at 26 weeks of gestation, who died of pneumonia on a background of

Table 6.1 Baseline characteristics, markers of microbial invasion and monocyte function

		Control group n=31	Glutamine group n=29	Difference glutamine vs. control (95% CI)	P-value
Males		18 (58%)	17 (59%)	0.006 (-0.24, 0.26)	1.0
Gestational age (weeks) at enrolment		36 (24 – 49)	37 (27 – 46)	1.7 (-0.87, 4.3)	0.23
Gestational age (weeks) at birth		35 (22 – 40)	35 (25 – 42)	1.2 (-1.6, 4.0)	0.51
Postnatal age (days)		6 (1 – 77)	6 (2 – 79)	2.7 (-8.1, 13.5)	0.31
Weight at enrolment (g)		2160 (620 – 3670)	2360 (660 – 4620)	173 (-350, 696)	0.43
Diagnosis	Congenital bowel obstruction	13 (42%)	12 (41%)	0.006 (-0.24, 0.26)	1.0
	Abdominal wall defect	10 (32%)	9 (31%)	0.01 (-0.22, 0.25)	1.0
	NEC	6 (19%)	7 (24%)	0.05 (-0.16, 0.26)	0.76
	Other	2 (6%)	1 (3%)	0.03*	1.0
Patients with intact ileo-caecal valve		31 (100%)	27 (93%)	0.07*	0.23
Patients with ileo-caecal valve in use		19 (61%)	19 (66%)	0.04 (-0.2, 0.29)	0.79
Patients with stoma		14 (45%)	11 (38%)	0.07 (-0.18, 0.32)	0.61
Length of small bowel after resection	no resection	26 (84%)	23 (79%)	0.05*	0.74
	≥30cm remaining	5 (16%)	5 (17%)	0.01*	1.0
	<30cm remaining	0 (0%)	1 (3%)	0.03*	0.48
Bowel perforation prior to enrolment		12 (39%)	9 (31%)	0.08 (-0.16, 0.32)	0.6
Microbial invasion		6 (19%)	5 (17%)	0.02*	1.0
Clinical sepsis		2 (6%)	0 (0%)	0.06*	0.49
Positive blood culture		0 (0%)	0 (0%)		
Positive PCR		2 (6%)	2 (7%)	0*	1.0
Plasma Endotoxin (EU/mL)		0.2 (0 - 0.9)	0.19 (0.01 - 0.3)	-0.03 (-0.09, 0.03)	0.49
Plasma LBP (μg/mL)		31 (0 – 60)	22 (0 – 60)	-8 (-16, 0)	0.04
Plasma sCD14 (pg/mL)		1287 (814 - 2139)	1067 (553 - 2072)	-119 (-306, 69)	0.25
% monocytes positive for HLA-DR		55 (14 - 91)	59 (11 - 96)	3.9 (-9.4, 17.1)	0.59
Monocyte HLA-DR (MFI)		10 (4 - 71)	14 (5 - 43)	0.7 (-5.1, 6.5)	0.22

Values given as median (range) or n (%). Continuous data were compared using Mann-Whitney test and dichotomous data using Fisher's exact test. EU: endotoxin units; LBP: lipopolysaccharide binding protein; sCD14: soluble CD14; MFI: median fluorescence intensity. *95% CIs cannot be calculated as some cells 5 or less.

chronic lung disease, 23 days after laparotomy for necrotising enterocolitis. The third was another premature infant (in the control group) born at 25 weeks gestation, who also died of pneumonia on a background of chronic lung disease, 32 days after laparotomy for ileal perforation.

6.4.4 Primary Outcome

There was no significant difference between the two groups in the primary outcome: evidence of microbial invasion after five days was found in 9/31 in the control group and 8/29 in the glutamine group. This was not significant using Fisher's Exact test [relative risk 1.05 (0.47 – 2.36; p = 1.0)], or by binary logistic regression analysis adjusting for the minimisation criteria (p = 0.77, odds ratio 0.83 (95% CI 0.24-2.86). The individual components of the primary outcome are also shown in Table 6.2.

Table 6.2 Primary outcome: evidence for microbial invasion on day 5

	Control group n=31	Glutamine group n=29	Difference glutamine vs. control (95% CI)	P-value
Infants with microbial invasion	9 (29%)	8 (28%)	0.01 (-0.21, 0.24)	1.0
Positive blood culture only	3 (10%)	1 (3%)		
Positive PCR only	3 (10%)	1 (3%)		
Positive blood culture and positive PCR	1 (3%)	1 (3%)		
LBP ≥50 µg/mL	1 (3%)	5 (17%)		
Endotoxin ≥0.5 EU/mL	1 (3%)	0 (0%)		

Values given as n (%). Data compared using Fisher's exact test. EU: endotoxin units; LBP: lipopolysaccharide binding protein;

Thirty six infants showed evidence of microbial invasion at any time during the study: 17 of these were not detected by conventional blood culture (Figure 6.7). There was no difference in the incidence rate of microbial invasion between the two groups, with median values of 4.2 episodes of microbial invasion/100 days in the control group and 2.9 episodes of microbial invasion/100 days in the glutamine group (p = 0.64, Mann-Whitney – see Table 6.3).

6.4.5 Planned Secondary Outcome Analysis

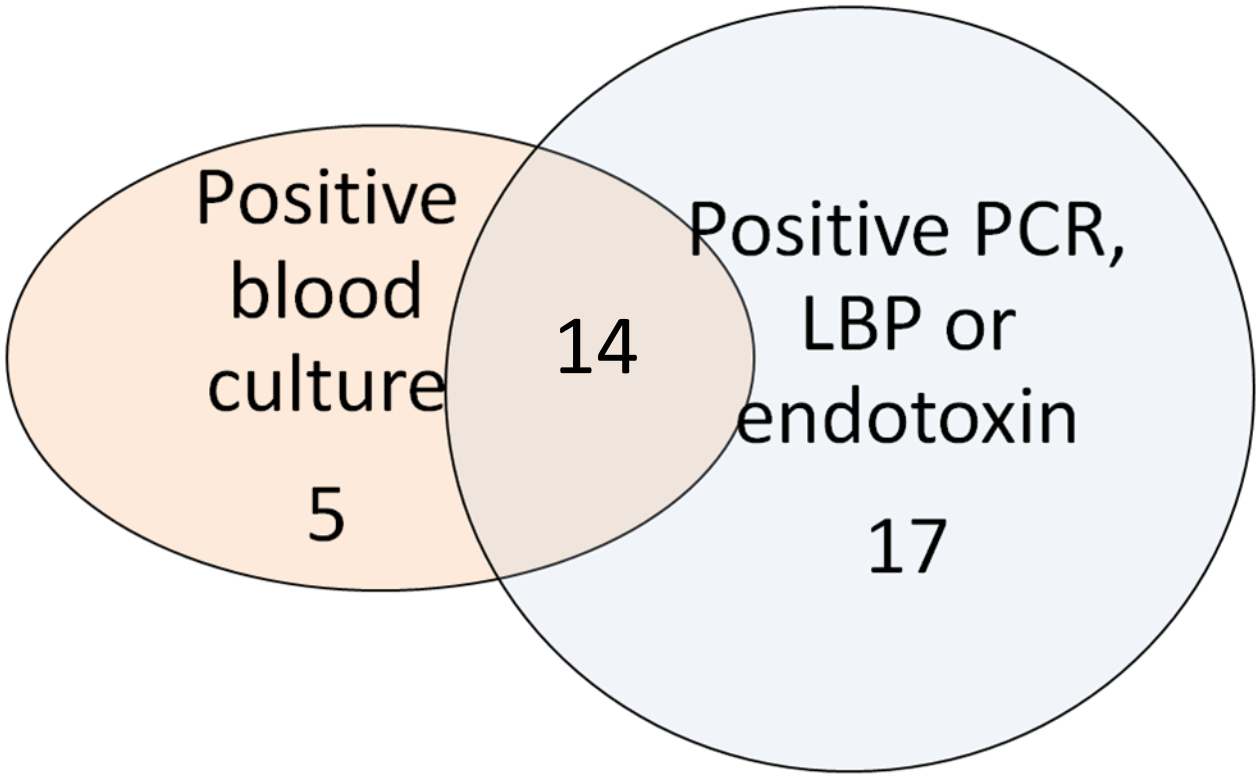
6.4.5.1 Sepsis

There were 66 clinical episodes of sepsis in 31 patients, of which 26 (in 20 patients) showed evidence of microbial invasion, including 15 episodes (in 13 patients) with positive blood cultures. There was no difference between the two randomised groups in the proportion of infants who developed sepsis, or in the incidence of sepsis, with mean values of 6.0 episodes of sepsis/100 days in the control group and 4.2 episodes of sepsis/100 days in the glutamine group (p = 0.49, Mann-Whitney).

6.4.5.2 Intestinal Function

The time to first enteral feeding and time to full enteral feeding were not significantly different between the two groups (Table 6.4). There was also no significant difference between the two groups in the proportions of infants who developed intestinal failure or IFALD.

Figure 6.7 Microbial invasion during the study



Thirty six infants showed evidence of microbial invasion at any time during the study; 17 of these did not have any growth of micro-organisms from conventional blood culture.

Table 6.3 Secondary outcomes (microbial invasion and sepsis)

	Control group n=31	Glutamine group n=29	Difference glutamine vs. control (95% CI)	P-value
Infants with microbial invasion at any time	20 (65%)	16 (55%)	0.09 (-0.15, 0.34)	0.60
Episodes of microbial invasion/100 days	4.2 (0 – 33.3)	2.9 (0 – 22.2)	-1.2 (-5.1, 2.7)	0.64
Infants with clinical sepsis on day 5	3 (10%)	2 (7%)	0.03*	1.0
Infants with clinical sepsis at any time	18 (58%)	14 (48%)	0.10 (-0.15, 0.35)	0.6
Episodes of sepsis/100 days	4.3 (0 – 33.3)	0 (0 – 18.2)	-1.8 (-5.2, 1.6)	0.49
Plasma endotoxin on day 5 (EU/mL)	0.24 (0.01 – 0.67)	0.23 (0.09 – 0.32)	-0.03 (-0.08, 0.02)	0.44
Plasma endotoxin at end of study (EU/mL)	0.22 (0.02 – 4.8)	0.21 (0.13 – 0.48)	-0.19 (-0.52, 0.13)	0.16
LBP on day 5 (µg/mL)	23 (5 – 60)	29 (0 – 60)	4 (-4, 12)	0.38
LBP at end of study (µg/mL)	16 (2 – 48)	19 (2 – 60)	3 (-4, 10)	0.56
Infants with positive PCR	8 (26%)	9 (31%)	0.05 (-0.18, 0.28)	0.78
Infants with positive blood culture	11 (35%)	8 (28%)	0.08 (-0.16, 0.31)	0.59
sCD14 on day 5	1206 (640 – 1989)	1385 (625 – 3061)	232 (-9, 473)	0.10
sCD14 at end of study	1209 (590 – 2188)	1196 (456 – 2050)	12 (-196, 219)	0.97
% monocyte HLA-DR on day 5	60 (18 – 95)	72 (48 – 91)	14 (4, 23)	0.01
% monocyte HLA-DR at end	70 (18 – 100)	81 (45 – 98)	13 (3, 24)	0.04
Monocyte HLA-DR (MFI) day 5	14 (4 - 44)	16 (7 – 56)	5 (-1, 12)	0.11
Monocyte HLA-DR (MFI) at end	15 (5 - 215)	31 (10 – 78)	3 (-15, 21)	0.01

Values given as median (range) or number (%). Continuous data were compared using Mann- Whitney test and dichotomous data using Fisher's exact test. EU: endotoxin units; LBP: lipopolysaccharide binding protein; sCD14: soluble CD14; MFI: median fluorescence intensity.

Table 6.4 Secondary outcomes (other clinical outcomes)

	Control group n=31	Glutamine group n=29	Difference glutamine vs. control (95% CI)	P-value
Time to start enteral feeds (days)	6 (0 – 25)	5 (0 – 26)	0.2 (-3.0, 3.3)	0.85
Time to full enteral feeds (days)	12 (7 – 159)	13 (5 – 88)	-5 (-21, 11)	0.85
Time in study	12 (6 – 159)	11 (5 – 88)	-6 (-19, 7)	0.60
Infants with intestinal failure	11 (35%)	9 (31%)	0.04 (-0.19, 0.28)	0.79
Infants with IFALD	6 (19%)	6 (21%)	0.01 (-0.19, 0.22)	1.0
Reached full enteral feeds	25 (81%)	22 (76%)	0.05 (-0.16, 0.26)	0.76
Transferred on mixed parenteral-enteral feed	3 (10%)	5 (17%)	0.08	0.47
Transferred on total parenteral nutrition	1 (3%)	1 (3%)	0.002	1.0
Died	2 (6%)	1 (3%)	0.03	1.0

Values given as median (range) or number (%). Continuous data were compared using Mann-Whitney test and dichotomous data using Fisher's exact test.

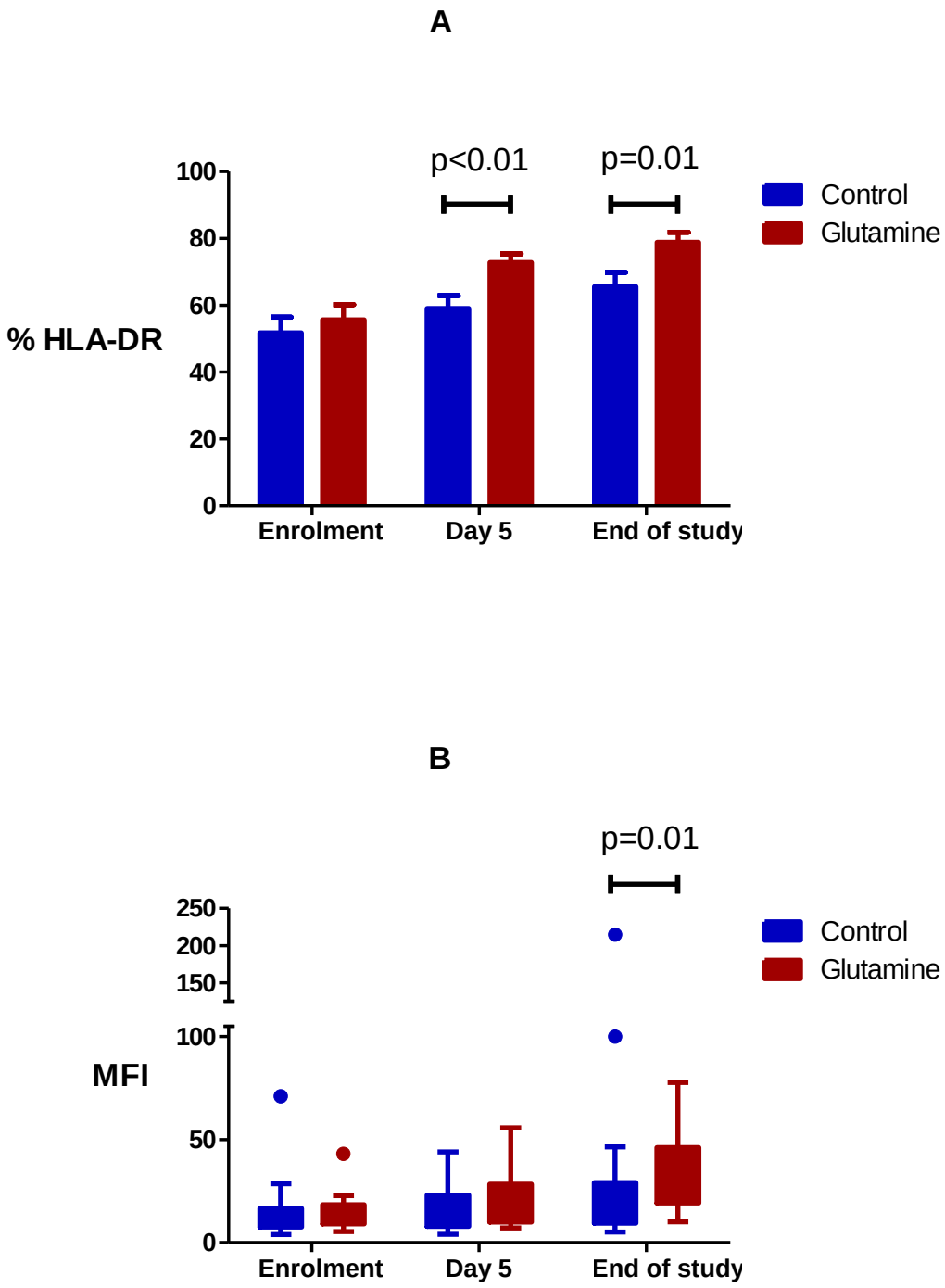
6.4.5.3 Monocyte HLA-DR expression

While there was no difference between the two groups at enrolment, monocyte HLA-DR expression was significantly higher among the infants receiving glutamine supplementation, both after five days and at the end of the study (Table 6.1 and Table 6.3, Figure 6.8).

6.4.5.4 Soluble CD14

Plasma levels of soluble CD14 were not found to be significantly different between the two groups, though there was a trend to higher levels of soluble CD14 in the glutamine group after five days of supplementation.

Figure 6.8 Monocyte HLA-DR expression in control and glutamine groups



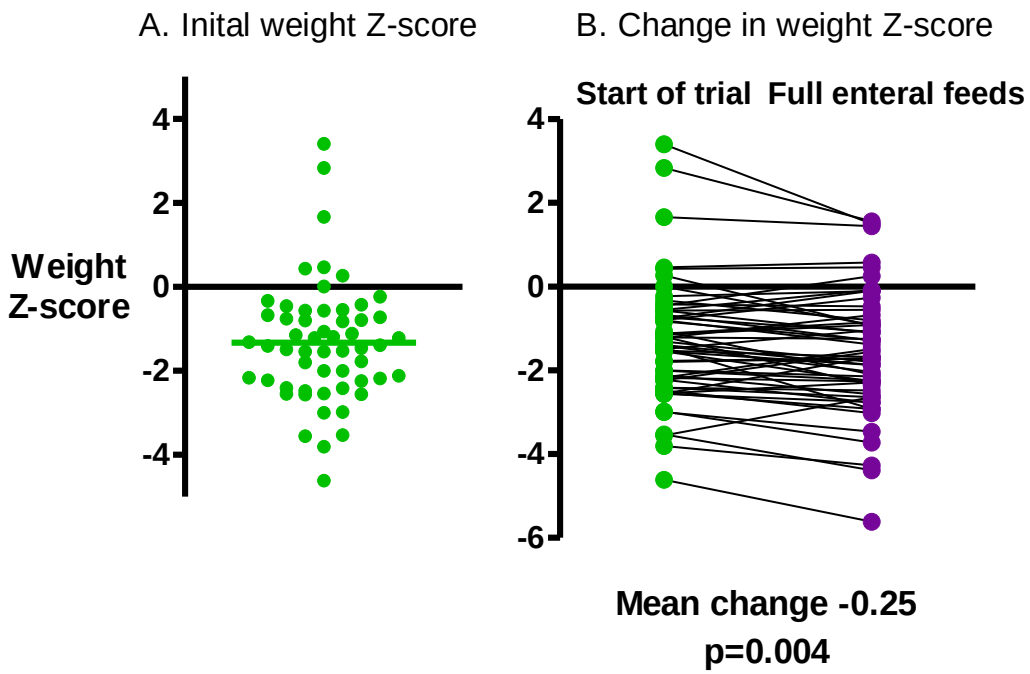
A. % Monocyte HLA-DR expression, plotted as mean ± SEM and compared using unpaired *t* test (with Welch correction). B. Median fluorescence intensity (MFI), plotted as median, range and interquartile range, and compared using Mann-Whitney test.

6.4.5.5 Growth

The mean weight Z-score at the time of enrolment was -1.34 ± 0.19 (Figure 6.9). From start of trial to reaching full enteral feeds, there was a mean decrease in weight Z-score of 0.25 (95% confidence interval 0.08 to 0.41), p = 0.004 (unpaired *t* test).

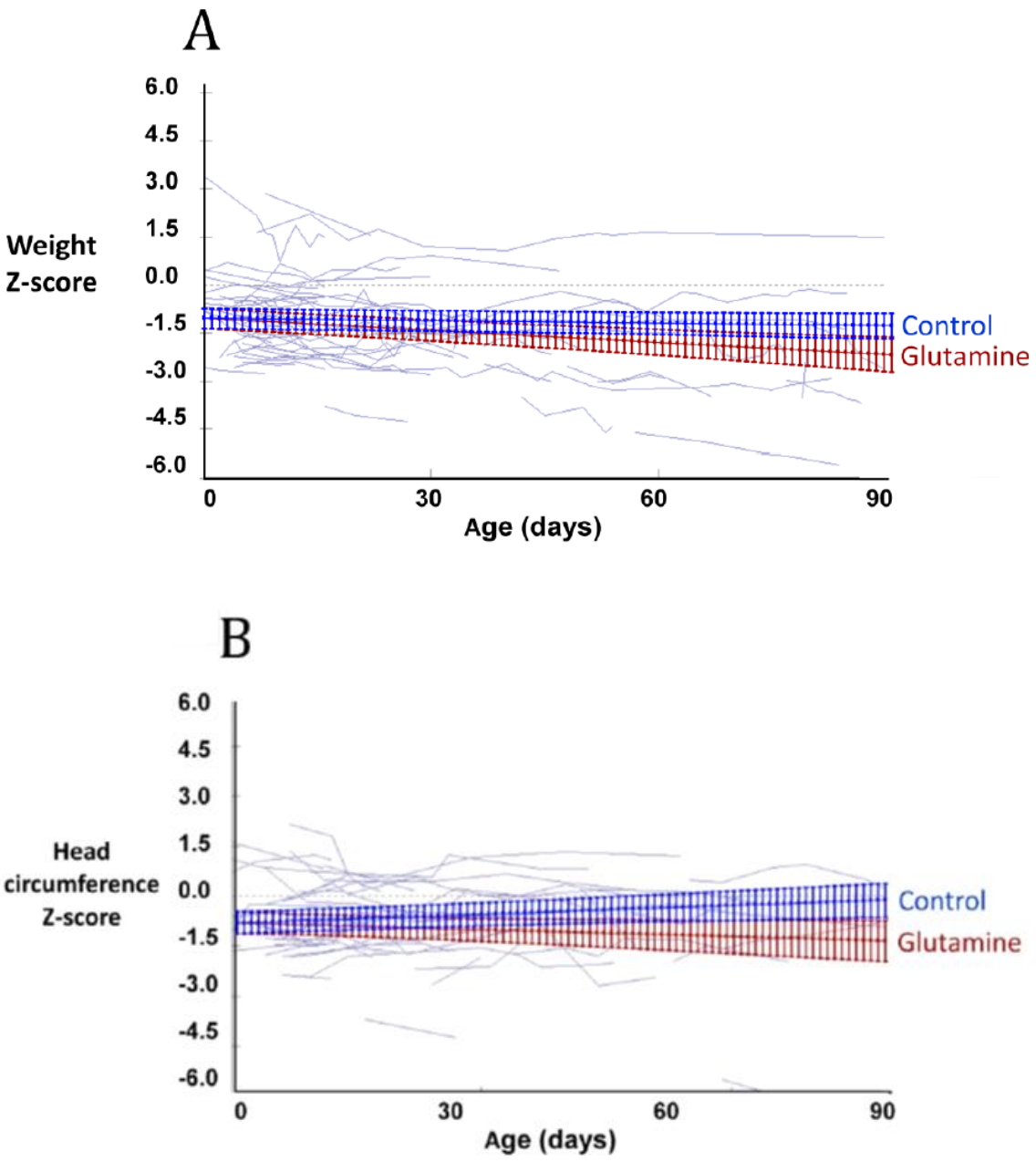
Control infants grew along weight centiles during PN (-0.021 ± 0.014 Z-scores per week of PN, p = 0.10; see Figure 6.10, 6.11) but glutamine supplementation led to a clinically small but statistically significant loss in weight Z-score (-0.07 ± 0.021 per week of PN, p = 0.003). Similarly, control infants increased head circumference Z-score (+0.024 ± 0.021 per week of PN, p = 0.003) whereas glutamine supplementation was associated with impaired head growth (-0.098 ± 0.028 per week of PN, p = 0.002).

Figure 6.9 Weight Z-scores at baseline and during the study



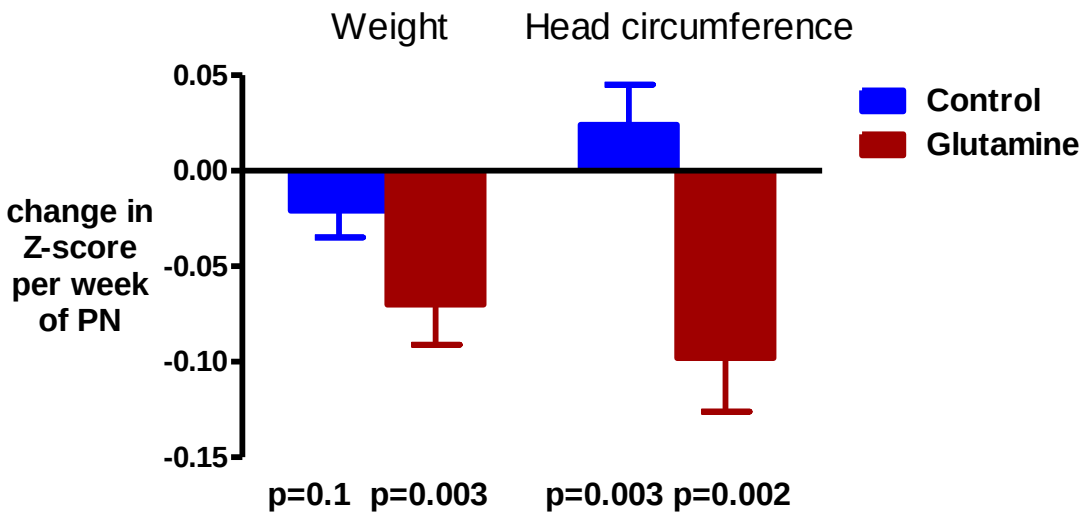
A. Weight Z-score at time of enrolment (green line at mean). B. Change in weight Z-score during study. Mean change -0.25, p = 0.004 (unpaired *t* test).

Figure 6.10 Modelling growth in control and glutamine groups during the study



Individual lines showing serial data for individuals, with superimposed mean ± SEM for groups. A. Weight Z-scores. B. Head circumference Z-scores.

Figure 6.11 Growth in control and glutamine groups during the study



Growth in the two groups was compared by multilevel modelling adjusting for diagnosis. P-values are for trend over time.

6.4.6 Exploratory Analyses (Hypothesis-Generating)

There was highly significant correlation between plasma levels of endotoxin and LBP, Spearman r 0.24 (0.11 – 0.36), p = 0.0002.

Levels of soluble CD14 were found to correlate significantly with other markers of microbial invasion (plasma endotoxin level and lipopolysaccharide binding protein) and were significantly higher in samples with evidence of microbial invasion as defined in the trial protocol (Table 6.5). Levels of monocyte HLA-DR expression were no different in samples with evidence of microbial invasion.

Samples taken during episodes of clinically suspected sepsis, showed significantly higher levels of endotoxin and LBP, and significantly lower levels of monocyte HLA-DR expression, compared to other samples (Table 6.6, Figure 6.12). There was no difference in levels of soluble CD14 during sepsis.

Those infants who showed evidence of microbial invasion during the study were found to have a significantly higher baseline level of LBP at enrolment. Baseline monocyte HLA-DR expression did not appear to predict microbial invasion (Table 6.7).

Infants who developed clinical sepsis during the study were found to have a significantly higher baseline level of LBP, and significantly lower baseline monocyte HLA-DR expression, compared to those who did not (Table 6.8, Figure 6.13).

Table 6.5 Soluble CD14 and monocyte HLA-DR expression in samples with and without evidence of microbial invasion

	No MI (n = 175)	MI (n = 61)	Difference MI vs. No MI (95% CI)	P-value
sCD14	1149 (456 – 2970)	1301 (590 - 3061)	193 (72, 314)	0.003
% monocyte HLA-DR	66 (12 – 100)	61 (9 – 96)	-4 (-12, 3)	0.28
Monocyte HLA-DR (MFI)	15 (4 – 215)	15 (6 - 100)	-3, (-9, 3)	0.72

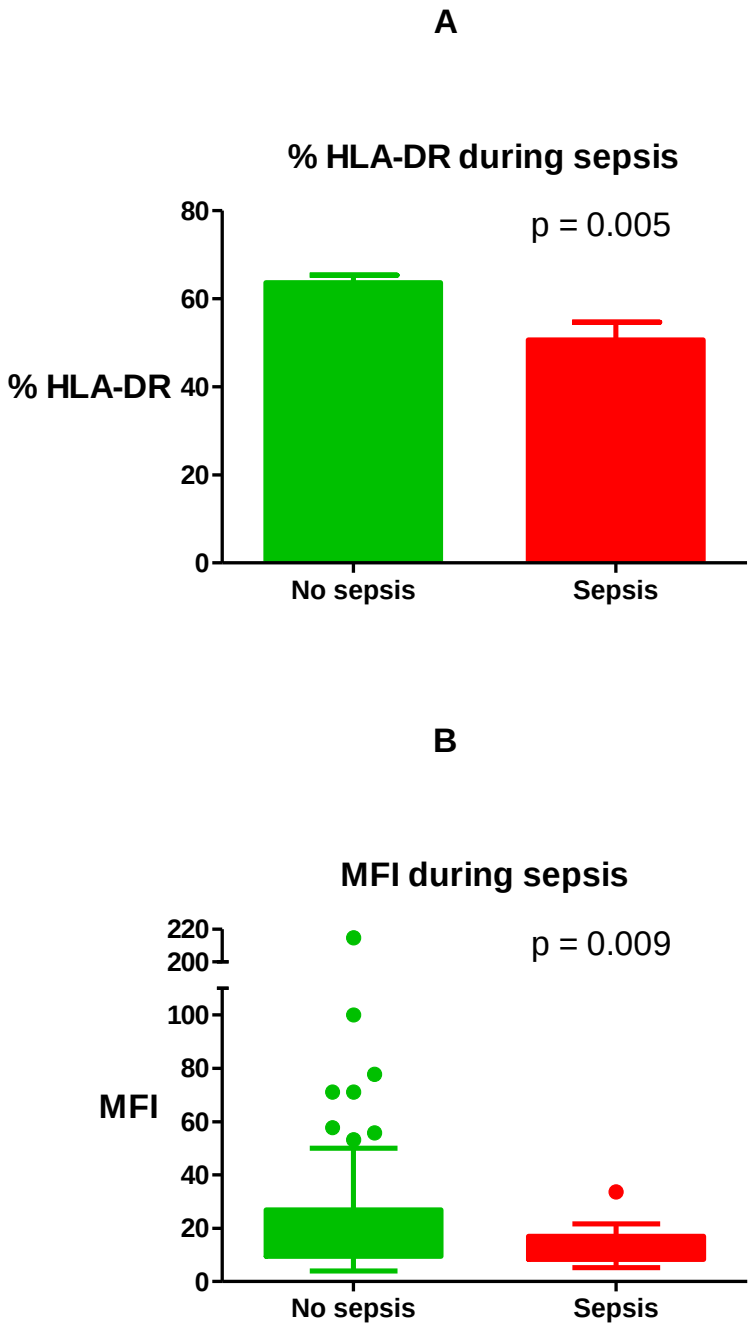
Data presented as median (range) compared by Mann-Whitney test.

Table 6.6 Levels of endotoxin, LBP, soluble CD14 and monocyte HLA-DR expression in samples taken with and without clinical signs of sepsis

	No sepsis (n = 187)	Sepsis (n = 53)	Difference Sepsis vs. No sepsis (95% CI)	P-value
Endotoxin (EU/mL)	0.21 (0 - 4.8)	0.24 (0 - 1.8)	0.002 (-0.09, 0.09)	0.04
LBP (µg/mL)	21 (0 - 60)	29 (9 - 60)	10 (4, 15)	0.0006
sCD14	1178 (456-3061)	1233 (550-2574)	70 (-60, 159)	0.33
% monocyte HLA-DR	68 (11 – 100)	50 (9 – 84)	-13 (-22, -4)	0.005
Monocyte HLA-DR (MFI)	16 (4 – 215)	10 (5 - 34)	-9 (-13, -5)	0.009

Data presented as median (range) compared by Mann-Whitney test.

Figure 6.12 Monocyte HLA-DR expression in samples taken during sepsis compared to other samples



A % monocyte HLA-DR expression, plotted as mean ± SEM, compared using unpaired *t* test. B Median fluorescence intensity (MFI), plotted as median, range and interquartile range, compared using Mann-Whitney test.

Table 6.7 Baseline values and subsequent microbial invasion

	No microbial invasion (n = 24)	Microbial invasion (n = 36)	Difference MI vs. No MI (95% CI)	P-value
Endotoxin (EU/mL)	0.18 (0.01 - 0.31)	0.22 (0 – 0.91)	0.05 (-0.01, 0.1)	0.09
LBP (μg/mL)	21 (0 – 55)	31 (0 – 60)	11 (3, 19)	0.01
sCD14	1167 (580 – 2072)	1166 (553 – 2139)	-12 (-207, 182)	0.90
% monocyte HLA-DR	58 (12 – 96)	56 (11 – 91)	-4 (-18, 9)	0.56
Monocyte HLA-DR (MFI)	14 (5 – 29)	11 (4 – 71)	0 (-6, 5)	0.29

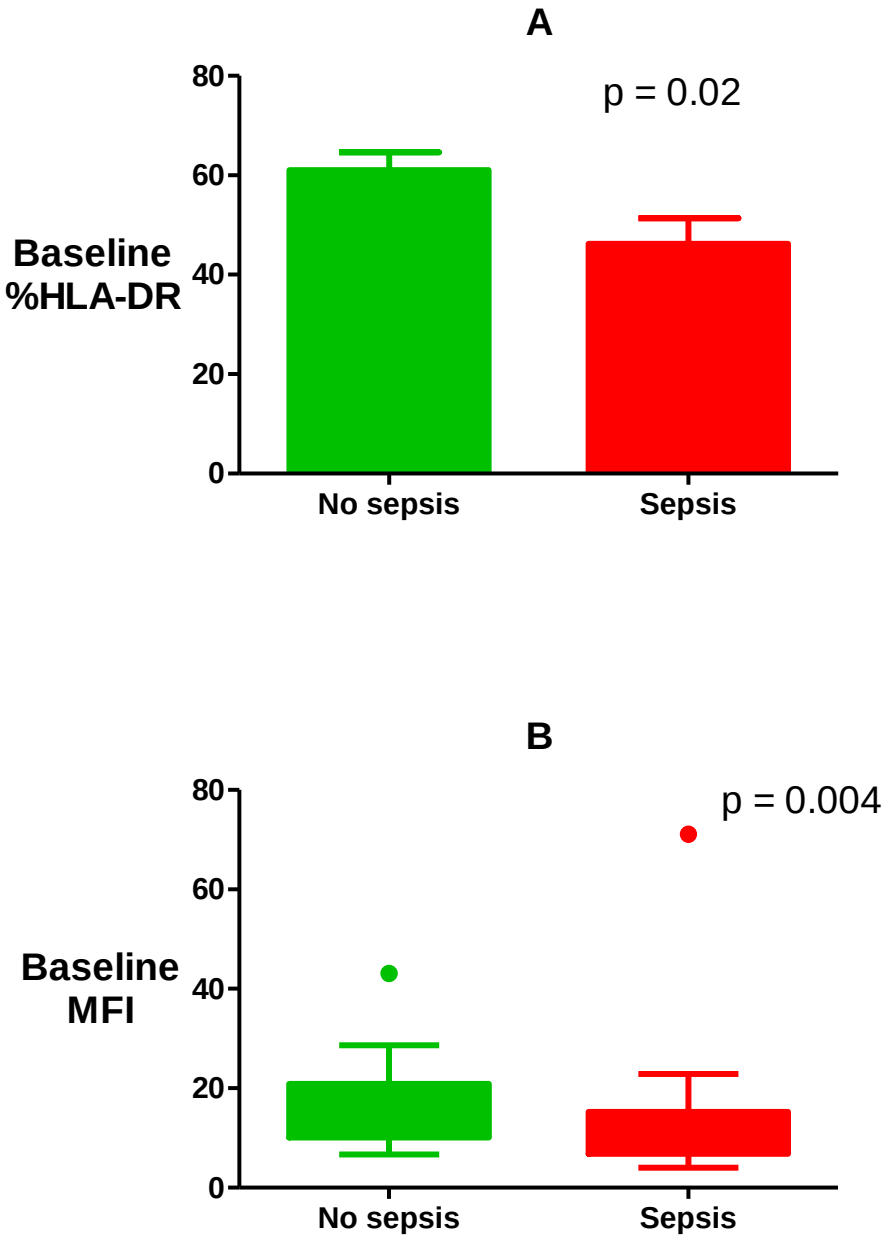
Data presented as median (range) compared by Mann-Whitney test.

Table 6.8 Baseline values and subsequent sepsis

	No sepsis (n = 28)	Sepsis (n = 32)	Difference Sepsis vs. No sepsis (95% CI)	P-value
Endotoxin (EU/mL)	0.20 (0 – 0.32)	0.19 (0.01 – 0.91)	0.02 (-0.04, 0.08)	0.85
LBP (μg/mL)	22 (0 – 55)	39 (0 – 60)	13 (6 – 21)	0.002
sCD14	1167 (589 – 2072)	1166 (553 – 2139)	-7 (-198, 184)	0.94
% monocyte HLA-DR	61 (12 – 96)	43 (11 – 91)	-15 (-28, -2)	0.02*
Monocyte HLA-DR (MFI)	16 (7 – 43)	9 (4 – 71)	-4 (-9, 2)	0.004

Data presented as median (range) compared by Mann-Whitney test.
*Compared by unpaired *t* test (with Welch correction).

Figure 6.13 Baseline monocyte HLA-DR expression predicts clinical sepsis



A. % monocyte HLA-DR expression, plotted as mean ± SEM and compared using unpaired *t* test (with Welch correction). B. Median fluorescence intensity (MFI), plotted as median, range and interquartile range, and compared using Mann-Whitney test.

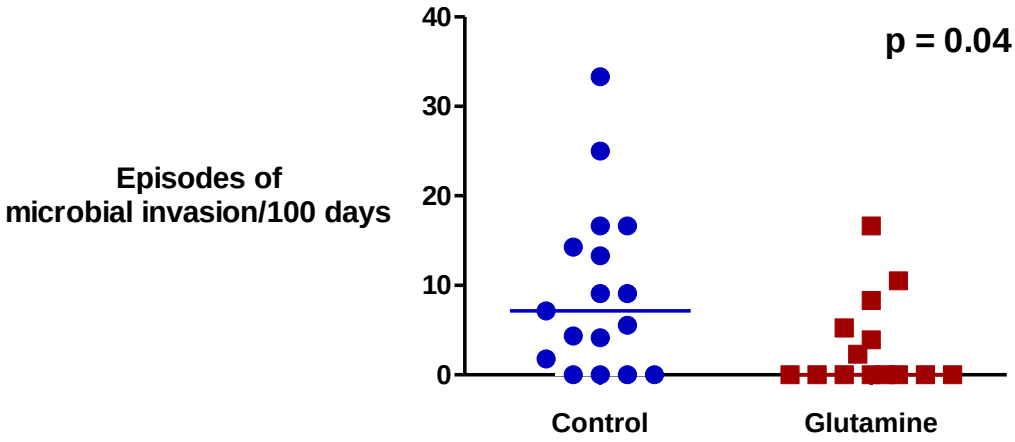
This raises the question of whether these baseline parameters can be used to identify a high-risk category of infants who may particularly benefit from interventions such as glutamine supplementation to prevent microbial invasion. Allen et al. calculated that HLA-DR <60% in the first 72 hours after surgery was predictive of sepsis and prolonged ICU stay in infants and children undergoing elective cardiac surgery (Allen et al. 2002). Applying this threshold to the current data, I find that 31 of the infants had HLA-DR <60% at enrolment. Among this subgroup, glutamine supplementation does appear to be protective against microbial invasion (Table 6.8 & Figure 6.14).

Table 6.9 Microbial invasion in control and glutamine groups among the subgroup of patients with monocyte HLA-DR <60% at enrolment

	Control group n=17	Glutamine group n=14	Difference glutamine vs. control (95% CI)	P-value
Infants with microbial invasion at any time	13	6	RR 0.41 (0.16 , 1.09)	0.08
Episodes of microbial invasion/100 days	7.1 (0 – 33.3)	0 (0 – 16.7)	-6.1 (-11.6, -0.54)	0.04

Data presented as median (range) or number (percentage) compared by Mann-Whitney test or Fisher's exact test. RR = relative risk

Figure 6.14 Rate of microbial invasion in control and glutamine groups among the subgroup of patients with monocyte HLA-DR <60% at enrolment



Rate of microbial invasion in control and glutamine groups compared by Mann-Whitney test. Line at median.

6.5 Discussion

This randomised controlled trial did not find any effect of enteral and parenteral glutamine supplementation in surgical infants on the incidence of microbial invasion. There was a high incidence of microbial invasion in these patients, with 60% showing evidence of microbial invasion at some point. Only half of this was detectable by conventional blood culture. To my knowledge, this is the first study to apply these techniques in this group of patients. The infants in this study also showed a high incidence of clinical sepsis, which is in keeping with previous studies (Bishay et al. 2011b; Donnell et al. 2002; Pichler et al. 2010).

Glutamine supplementation was associated with impaired growth in this study. Because the groups were isonitrogenous, the glutamine group in this study received a lower intake of other amino acids. It could be that this lower intake of other amino acids was sub-optimal and may account for the observed difference in growth. A previous small pilot trial of enteral glutamine supplementation in surgical infants found no difference in weight gain between the two groups (Duggan et al. 2004).

The limited concordance between positive blood cultures by conventional microbiology and positive biomarkers of microbial invasion (broad range or pathogen specific PCR, LBP and endotoxin) highlights the problems in diagnosis and targeted therapy of infants with clinically suspected sepsis. Five infants with a positive blood culture did not have elevated markers of microbial invasion, whereas 17 infants who were blood culture negative did have raised markers of microbial invasion. The limited ability of conventional microbiology to detect all pathogens has long been known, and these patients are generally treated empirically with broad-spectrum antibiotics, based on

clinical findings. The advent of more advanced molecular microbiological techniques such as 16S rDNA broad-range PCR, single species PCR or more recently, pyrosequencing, has improved the ability to detect and treat infectious organisms. However, these techniques are very sensitive to contamination so may suffer from detection of false positive signals. In addition to the markers of microbial invasion that formed the primary outcome, I also measured levels of plasma soluble CD14, which is an established marker of monocyte response to endotoxin that has been shown to prognosticate mortality in HIV-infected subjects (Brenchley et al. 2006; Sandler et al. 2011). Although soluble CD14 levels correlated with other markers of microbial invasion, plasma levels were not able to differentiate those samples taken during clinical sepsis from those taken at other times, suggesting that soluble CD14 offers no advantage over other markers in this group of patients.

Glutamine supplementation of parenteral and enteral nutrition was not effective in decreasing microbial invasion in the current trial, despite our previous study which showed that glutamine was effective in reducing sepsis during the period of total parenteral nutrition, although when the whole period of parenteral feeding (including partial enteral feeding) was considered, there was no benefit of parenteral glutamine supplementation (Ong et al. 2012). In the current study, I hypothesized that maintaining the difference in glutamine content between the two groups during the period of parenteral feeding, by supplementing the enteral feed as well as the parenteral feed, would allow the beneficial effect of glutamine to be extended into the period of partial enteral feeding. However, there was no significant effect of parenteral plus enteral glutamine supplementation on microbial invasion.

Despite this overall negative finding of glutamine supplementation, on planned secondary outcome analysis glutamine did result in significantly higher monocyte HLA-DR expression post-operatively compared with the control group. Monocyte HLA-DR expression has been shown to be reduced following surgery (Allen et al. 2002) and all of the infants in this study were recruited soon after undergoing surgery. Glutamine supplementation led to a faster recovery of monocyte HLA-DR expression towards normal healthy levels. Glutamine has previously been shown to improve recovery of monocyte HLA-DR following trauma (Boelens et al. 2002; Spittler et al. 2001). The mechanism of this effect of glutamine on monocyte HLA-DR expression is not completely understood, although glutamine is known to be a preferred nutrient source for monocytes and other cells of the immune system (Newsholme 2001; Roth 2013).

As in previous studies in children undergoing cardiac surgery, in which low monocyte HLA-DR expression was associated with infection and increased length of post-operative intensive care unit stay (Allen et al. 2002), in the current study low HLA-DR expression at baseline was found to be predictive of clinical sepsis, and low HLA-DR expression was also found at the time of clinical sepsis (low monocyte function may be directly a mediator of the risk of infection, or it may simply be a marker for associated immunoparesis). Exploratory analysis of the data from the current trial shows that monocyte HLA-DR expression in the early postoperative period can be used to select a subgroup of infants in whom glutamine supplementation appears to be beneficial in preventing microbial invasion. A future study could therefore be designed to prospectively identify those infants with the lowest HLA-DR expression (i.e. those most at risk of microbial invasion) and specifically target those infants with glutamine and/or

other immunomodulatory therapies. Although HLA-DR expression requires flow cytometric measurement, protocols have been suggested for standardisation of HLA-DR measurement that might allow identification of patients most at risk from post-operative sepsis (Docke et al. 2005). Such techniques may aid the development of biomarker-guided immunomodulatory therapy, which holds great promise in this context. A number of different cytokine-based immunotherapies have been shown to reverse immunosuppression in sepsis in both animal models and clinical trials (Hotchkiss et al. 2013). For example, in a randomised controlled trial in adults with immunosuppression following oesophageal or pancreatic resections, patients with low baseline monocyte HLA-DR expression were selected, and it was demonstrated that administration of granulocyte-macrophage colony-stimulating factor (GM-CSF) restored monocyte HLA-DR expression, and led to significantly reduced infections ($p < 0.001$) (Spies et al. 2015).

In addition to effects on the immune system, as glutamine is also a preferential fuel for enterocytes (Ashy et al. 1988; Windmueller and Spaeth 1974), it has been suggested that glutamine supplementation, particularly via the enteral route, may protect mucosal integrity and prevent or reverse gut barrier dysfunction (van der Hulst et al. 1993). In the current study, glutamine supplementation was given both parenterally and enterally, in order to maintain the difference between the randomised groups during the period of partial enteral feeding that occurs in all patients during their transition to full enteral feeds. Most studies of glutamine supplementation have used either the parenteral or the enteral route rather than a combination, although there are some studies in critically ill adults that have supplemented with enteral and parenteral glutamine and antioxidants (Heyland et al. 2013). Despite parenteral and enteral supplementation, in the current

study there was no evidence for a beneficial effect of glutamine in time to full enteral feeds. In addition, plasma endotoxin levels are a proxy measure for gut barrier dysfunction, and glutamine-supplemented infants had similar levels of plasma endotoxin to the control group, suggesting that gut barrier integrity was similar between the groups.

As well as gut barrier integrity and immune function, differences in gut flora may be important in determining the risk of infection. There is evidence of a significant role of bacterial overgrowth in infections in surgical infants (Pierro et al. 1998; van Saene et al. 2003). Modern high-throughput molecular sequencing techniques allow a more in-depth analysis of the microbiome which may shed more light on the aetiology of bloodstream infections and microbial invasion in these infants. Such techniques have already been applied with some success to the study of inflammatory bowel disease, obesity and bowel cancer, and are being applied to surgical patients with the aim of understanding and preventing infections and other complications such as anastomotic failure (Morowitz et al. 2011; Stavrou and Kotzampassi 2017).

One limitation of this study is the use of a composite endpoint to assess microbial invasion for the primary outcome. The individual aspects of this endpoint are not validated as being equivalent in their effect on clinical outcome, and it is possible that changing the cut-off points used for these assays could potentially affect outcome. Furthermore, the cut-off value for the LBP assay was altered from that specified in the original trial protocol, as described in section 6.3.4.7. However, had I, for example, only used blood cultures to assess the primary outcome, there would clearly not have been enough evidence of microbial invasion to detect a difference (only six infants out of 60 had a positive blood culture on day 5). The composite endpoint was formulated to try and

improve the sensitivity of detection of microbial invasion. It is of note therefore that in four patients (two in each group), blood cultures were positive on day 5 while PCR, endotoxin and LBP were negative. These four cultures were positive for staphylococci or streptococci which may potentially have been contaminants.

Another limitation is that the study is not strictly an intention-to-treat analysis in the sense that the four patients who did not receive trial supplementation for five days did not have the primary outcome assessed. Two patients (both turned out to be in the glutamine group) were transferred away to non-participating hospitals (in which Ihad no approvals in place for blood sampling). I did amend the trial protocol and obtain the necessary approvals to extend the trial to a large nearby neonatal unit so that infants transferred there could continue trial supplementation and have the relevant blood samples taken and analysed. A further two patients (one in each group) reached full enteral feeds in less than five days, and therefore stopped trial supplementation and did not have blood samples taken on day 5. It would not be appropriate to assume that these four infants had no evidence of microbial invasion, as this might introduce a significant bias. The decision not to sample was taken whilst I was blinded to patient allocation. Hence, the analysis cannot be regarded as a pure intention to treat analysis.

It should also be noted that the definition of sepsis used in this study (see section 6.3.4) predates the more recent consensus definitions (see section 2.5 and figure 2.3). The definition used in this study may encompass conditions defined by the consensus as severe sepsis/septic shock as well as sepsis, since it includes hypotension, lethargy and poor perfusion.

Study in this group of patients has always been challenging due to the relative rarity of the individual conditions, leading to studies such as the present one, which are limited by a degree of heterogeneity. Application of the laboratory techniques used here can allow the selection of a subgroup of patients who may be similar in terms of their immune status and vulnerability to microbial invasion, allowing us to demonstrate effects which were not apparent in the larger group.

6.6 Conclusions

More than half of surgical infants requiring parenteral nutrition showed evidence of microbial invasion. Approximately half of this was not detectable by conventional blood cultures. Parenteral plus enteral glutamine supplementation had no effect on the incidence of microbial invasion in surgical infants. However, glutamine supplementation did assist recovery of post-operative immunoparesis, and glutamine did prevent microbial invasion in those with the most severe immunoparesis, suggesting that a targeted population may benefit from supplementation.

Chapter 7. Hypercapnia and Acidosis during Open and Thoracoscopic Repair of Congenital Diaphragmatic Hernia and Oesophageal Atresia: a Pilot Randomised Controlled Trial

7.1 Summary of Chapter 7

7.1.1 Aims

Congenital diaphragmatic hernia (CDH) and oesophageal atresia with tracheo-oesophageal fistula (OA/TOF) can be repaired thoracoscopically, but this may cause hypercapnia and acidosis which are potentially harmful. I aimed to evaluate the effect of thoracoscopy in neonates on intra-operative arterial blood gases, compared to open surgery.

7.1.2 Methods

This was a pilot randomised controlled trial. The target number of 20 neonates (weight >1.6 kg) were randomised to either open (5 CDH, 5 OA/TOF) or thoracoscopic (5 CDH, 5 OA/TOF) repair. Arterial blood gases were measured every 30 minutes intra-operatively, and compared by multilevel modelling, presented as mean and difference (95% confidence interval) from these predictions. ClinicalTrials.gov Identifier: NCT01467245

7.1.3 Results

Overall the intraoperative $PaCO_2$ was 8·1 kPa in open and 11·0 kPa (difference 2·9 [0·2, 5·6]; p = 0·036) in thoracoscopy and the pH was 7·24 in open and 7·13 (difference -0·11 [-0·20, -0·01]; p = 0·025) in thoracoscopy. Duration of hypercapnia and acidosis was longer in thoracoscopy compared to open. For patients with CDH, thoracoscopy was associated with a significant increase in intra-operative hypercapnia (open 9·1; thoracoscopy 12·8; difference 3.7 [1·0, 6·4]; p = 0·008) and severe acidosis (open 7·21;

thoracoscopy 7·08; difference -0.13 [-0·24, -0·02]; p = 0·018). No significant difference in $PaCO_2$, pH or PaO_2 was observed in patients undergoing thoracoscopic repair of OA/TOF.

7.1.4 Conclusions

This pilot randomised controlled trial shows that thoracoscopic repair of CDH is associated with prolonged and severe intraoperative hypercapnia and acidosis, compared to open surgery. These findings do not support the use of thoracoscopy with carbon dioxide insufflation and conventional ventilation for the repair of CDH, calling into question the safety of this practice. The effect of thoracoscopy on blood gases during repair of OA/TOF in neonates requires further evaluation.

7.2 Introduction

The use of minimally invasive surgical techniques has been increasingly applied in infants and children leading to the use of thoracoscopy in neonates (Ponsky and Rothenberg 2008; Ure et al. 2005) for congenital anomalies such as congenital diaphragmatic hernia (CDH) (Yang et al. 2005) and oesophageal atresia with distal tracheo-oesophageal fistula (OA/TOF) (Holcomb et al. 2005).

Minimally invasive surgery for these conditions involves insufflation of exogenous carbon dioxide (CO_2) into the pleural cavity and collapse/reduced ventilation of the ipsilateral lung to enable clear visualisation and thoracoscopic repair of the anomalies.

During the early experience of thoracoscopic procedures in neonates in our institution, a degree of intraoperative hypercapnia and acidosis was noted – presumably due to CO_2 insufflation into the pleural cavity and collapse/hypoventilation of the lung in an immature and often pathological neonatal cardiopulmonary system. This was naturally a matter of great concern to the surgeons, anaesthetists, and neonatal intensivists involved. Relatively few studies reporting on the cardiorespiratory consequences of thoracoscopy in neonates had been published at the time of planning this study (Bliss et al. 2009; Fishman et al. 2011; Gourlay et al. 2009; Krosnar and Baxter 2005; McHoney et al. 2010) and these studies relied on retrospective review of anaesthetic charts. Krosnar and Baxter described anaesthetic management of a series of eight neonates with OA/TOF (Krosnar and Baxter 2005). They observed intraoperative periods of raised arterial CO_2 and reduced arterial pH (with a 'falsely low' end-tidal CO_2), but they do not provide any numerical data.

There were also a small number of studies reporting intraoperative blood gases during thoracoscopic repair of CDH. Bliss et al. reported a series of 31 patients undergoing thoracoscopic repair of CDH (Bliss et al. 2009), and describe a degree of intraoperative hypercapnia and acidosis, but did not provide any comparison to open surgery. Gourlay et al. reported 33 neonates who underwent thoracoscopic repair of CDH and compared them to 18 matched historic controls (Gourlay et al. 2009). They found that the thoracoscopic group experienced a more acidotic lowest median arterial pH (7.15 vs. 7.27, $p = 0.04$) and required higher peak inspiratory ventilator pressures (27.1 vs. 24.8, $p = 0.04$). McHoney et al. retrospectively compared 13 infants who underwent thoracoscopic repair of CDH to 35 patients who underwent open repair (McHoney et al. 2010). They found that the thoracoscopy group experienced a significantly higher peak and mean intra-operative end-tidal CO_2, but this was not reflected in arterial CO_2 or pH. These studies are all limited by the facts that they relied on retrospective review of anaesthetic charts, and blood gas measurements were not taken systematically, but only as deemed necessary by the anaesthetist.

Because of these concerns about hypercapnia and acidosis during the thoracoscopic repair of CDH and OA/TOF, I carried out a prospective pilot randomised controlled trial to evaluate the effect of thoracoscopy with CO_2 insufflation on (i) arterial blood gases, and (ii) absorption of insufflated CO_2. The main aim was to determine whether thoracoscopic repair of CDH and OA/TOF is associated with hypercapnia and acidosis, compared to open surgery. The primary outcome measure was intra-operative arterial blood carbon dioxide level.

One of the secondary outcome measures I investigated was regional cerebral oxygen saturation (cSO_2) measured using near-infrared spectroscopy (NIRS).

7.3 Methods

7.3.1 Randomisation, Masking and Sample Size

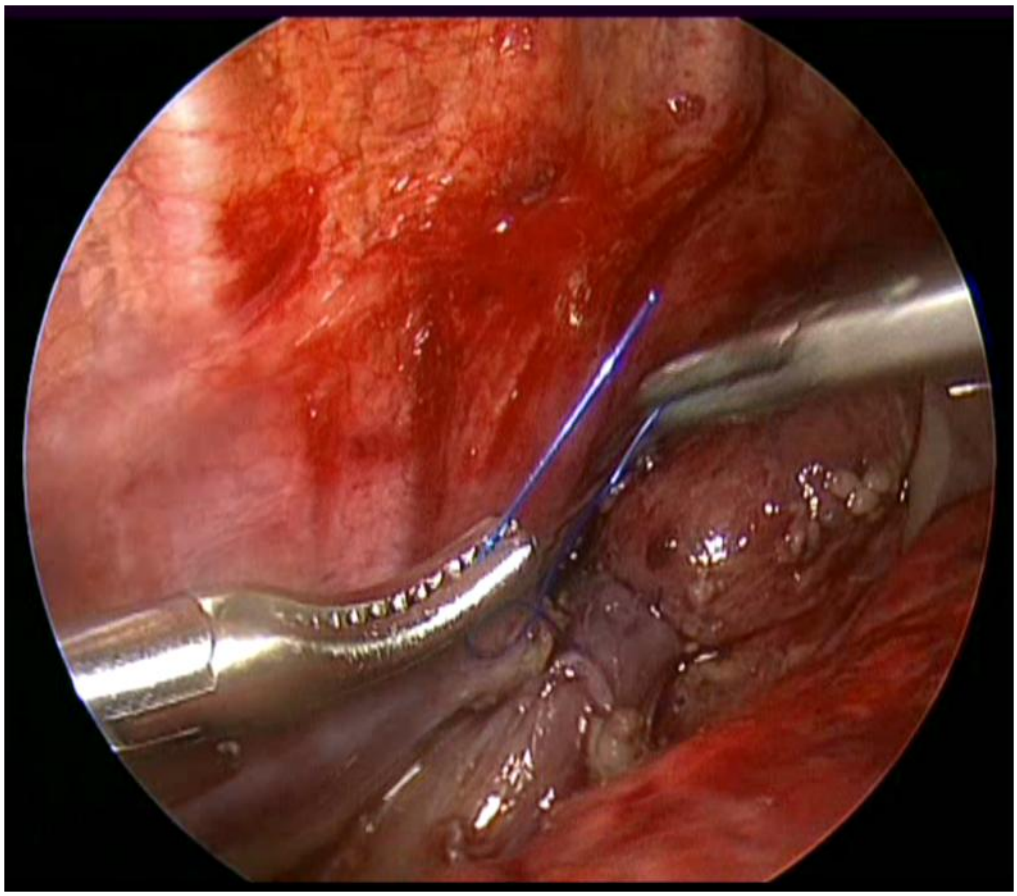
This was a pilot randomised controlled trial to establish the safety of thoracoscopy and to plan a larger trial if necessary (for the full trial protocol, see Appendix 3). The target number of twenty patients was chosen based on feasibility and no power calculation was performed. Ethical approval (09/H0714/2) for this study was obtained and written informed consent was obtained from parents or guardians of all participants. Eligible patients were neonates (i.e. in the first month of life), diagnosed with CDH or OA/TOF, and not requiring high frequency oscillatory ventilation, inhaled nitric oxide or inotropic support for at least 24 hours. Patients weighing less than 1·6 kg, or requiring more than 40% oxygen were excluded, as were neonates who had required extra-corporeal membrane oxygenation (ECMO), or had major congenital heart defects, pulmonary hypertension, or bilateral grade IV intraventricular haemorrhage. Subjects were randomly allocated to open or thoracoscopic surgery in a 1:1 ratio using computerised balanced minimisation (Wade et al. 2006) with the following criteria: i) diagnosis (congenital diaphragmatic hernia / oesophageal atresia with trachea-oesophageal fistula); ii) weight at the time of enrolment in the study (<2·5 kg / >2·5 kg); iii) age at time of enrolment (less than seven days / seven days or older). There was no blinding of clinicians, parents, or investigators. Enrolment and randomisation were performed by members of the research team (using SIMIN software), who then informed the clinical team of the allocation. The study was performed at a single UK centre (Great Ormond Street Hospital).

7.3.2 Surgery

Thoracoscopy was performed with both lungs ventilated. Patients were positioned semi-prone. A 3- or 5-mm Hasson cannula was inserted through the third or fourth intercostal space in the posterior axillary line and the chest was insufflated using CO_2 and if possible the initial pressure reduced after reduction of hernia content (CDH) or after collapse of the lung (OA/TOF). If necessary to maintain visualisation, the insufflation pressure was increased for a transient period. Surgery was performed with the aid of 2 or 3 working ports. For thoracoscopic repair of CDH, the hernia contents were reduced into the abdomen, and if a hernia sac was present, this was not resected. The defect was closed with interrupted non-absorbable stitches. If necessary, a polyester patch (Bard Sauvage Filamentous Fabric; Bard, Billerica, MA, USA) was used to close the defect. When necessary, the posterolateral stitches were ligated extra-corporeally using small skin incisions. For the thoracoscopic repair of OA/TOF the azygos vein was divided and the tracheo-oesophageal fistula was transfixed and ligated close to its entry into the trachea with a fine non-absorbable suture. The oesophageal anastomosis was performed with interrupted non-absorbable sutures, with minimal handling of the oesophageal ends.

For patients randomised to open surgery, CDH was repaired via an upper transverse laparotomy, and the defect was closed using non-absorbable sutures, with a polyester patch if necessary. OA/TOF was repaired via right posterolateral thoracotomy in the third or fourth intercostal space, using an extrapleural approach when possible. The TOF was

Figure 7.1 Thoracoscopic view of repair of oesophageal atresia



Thoracoscopic view of suturing of oesophageal anastomosis.

divided and the trachea closed with interrupted stitches. The oesophageal anastomosis was performed using interrupted stitches.

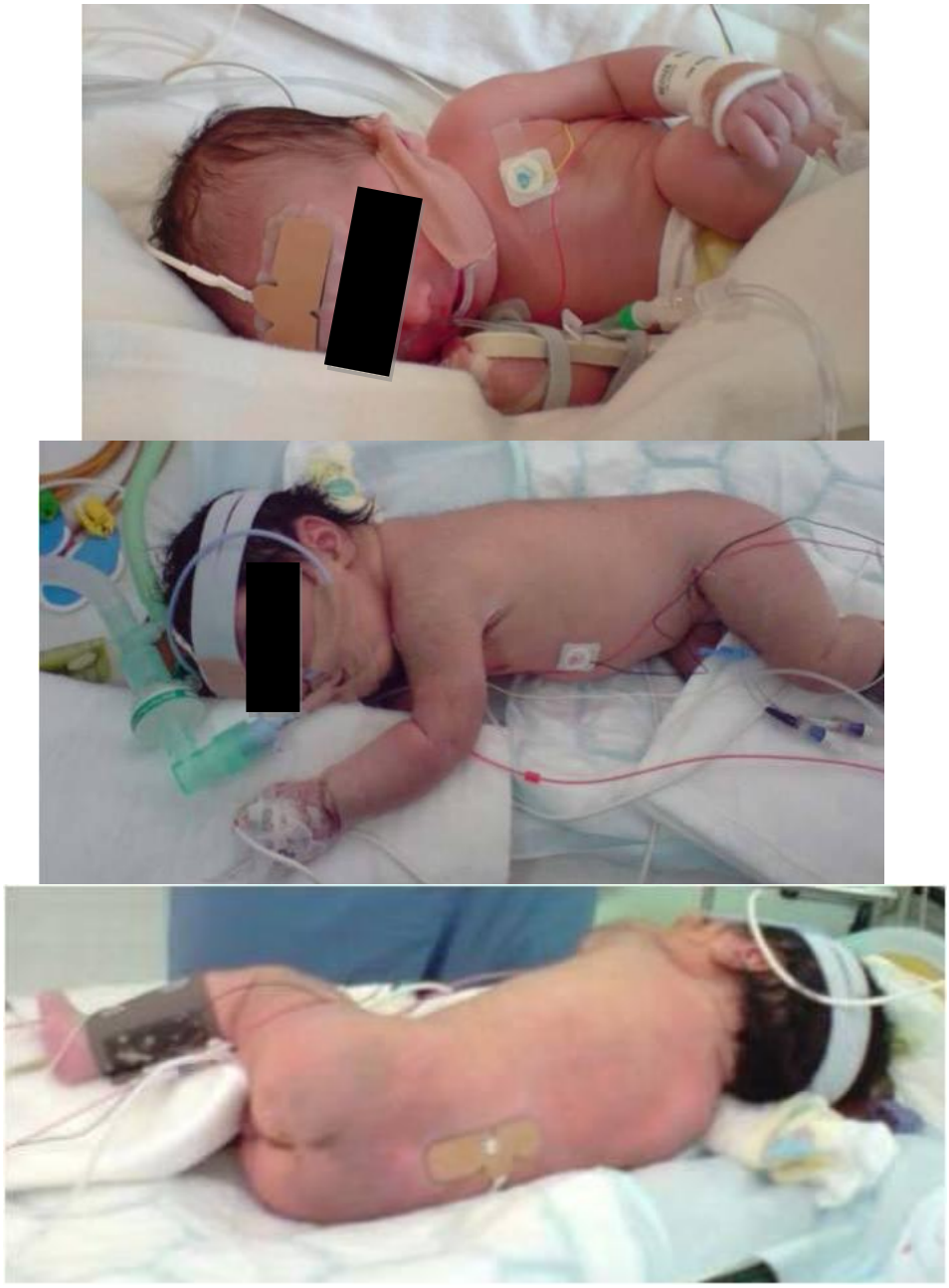
The trial protocol specified that if ventilation was felt to be a significant concern during thoracoscopy, insufflation of CO_2 would be temporarily paused to allow improvement in ventilation. If this was not successful, the operation would be converted to open surgery.

7.3.3 Data Collection and Sample Analysis

Arterial blood gases were measured in all patients, pre-, intra-, and post-operatively, at predetermined intervals (every 30 minutes intraoperatively, and at twelve and twenty four hours post operation). Breath samples were collected at 15-minute intervals using a 10 mL syringe connected to a 3-way valve at the sampling line for measurement of end tidal CO_2. The air was aspirated into a 10 mL syringe and immediately transferred into 10 mL vacuum test tubes (Labco Limited, High Wycombe, United Kingdom) for analysis. Samples were collected before the start of the operation, and every 15 minutes during the operation. In addition, samples of medical CO_2 used for insufflation were obtained for each thoracoscopic operation. These breath and medical gas samples were analysed for $^{13}CO_2/^{12}CO_2$ enrichment by isotope ratio mass spectrometry as previously described (Pacilli et al. 2006). Using the $^{13}CO_2/^{12}CO_2$ of the medical CO_2 used for insufflation to represent 100% of exhaled CO_2 originating from insufflation and baseline breath $^{13}CO_2/^{12}CO_2$ at the start of operation to represent 0%, the percentage of exhaled CO_2 originating from insufflation at each time point was calculated as previously described (Pacilli et al. 2006).

Regional cerebral oxygen saturation (cSO_2) was measured using near-infrared spectroscopy (NIRS) with an In Vivo Optical Spectroscopy (INVOS®) oximeter model 5100C (Somanetics Corporation, Troy, MI, USA) via a cerebral neonatal sensor applied to the forehead, secured using a phototherapy eyeshield. A somatic (renal) sensor was applied for reference to one of the loins overlying the kidney. Positioning of sensors is illustrated in Figure 7.2. Measurements were taken continuously preoperatively, during operation, and for 24 hours after the end of operation. Readings were averaged over periods of ten minutes at set time points: preoperatively, prior to transfer to theatre, at start of operation, intraoperatively at the time of nadir pH, at the end of operation, and at 12 hours and 24 hours postoperatively. Near-infrared spectroscopy (NIRS) is used to noninvasively measure and monitor changes in the approximate regional haemoglobin oxygen saturation in the blood. The device uses a light emitting diode (LED) which emits near-infrared light (650 to 950 nm wavelengths) and uses two sensors to detect light reflected back from the underlying tissue. The amount of light absorbed by the tissue will vary according to the ratio of oxygenated to total (oxygenated plus deoxygenated haemoglobin) in the tissue. An algorithm is therefore used to calculate this ratio (the tissue oxygenation index). Typically, the haemoglobin in the sensor's field is made up of approximately 75% venous blood, 20% arterial blood and 5% capillary blood. NIRS is widely used in paediatric cardiac surgery, where there is evidence that it appears to be more sensitive than other monitoring techniques for detecting cerebral oxygen desaturation (Austin et al. 1997). There is further evidence from adult aortic surgery that NIRS-measured duration of cerebral oxygen desaturation was the most accurate available predictor of post-operative neurological injury (Orihashi et al. 2004).

Figure 7.2 Positioning of sensors to monitor tissue oxygenation



Photographs taken to illustrate the positioning of sensors used to monitor tissue oxygenation by near-infrared spectroscopy. One sensor was placed on the forehead (top and middle pictures) to monitor cerebral oxygenation. Another sensor was placed on the loin to monitor renal oxygenation (bottom picture).

This technique is therefore appropriate for monitoring of patients for whom there is concern regarding the potential for decrease in cerebral oxygenation. However, normal values in this group of infants are not well established or validated. Among infants undergoing cardiac surgery (Norwood procedure), cerebral oximetry readings (using Somanetics INVOS 5100A oximeter) <45% for >180 min were found to be associated with the development of abnormalities on magnetic resonance imaging (MRI) of the brain (Dent et al. 2006). A study of adult patients undergoing cardiac surgery suggests that cerebral NIRS monitoring is more reliable in monitoring trends in tissue oxygenation rather than absolute values (Dullenkopf et al. 2007). Furthermore, the use of different devices affects the validity of NIRS measurements – for example, in a study of awake hyperventilating adult volunteers, the Somanetics INVOS 3100 oximeter was found to reflect changes in cerebral haemoglobin oxygenation than the Hamamatsu NIRO 500 oximeter (Grubhofer et al. 1999). Austin et al. in the study mentioned above defined desaturation signifying cerebral ischaemia as a decrease of more than 20% in the regional cerebral tissue oxygenation measured by NIRS (using Somanetics INVOS 3100A oximeter), which persisted for more than 3 minutes (Austin et al. 1997). They used cerebral oximetry alongside electroencephalography (EEG) and transcranial Doppler ultrasonography to monitor 250 children undergoing cardiac surgery, and found that cerebral oximetry using NIRS accounted for 58% of monitoring events. The 20% figure was derived from a study in adults were NIRS changes were shown to be associated with other signs of reduced cerebral perfusion detected by Doppler studies and quantitative EEG during ventricular tachycardia (Singer and Edmonds, Jr. 1994). In view of these findings, I decided to measure the amount of time each patient had tissue oxygenation

below a threshold of 45%, and below 80% of baseline pre-operative measurements, thus examining trends as well as absolute values.

7.3.4 End Points and Monitoring

The primary end point of the trial was intraoperative arterial CO_2 level. The secondary end points included acidosis (arterial pH), arterial oxygenation, percentage of exhaled CO_2 derived from the medical CO_2 used to inflate the chest in thoracoscopy, cerebral oxygenation measured by near-infrared spectroscopy, type and duration of postoperative ventilation, postoperative analgesia, pause of operation or conversion to open surgery, intraoperative and postoperative complications. The trial was monitored by the Data Monitoring and Ethics Committee (DMEC) comprising paediatric specialists not involved in the trial: anaesthetist, surgeon, intensivist, and neonatologist. The DMEC met 3 times during the trial to evaluate progress and review interim analysis, and reported recommendations to the Trial Steering Committee.

7.3.5 Statistical Analysis

Data are presented as mean ± SEM, unless stated otherwise. Normally distributed data were analysed using a *t* test, and non-parametric data were analysed using a Mann-Whitney test. Proportions were compared using a Fisher's exact test. The effects of operative approach (minimally invasive or open) on intraoperative $PaCO_2$, pH and PaO_2 were compared by multilevel regression modelling of each arterial blood gas measurement using MLWin v2.11. In the regression model, individual blood gas measurements were analysed taking into account that these were repeated measures of each patient. Diagnosis (CDH/OA-TOF) and operative approach (MIS/open) were used

as co-variates. A P-value ≤ 0·05 was considered statistically significant. This trial is registered with ClinicalTrials.gov, number NCT01467245.

7.3.6 Funding

This work was kindly supported by the Mittal Paediatric Surgery Research Fund and Great Ormond Street Hospital Children's Charity. No sponsors played any role in study design; in the collection, analysis, or interpretation of the data; or in the writing or publication of the report.

7.4 Results

The target number of twenty neonates underwent surgery between August 2009 and February 2011. Patients were randomised to either open (5 CDH, 5 OA/TOF) or thoracoscopic (5 CDH, 5 OA/TOF) repair (Figure 7.3). There was no significant difference between the open and thoracoscopic groups in demographics, pre-operative ventilation, or duration of operation (Table 7.1). In thoracoscopy, initial insufflation pressure was 7·1 ± 0·5 mmHg; this was reduced or maintained in all except one neonate undergoing repair of CDH where an increase from 6 to 9 mmHg was necessary to achieve visualisation.

7.4.1 Arterial Blood Gas Analysis

Using multilevel regression modelling to compare intraoperative blood gases, thoracoscopy was found to be associated with a significant increase in intraoperative hypercapnia (the primary outcome) and acidosis, while oxygenation was no different (Table 7.2).

As advised by the trial DMEC, I compared the duration of different levels of hypercapnia and acidosis between the two groups. The thoracoscopic patients experienced a markedly longer duration of the most extreme levels of hypercapnia and acidosis, compared to the open group (Table 7.3). In the thoracoscopic group, four out of ten patients experienced pH <7, up to a maximum duration of 135 minutes. No patients in the open group experienced pH <7. With regard to hypercapnia, six out of ten patients in the thoracoscopic group experienced hypercapnia with $PaCO_2$ >14 kPa (maximum

Figure 7.3 Trial profile – CONSORT flow chart

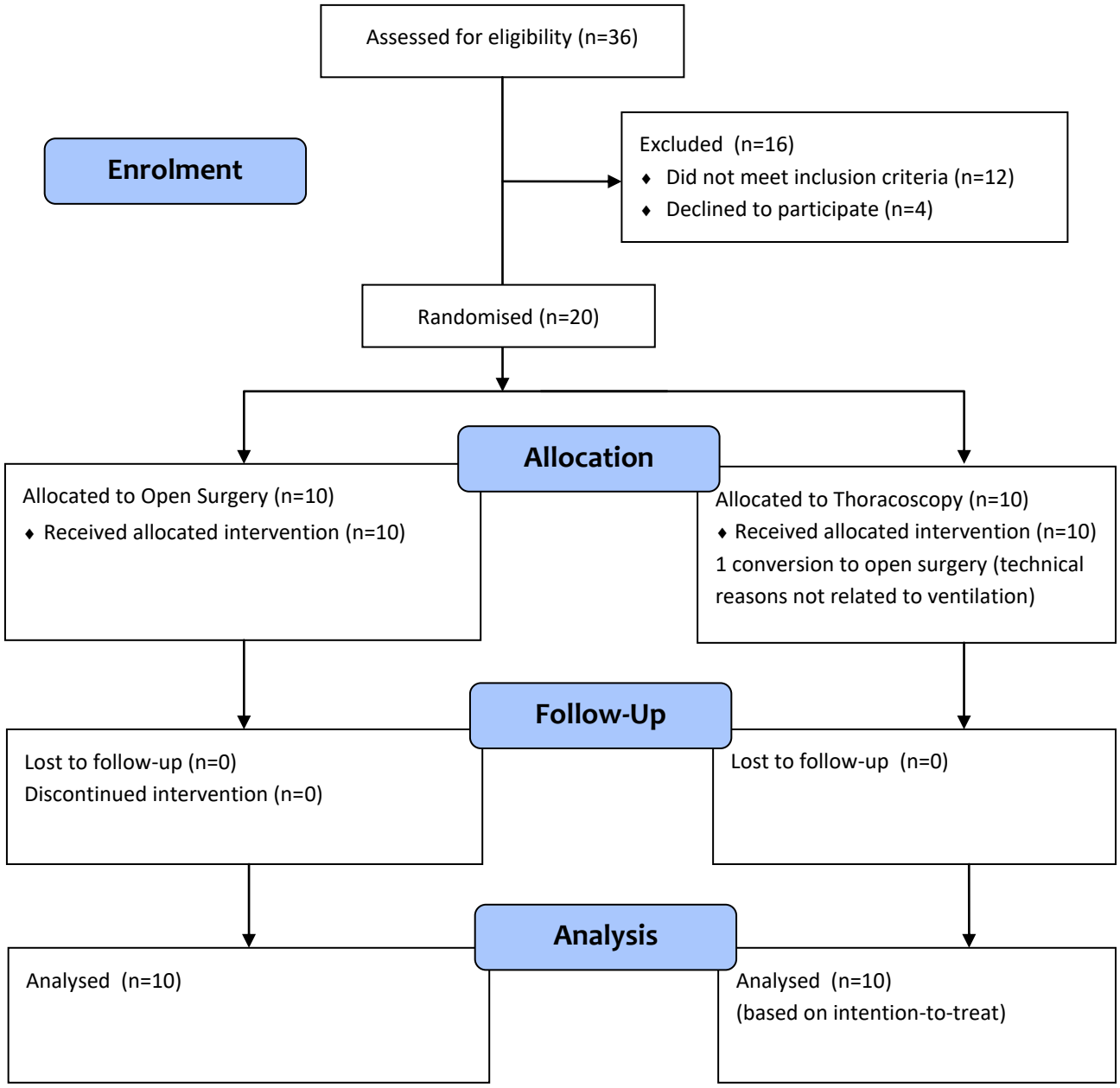


Table 7.1 Patient characteristics, preoperative ventilation, duration of operation and postoperative outcomes

	Open	Thoracoscopic	P-value
A. Congenital Diaphragmatic Hernia Patients			
Weight (kg)	3·0 (2·7 - 3·5)	3·4 (2·5 – 4·0)	0·69
Age (days)	5 (2 - 25)	4 (1 – 6)	0·53
Gestational Age (weeks)	38 (36 - 41)	39 (37 – 42)	0·40
Sex (M:F)	3:2	4:1	N/A
Duration of preoperative ventilation (days)	3 (1 - 11)	2 (1 – 6)	0·67
Preoperative Oxygenation Index	0·31 (0·19 – 0·47)	0·38 (0·16 – 0·85)	1·0
Preoperative HFOV	2/5	2/5	N/A
Preoperative iNO	1/5	1/5	N/A
Operating time (minutes)	79 (45-130)	91 (78-148)	0·56
PIP (cmH_2O)	22 (16 - 25)	15 (0 – 16)	0·02
Postoperative HFOV	2/5	0/5	N/A
ICU stay (days)	5 (3 - 13)	2 (2 – 6)	0·12
IV morphine (days)	3 (2 - 11)	1 (1 – 2)	0·02

Data as median (range); compared by Mann Whitney Test. HFOV = High-frequency oscillatory ventilation (used at any time prior to operation). iNO = inhaled nitric oxide (used at any time prior to operation). PIP = Peak inspiratory pressure supplied at 24 hours post operation; PIP recorded as 0 if patient extubated within 24 hours. ICU = Intensive Care Unit. IV = intravenous.

Table 7.1 (continued)

	Open	Thoracoscopic	P-value
B. Oesophageal Atresia Patients			
Weight (kg)	3·3 (2·6 - 3·5)	3·3 (2·9 – 3·7)	0·60
Age (days)	1 (1 - 2)	1 (1 – 5)	0·92
Gestational Age (weeks)	40 (38 - 41)	40 (39 – 41)	0·40
Sex (M:F)	4:1	3:2	N/A
Operating time (minutes)	150 (95-213)	180 (143-225)	0·41
PIP (cmH_2O)	16 (0 - 20)	0 (0 – 18)	0·12
Extubated within 24h	1/5	3/5	N/A
ICU stay (days)	5 (1 - 8)	3 (1 – 13)	0·92
IV morphine (days)	5 (1 - 10)	2 (1 – 11)	1·0

Table 7.2 Intraoperative arterial blood gases

Data were compared by multilevel modelling; average intraoperative blood gas values and the mean difference with 95% confidence intervals are reported.

	Open	Thoracoscopic	Difference (95% CI)	P-value
All Patients				
$PaCO_2$ (kPa)	8·1	11·0	2·9 (0·2, 5·6)	0·036
pH	7·24	7·13	-0·11 (-0·20, -0·01)	0·025
PaO_2 (kPa)	24·3	20·1	-4·3 (-10·9, 2·4)	0·21
CDH				
$PaCO_2$ (kPa)	9·1	12·8	3·7 (1·0, 6·4)	0·008
pH	7·21	7·08	-0·13 (-0·24, -0·02)	0·018
PaO_2 (kPa)	28·5	24·7	-3·8 (-13·3, 5·7)	0·43
OA/TOF				
$PaCO_2$ (kPa)	7·4	9·3	1·9 (-1·9, 5·8)	0·33
pH	7·26	7·18	-0·08 (-0·22, 0·05)	0·22
PaO_2 (kPa)	21·1	16·9	-4·1 (-11·3, 3·1)	0·26

Table 7.3 Duration of hypercapnia and acidosis in each patient, in minutes

	Open	Thoracoscopic
$PaCO_2$		
>14 kPa	0,0,0,0,0,0,0,0,0, 30	0,0,0,0, 30,45,107,120,125,225
>12 kPa	0,0,0,0,0, 15,30,30,30,60	0,0,0, 23,30,105,107,120,125,225
>10 kPa	0,0,0,0,0, 15,60,60,60,198	0,0, 30,53,111,120,125,183,218,225
pH		
<7·0	0,0,0,0,0,0,0,0,0,0	0,0,0,0,0,0,0, 22,30,125,135
<7·1	0,0,0,0,0,0, 15,30,30,198	0,0,0, 30,30,53,111,125,135,225
<7·2	0,0,0,0,0, 45,60,60,162,298	0, 30,61,107,111,120,120,241,248,255

duration 225 minutes), compared to one out of ten patients in the open group (duration 30 minutes).

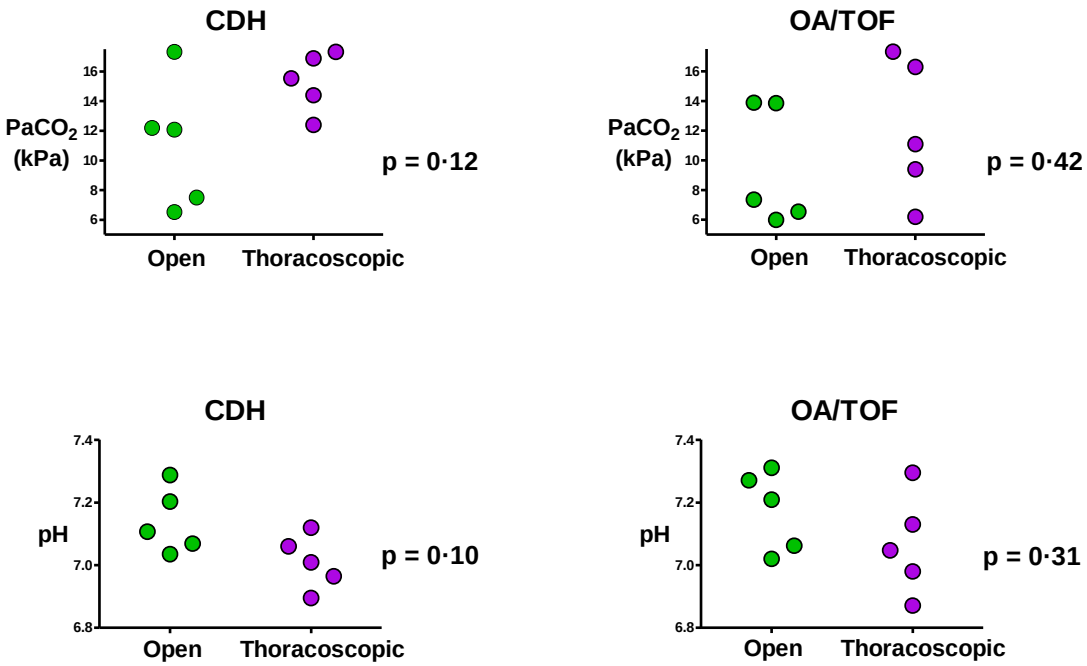
7.4.2 Congenital Diaphragmatic Hernia

All ten neonates had left-sided diaphragmatic hernias. Eight were repaired primarily, with two cases requiring a patch repair (one in each group). There were no cases of thoracoscopy converted to open surgery. There was one case where the anaesthetist requested a pause of insufflation (7 minutes) during thoracoscopy due to concerns regarding hypercapnia (which improved after hand ventilation/aspiration of secretions).

Figure 7.4 illustrates the highest $PaCO_2$ and lowest pH reached for each patient with CDH. While there is evidence of hypercapnia and acidosis in both groups, more extreme values were attained in the thoracoscopic group, with four patients out of five attaining a $PaCO_2$ >14 kPa (compared to one out of five in the open group), and two patients experiencing extreme acidosis with pH <7.0 (compared to none in the open group). Using multilevel regression modelling, thoracoscopy was found to be associated with a significant increase in intra-operative hypercapnia and acidosis for patients with CDH, while oxygenation was no different (Table 7.2). There was no difference between the two groups in $PaCO_2$, pH or PaO_2 observed at 12 or 24 hours post operation.

Post-operative outcomes are given in Table 7.1: thoracoscopy was associated with significantly shorter duration of postoperative intravenous opiate analgesia, and lower peak inspiratory ventilatory pressure at 24 hours post operation. There were no surgical complications in the open group. In the thoracoscopic group, one patient had an intestinal

Figure 7.4 Intraoperative blood gases during open and thoracoscopic surgery



Plots of peak intraoperative $PaCO_2$ (top row) and nadir intraoperative pH (bottom row) during open and thoracoscopic repair of congenital diaphragmatic hernia (CDH) and oesophageal atresia with tracheo-oesophageal fistula (OA/TOF). Data compared using Mann-Whitney test.

perforation, while another had a recurrence of diaphragmatic hernia. Median follow up was 56 weeks (range 12 – 92).

7.4.3 Oesophageal Atresia with Tracheo-Oesophageal Fistula

There were no cases of thoracoscopy converted to open surgery due to ventilatory concerns. In one patient, thoracoscopic OA/TOF repair was converted to thoracotomy for technical reasons (lack of proper visualisation) unrelated to hypercapnia or acidosis. There was one case where the anaesthetist requested a pause of insufflation during thoracoscopy (6 minutes) due to concerns regarding hypercapnia (which improved after hand ventilation/aspiration of secretions).

Figure 7.4 illustrates the highest $PaCO_2$ and lowest pH reached for each patient. Again there is evidence of hypercapnia and acidosis in both groups, with more extreme values attained in the thoracoscopic group: two patients out of five attained a $PaCO_2$ >14 kPa (compared to none out of five in the open group), and two patients experienced extreme acidosis with pH <7 (compared to none in the open group). Using multilevel regression modelling, no significant change in $PaCO_2$, pH or PaO_2 was observed in patients undergoing thoracoscopic repair of OA/TOF (Table 7.2). There was no difference between the two groups in $PaCO_2$, pH or PaO_2 observed at 12 or 24 hours post operation.

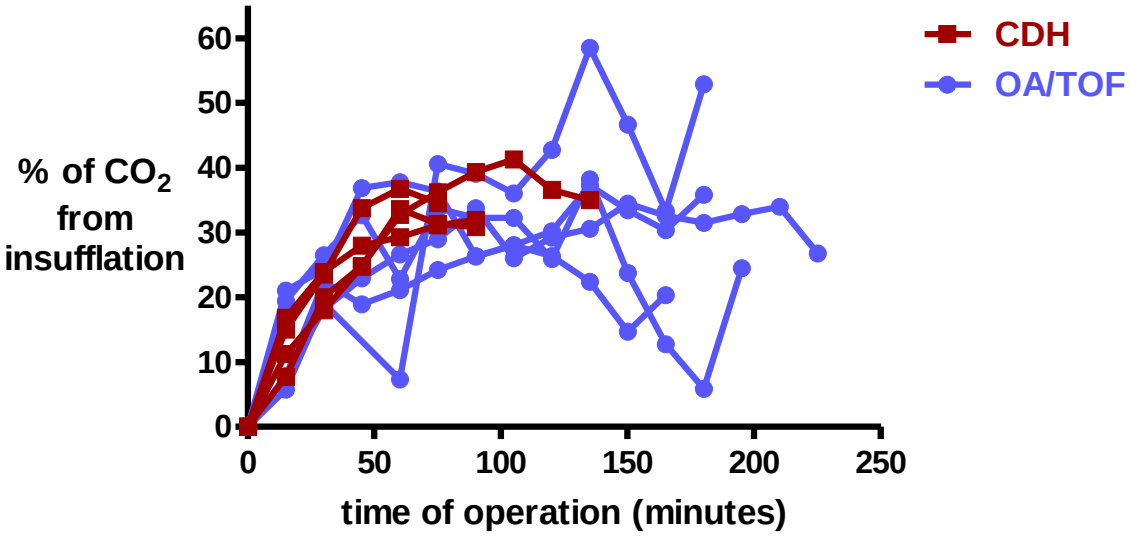
There was no significant difference in postoperative outcome between the two groups (Table 7.1), using intention to treat analysis (one patient in the thoracoscopic group was converted to open surgery). In the open group, one patient developed oesophageal stricture requiring two dilatations of the anastomosis. In the thoracoscopic group, three patients developed strictures requiring at least one dilatation (range 1-3). One of these

patients also developed an anastomotic leak repaired thoracoscopically on postoperative day 4 and was subsequently diagnosed as having an upper pouch tracheo-oesophageal fistula which required ligation via cervical approach (postoperative day 34). Median follow up was 80 weeks (range 31 – 113).

7.4.4 Analysis of Breath Samples

Analysis of $^{13}CO_2/^{12}CO_2$ enrichment in breath samples taken intra-operatively during thoracoscopy showed a progressive decrease in ppm $^{13}CO_2$ during operation, suggesting absorption of medical CO_2. No such change was observed in patients undergoing open surgery. The percentage of exhaled CO_2 originating from the insufflated medical gas was calculated, using baseline $^{13}CO_2/^{12}CO_2$ to represent zero, and the $^{13}CO_2/^{12}CO_2$ of the insufflation gas to represent 100%. Up to 39±3% (range 32 - 59%) of exhaled CO_2 was derived from insufflated gas rather than being of metabolic origin (Figure 7.5; Data are presented for 9 of the 10 thoracoscopic patients: samples from one case could not be analysed for technical reasons). There was no significant difference in absorption of insufflated CO_2 between CDH and OA/TOF.

Figure 7.5 Percentage of exhaled CO_2 originating from insufflated gas in patients undergoing thoracoscopy

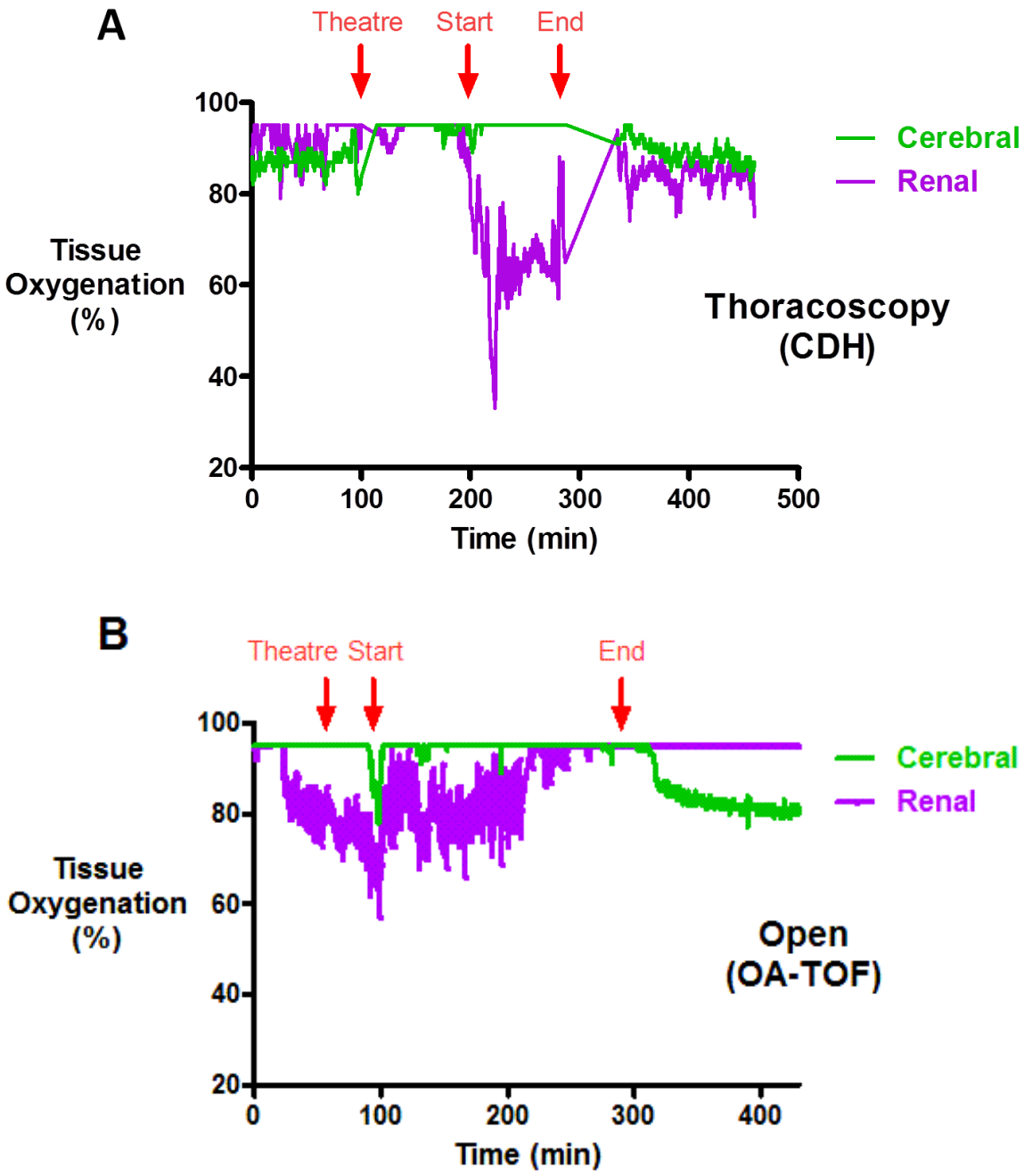


Analysis of $^{13}CO_2/^{12}CO_2$ enrichment in breath samples taken intra-operatively during thoracoscopic repair of congenital diaphragmatic hernia (CDH) or oesophageal atresia with distal tracheo-oesophageal fistula (OA/TOF) was used to calculate the percentage of exhaled CO_2 originating from the insufflated medical gas. Each line represents an individual patient.

7.4.5 Cerebral and Renal Oxygenation

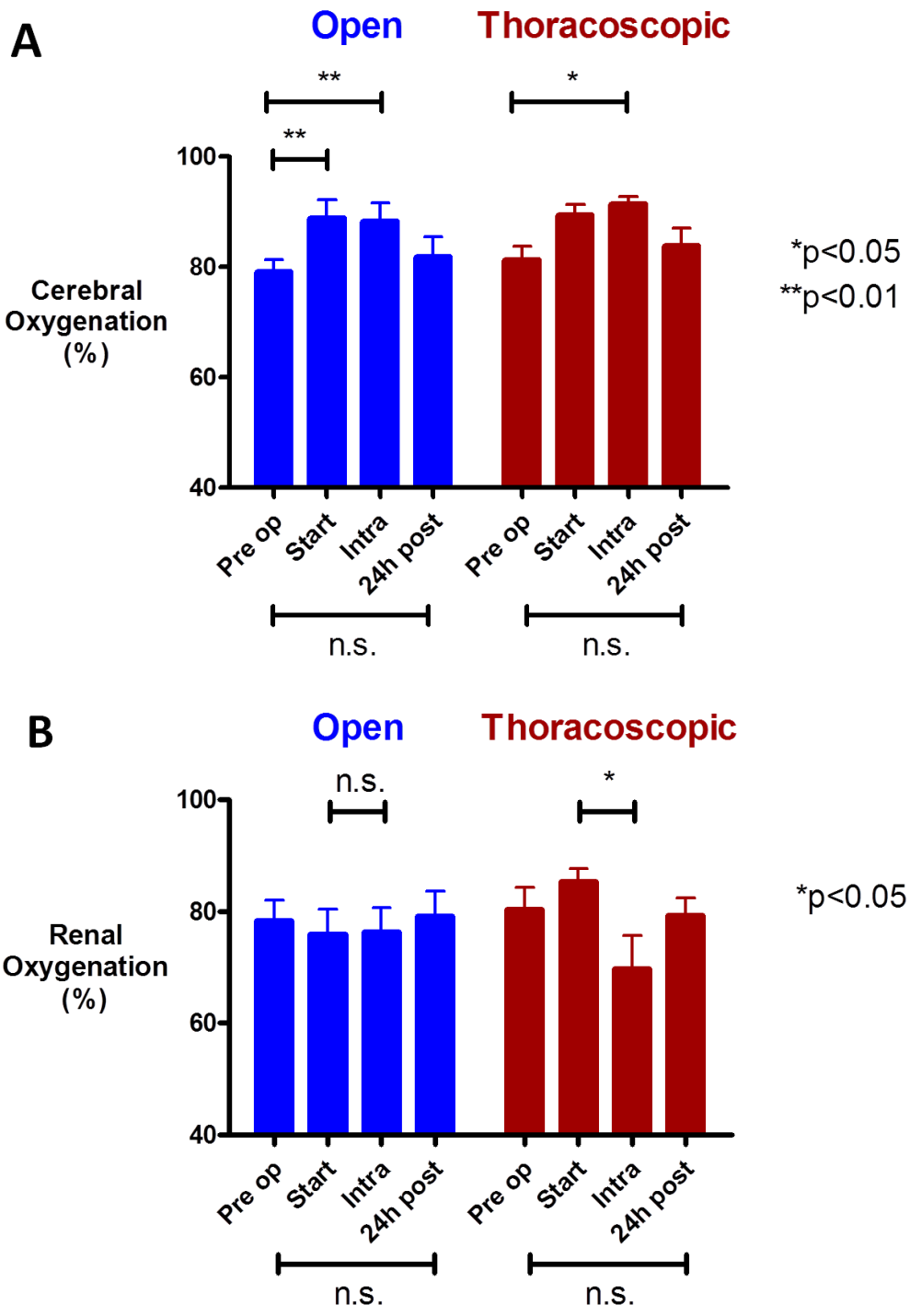
Examples of intraoperative NIRS traces are given in Figure 7.6. Levels of cerebral and renal oxygenation before, during and after operation were compared by repeated measures ANOVA with Tukey's multiple comparisons test (Figure 7.7). Both groups showed a significant increase in cerebral oxygenation during the operation compared to before transfer to theatre. Neither group showed a significant change in cerebral oxygenation from the start to the end of the operation. The open group showed no change in renal oxygenation during the operation. However the thoracoscopic group showed a statistically significant decrease in renal oxygenation during the operation ($p < 0.05$), with a mean decrease of 11±4%, which was significantly different to the open group ($p = 0.004$, unpaired *t* test; see Figure 7.8). This had recovered at 12 and 24 hours after operation. This unexpected finding of reduced renal oxygenation in the thoracoscopic group prompted me to then retrospectively compare indicators of postoperative renal function in the two groups using data from laboratory tests and intensive care records. There was no difference between the two groups in any indicators of postoperative renal function over the 24 hours following the procedure (Table 7.4).

Figure 7.6 Examples of perioperative near-infrared spectroscopy traces



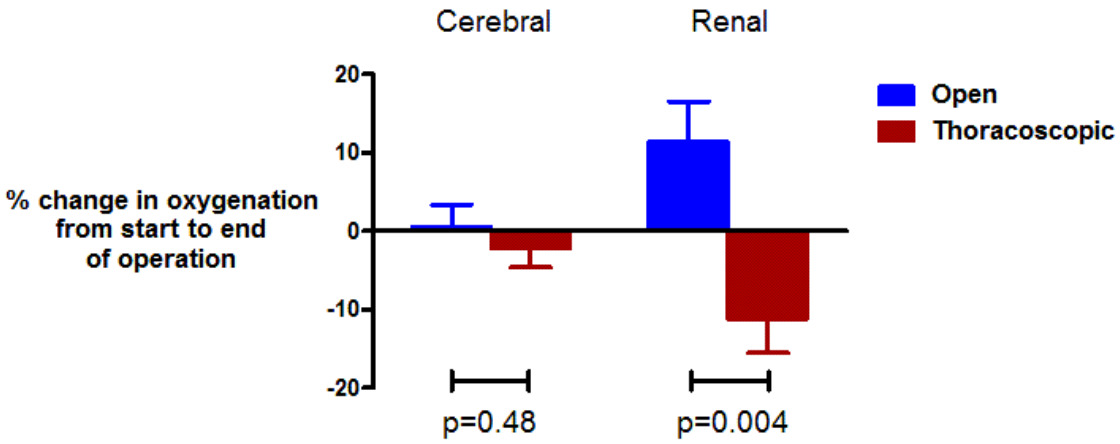
Recordings of cerebral and renal tissue oxygenation from individual neonates during (A) thoracoscopic repair of congenital diaphragmatic hernia (CDH) and (B) open repair of oesophageal atresia with distal tracheo-oesophageal fistula (OA/TOF).

Figure 7.7 Serial measurements of (A) cerebral and (B) renal oxygenation



Levels of (A) cerebral and (B) renal oxygenation before, during and after operation were compared by repeated measures ANOVA with Tukey's multiple comparisons test**.**

Figure 7.8 Percentage change in tissue oxygenation from start to end of operation



Percentage change in tissue oxygenation from start to end of operation. Data presented as mean ± SEM, compared by unpaired *t* test.

Table 7.4 Indicators of renal function in the 24 hours following operation

	Open	Thoracoscopic	P-value
Urine output (mL/kg/24hr)	78 ± 7	95 ± 13	0.27
Lactate (mmol/L)	1.8 ± 0.3	2.0 ± 0.4	0.80
Base excess (mEq/L)	-2.5 ± 1.4	-1.6 ± 1.7	0.69
Urea (mmol/L)	2.2 ± 0.4	2.4 ± 0.4	0.69
Creatinine (µmol/L)	47 ± 4	50 ± 3	0.63

Data given as mean ± SEM and compared using unpaired *t* test.

Table 7.5 shows the duration of time spent by each patient with decreases in tissue oxygenation below threshold values of either 45% tissue oxygenation index, or 80% of baseline (preoperative) tissue oxygenation. There were no decreases in cerebral oxygenation below these threshold values for more than ten minutes intraoperatively, and no decreases at all below the 45% threshold. The intraoperative data on renal oxygenation reflect the finding of a decrease in renal oxygenation in the thoracoscopic group, with periods over 30 minutes with renal oxygenation <45% in two thoracoscopic patients, and periods over 60 minutes with renal oxygenation <80% of baseline in three thoracoscopic patients. Looking at the data over the 24 hour period, there were no decreases in cerebral oxygenation below the 45% threshold for more than ten minutes. There were several patients in both groups who had long periods of tissue oxygenation <80% of baseline, but there was no difference between the two groups in the amount of time either cerebral or renal tissue oxygenation was below 80% of the baseline value (Table 7.6).

Table 7.5 Duration of decreases in tissue oxygenation

A. Duration of intraoperative decreases in tissue oxygenation in each patient, in minutes

	Open	Thoracoscopic
Cerebral oxygenation		
<80% of baseline	0,0,0,0,0,0,0,0,0,0	0,0,0,0,0,0,0,0, 1,10
<45%	0,0,0,0,0,0,0,0,0,0	0,0,0,0,0,0,0,0,0,0
Renal oxygenation		
<80% of baseline	0,0,0,0,0, 2,7,7,15,25	0,0,0,0, 1,6,11,62,68,178
<45%	0,0,0,0,0,0,0,0,0,0	0,0,0,0,0,0,0, 1,4,42,54

B. Duration of decreases in tissue oxygenation in each patient, in minutes, during the 24 hours from start of operation.

	Open	Thoracoscopic
Cerebral oxygenation		
<80% of baseline	0,0,1,1,4,8, 46,176,375,390	0,2,2,4,5, 54,99,805,1440,1440
<45%	0,0,0,0,0,0, 1,2,4,10	0,0,0,0,0, 1,1,1,1,1
Renal oxygenation		
<80% of baseline	1,3,12,47,115, 283,374,503,666,693	1,15,33,54,153, 223,280,292,1440,1440
<45%	1,1,1,1,5,6,7,7, 67,536	0,1,1,2,4,5,6,11, 44,81

Table 7.6 Comparison of duration of decreased tissue oxygenation

	Open	Thoracoscopic	P-value
Cerebral	6 (0-390)	29 (0-1742)	0.38
Renal	199 (1-693)	188 (1-1757)	0.91

Time (in minutes) that tissue oxygenation was below 80% of baseline value in the 24 hours from start of operation.
Data given as median (range), and compared using Mann-Whitney test.

7.5 Discussion

This pilot randomised controlled trial has demonstrated that thoracoscopic repair of congenital diaphragmatic hernia is associated with significantly increased hypercapnia and acidosis, compared to open surgery. Previous retrospective studies have reported hypercapnia and acidosis during thoracoscopic CDH repair (Bliss et al. 2009; Fishman et al. 2011; Gourlay et al. 2009; McHoney et al. 2010), but this is the first randomised trial to address this issue, and demonstrates a significantly more severe hypercapnia and acidosis in thoracoscopic repair compared to open surgery. In the UK, National Institute of Health and Clinical Excellence (NICE) guidelines on the use of thoracoscopy for the repair of CDH in neonates support the use of this procedure, but do not address safety concerns related to hypercapnia and acidosis (National Institute of Health and Clinical Excellence (NICE) 2011). However, in this report, NICE "encourages collection of data and publication of results" on this procedure. Although hypercapnia and acidosis are associated with adverse outcome in neonates with hypoxia, these neonates undergoing thoracoscopy did not show any decrease in arterial oxygenation, so the effects of these physiological changes on future brain development are unknown.

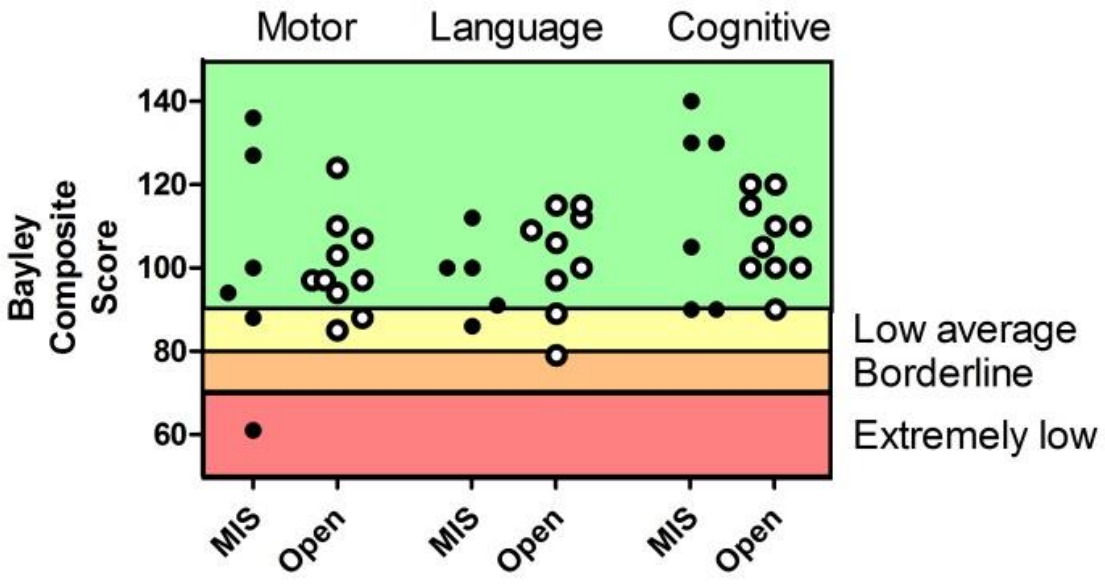
It has long been known that hypercapnia is associated with an increase in cerebral blood flow due to vasodilatation (Gibbs et al. 1935; Meng and Gelb 2015). Furthermore, during hypercapnia cerebral autoregulation (the maintenance of a constant cerebral blood flow across a range of cerebral perfusion pressures becomes impaired (Harper 1966; Meng & Gelb 2015). In severe hypercapnia, the relationship between cerebral perfusion pressure and cerebral blood flow becomes linear so that any fluctuation in cerebral perfusion pressure results in a corresponding fluctuation in cerebral blood flow. This may render

neonates more susceptible to brain injury (Del Toro et al. 1991). Hypercapnia has been shown to be a risk factor for intracranial haemorrhage in premature infants (Wallin et al. 1990). Even in adults, increases in cerebral blood flow have been found to be associated with neurological dysfunction including seizures, strokes and encephalopathy in the settings of pre-eclampsia (Barbosa et al. 2010) and cardiopulmonary bypass (Brillman et al. 1995).

My study did not demonstrate any significant effects of thoracoscopy on blood gases in neonates undergoing repair of OA/TOF, but this needs further evaluation with a larger study, given the extreme levels of hypercapnia and acidosis observed in 40% of thoracoscopic operations. As well as randomised studies, it would be helpful for data to be gathered prospectively in registries. Ideally all surgeons adopting these innovative techniques in relatively uncommon conditions should record a defined minimum dataset with an international registry. There is already a well established international registry for data on CDH (Tsao and Lally 2008).

Neurodevelopmental follow up of the children in this study was undertaken at 33 (29-36) months by a colleague (Nurain Sim), after I obtained the relevant ethical approval (as a substantial amendment to the approved protocol). Assessment using the Bayley scales of infant and toddler development – third edition (Bayley III) (Bayley 2006) was performed by a trained observer who was blinded to operative approach and intraoperative blood gases. The findings (see Figure 7.9) have been presented by N Sim at the annual meeting of the American Pediatric Surgical Association in 2014. Sixteen of the twenty patients attended for follow up. One patient from the MIS group scored ‘Extremely low’ for motor development, while one patient from the open group scored ‘Borderline low’ for

Figure 7.9 Neurodevelopmental assessment by Bayley III scores



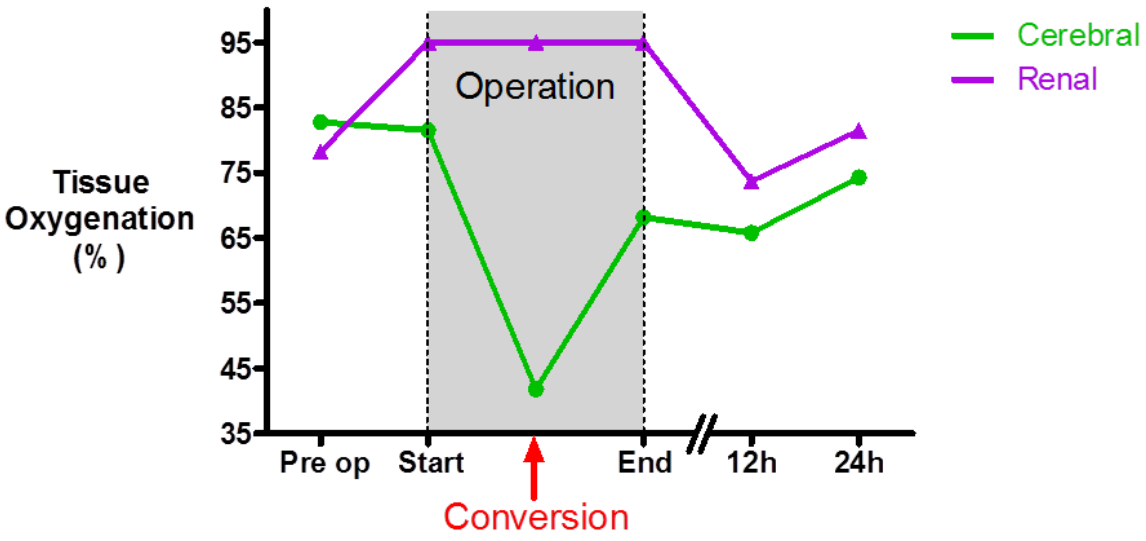
Neurodevelopmental assessment scores of the children in this study using the Bayley scales of infant and toddler development – third edition (Bayley III). Assessment was undertaken at median 33 (range 29-36) months Two patients could not be tested for language development (English not first language). Courtesy of N Sim.

language. Both of these patients had experienced prolonged intraoperative hypercapnia and acidosis. Other patients who experienced prolonged hypercapnia and acidosis scored within the normal range. There was no correlation between intraoperative acidosis or hypercapnia and motor, language or cognitive developmental scores. These findings highlight the importance of neurodevelopmental follow up in this group of patients.

This prospective study provides evidence that absorption of CO_2 from thoracic insufflation in neonates is much greater than previously reported from peritoneal insufflation during paediatric laparoscopy: reaching an average of 39% compared to 19% during laparoscopy (Pacilli et al. 2006).

This randomised trial shows no evidence of thoracoscopic repair of CDH and OA/TOF affecting cerebral oxygenation. Unexpectedly, thoracoscopy was associated with a significant decrease in renal oxygenation. This may reflect impaired venous return, or changes in haemodynamics related to acidosis and hypercapnia. Together with colleagues, I had previously performed a small preliminary observational study, in which cSO_2 was monitored in six infants, four with CDH and two with OA/TOF, undergoing thoracoscopy (Bishay et al. 2011a). This small observational study did seem to indicate an intraoperative decrease in cerebral oxygenation, associated with intraoperative hypercapnia and acidosis, though this study was too small to draw statistical conclusions. One neonate in that preliminary series undergoing repair of CDH did show a particularly striking decrease in cerebral oxygenation, which improved following conversion from thoracoscopy to open surgery (see Figure 7.10).

Figure 7.10 Changes in tissue oxygenation in a neonate undergoing thoracoscopic converted to open repair of congenital diaphragmatic hernia



Cerebral and renal tissue oxygenation were measured by near-infrared spectroscopy in a neonate undergoing thoracoscopic converted to open repair of congenital diaphragmatic hernia. There was a striking decrease in cerebral oxygenation during thoracoscopy which improved following conversion to open surgery.

A recent observational study reports a series of fifteen neonates undergoing thoracoscopic repair of OA/TOF with monitoring of cerebral oxygenation using NIRS (Tytgat et al. 2016). They did detect a very small but statistically significant drop in cSO2 following carbon dioxide insufflation: from 77% ± 10 to 73% ± 7 % ($p < 0.05$). They report that cerebral oxygenation never dropped below their designated 'safety threshold' of 55%. They do not give the reason for the designation of this threshold, but it is supported by the findings of Orihashi et al., who found that in adult patients undergoing aortic surgery with selective cerebral perfusion, a sustained drop in cerebral oxygenation (measured by NIRS) below 55% for over five minutes was associated with a significantly higher frequency of transient neurological events (44.4% versus 5.7%, $P = 0.0014$) (Orihashi et al. 2004). They do not report any measurements of renal or other somatic tissue oxygenation. The same group recently report that some children did show decreases in cerebral oxygenation below 55% during thoracoscopy for long gap oesophageal atresia (Stolwijk et al. 2017).

This pilot study was not powered to detect a significant difference in surgical complications, but I did observe more surgical complications requiring re-operation in patients undergoing thoracoscopic repair compared to those undergoing open repair. This has been shown in previous retrospective studies (Cho et al. 2009; Gander et al. 2011; Gourlay et al. 2009; McHoney et al. 2010), a meta-analysis of retrospective comparative studies (Lansdale et al. 2010), and data from an international CDH registry (Tsao et al. 2011): all demonstrate that thoracoscopic repair of CDH has a higher recurrence rate than open repair. It is difficult to ascertain how much of this is due to the effect of the learning curve. For thoracoscopic repair of OA/TOF, van der Zee et al. have reported evidence of

an institutional learning curve with a reduction in complications including anastomotic leak and stricture, and recurrent TOF in the period 2006 – 2010 compared to 2000 – 2005 (van der Zee et al. 2012). However, for CDH, the higher recurrence rate for thoracoscopy compared to open surgery persists even in recent studies from large centres. For example a recent retrospective comparative study from two large European centres (Costerus et al. 2016) found a recurrence rate of 18.9% in the thoracoscopic group (n = 75), compared to 5.9% in the open group (n = 34), p = 0.036. The same study also noted a statistically significant change in $PaCO_2$ and arterial pH in the thoracoscopic group (comparing postoperative to preoperative blood gases), which was not seen in the open group. This was not felt to be of a magnitude to cause clinical concern: the median pH decreased from 7.37 to 7.31 while the median pCO2 increased from 5.54 to 5.93 kPa. However no measure of spread and no intraoperative measurements are reported.

The current study does confirm that thoracoscopic repair of CDH may have some advantages, such as less postoperative requirement for narcotic analgesia and ventilation, as observed in a previous retrospective study (Gourlay et al. 2009). It also appears to be associated with a marked reduction in the rate of adhesional intestinal obstruction, compared to laparotomy. Comparative studies report no cases of bowel obstruction following thoracoscopic repair compared to rates of 15 to 25% following open repair by laparotomy (Gourlay et al. 2009; Inoue et al. 2016; Nam et al. 2013). Therefore if strategies can be found to minimise hypercapnia and acidosis, there may be advantages to the minimally invasive technique. One such strategy which has been reported is the use of high-frequency oscillatory ventilation during thoracoscopic repair (Liem et al. 2010; Mortellaro et al. 2011). Mortellaro et al. report 17 patients (12 with oesophageal atresia

and five with CDH) who underwent thoracoscopic repair using high-frequency oscillatory ventilation (Mortellaro et al. 2011). They report that this was successful in avoiding hypercapnia and acidosis. However, the oscillation may potentially make thoracoscopic surgery even more technically challenging than it is with conventional ventilation: the authors report a mean operating time of 208±72 minutes, compared to a median of 91 minutes (range 78 – 148) for thoracoscopic procedures in the current study. They provide no data regarding rates of surgical complications such as recurrence of CDH or oesophageal anastomotic leak. Intraoperative percussive ventilation (IPV) has been reported to be effective in preventing hypercapnia and acidosis during thoracoscopic repair of CDH (Inoue et al. 2016). In this retrospective study comparing a series of eight patients undergoing thoracoscopic repair of CDH to sixteen previous patients undergoing open surgery, the authors mention in the discussion that they "encountered temporary but severely abnormal arterial blood pH and pCO_2 values in two early cases". They report that "after implementing IPV, no study patients developed severe acidosis or hypercapnia", though they provide no quantitative data or definitions of severity. This may warrant further evaluation. Another possible strategy may be to use an alternative insufflation gas such as helium, argon, nitrogen or air for insufflations, but none of these dissolve as rapidly as carbon dioxide, resulting in a potential risk of embolisation, as well as issues of cost (Badger et al. 2008; Menes and Spivak 2000). Nitrous oxide has been used successfully for laparoscopy in adults in prospective studies (Rammohan et al. 2011; Tsereteli et al. 2002), despite earlier anecdotal reports of intra-peritoneal explosions (El-Kady and Abd-El-Razek 1976; Gunatilake 1978).

In patients with OA/TOF, thoracoscopy has a significant potential benefit in avoiding long term musculoskeletal sequelae of open thoracotomy: open repair of OA/TOF has been reported to be associated with subsequent scoliosis in up to 56% of cases (Rintala et al. 2011), while thoracoscopy has been shown to be associated with a lower incidence of musculoskeletal sequelae (including scoliosis) compared to open surgery, in infants and children requiring thoracic surgery for various indications (Lawal et al. 2009). A recent meta-analysis of studies comparing thoracoscopic versus open repair of OA/TOF (Yang et al. 2016) pooled findings from eight studies (452 patients) and found that the rates of surgical complications of anastomotic leak (OR 1.57; 95% CI 0.77–3.20; p = 0.22) and stricture (OR 0.90; 95% CI 0.27–2.97; p = 0.86) were not significantly different between the two groups. This meta-analysis also showed that although thoracoscopic repair was associated with a longer operating time, it did have some benefits in terms of time to extubation, time to first oral feed, and length of hospital stay (MD 10.76 days; 95% CI 16.39 to 5.12; p <0.001). However this was a meta-analysis of non-randomised observational studies and these apparent advantages are likely to be heavily influenced by selection bias.

The main limitation of this study is its small size, being a pilot study of relatively uncommon anomalies requiring lifesaving neonatal surgery. Nonetheless, it is a prospective randomised trial and I believe that the significant findings – of increased hypercapnia and acidosis associated with thoracoscopic repair of CDH – are valid and relevant to other institutions performing neonatal thoracoscopy. It is also of note that the main outcome was measured and managed by the anaesthetists and the findings could be interpreted as signifying that the anaesthetists simply had greater tolerance of

hypercapnia and acidosis in patients undergoing thoracoscopic surgery. Clearly this was a pragmatic study and it was not considered ethical to blind anaesthetists to blood gas results which they would normally make use of in their clinical management of the patient. It should be noted that there were two cases where the anaesthetist requested a pause of insufflation during thoracoscopy due to concerns regarding hypercapnia. It should also be noted that the study came about partly due to concerns from anaesthetists regarding the significant levels of hypercapnia and acidosis encountered during the early experience of neonatal thoracoscopy in our unit.

This pilot study shows that thoracoscopic repair of CDH is associated with profound and prolonged hypercapnia and acidosis compared to open surgery. The levels of hypercapnia and acidosis were of such concern that the trial's DMEC have advised that thoracoscopic repair of CDH should no longer be performed with this type of conventional insufflation and ventilation. The trial steering committee accepted these recommendations and the thoracoscopic repair of CDH in this way was discontinued in our institution.

7.6 Conclusions

This pilot randomised controlled trial shows that thoracoscopic repair of CDH is associated with prolonged and severe intraoperative hypercapnia and acidosis, compared to open surgery. These findings do not support the use of thoracoscopy with carbon dioxide insufflation and conventional ventilation for the repair of CDH, calling into question the safety of this practice. The effect of thoracoscopy on blood gases during repair of OA/TOF in neonates requires further evaluation.

Chapter 8. Overview, Conclusions and Future Work

These studies demonstrate that surgery in infants, while essential for these children, is associated with important complications. The use of parenteral nutrition (PN) in infants requiring gastrointestinal surgery, while it allows delivery of vital nutrients, can have significant complications, most notably infections (Chapters 2,3,4 and 6), liver disease (Chapter 5), and poor growth (Chapters 4 and 6). Meanwhile repair of oesophageal atresia and congenital diaphragmatic hernia in neonates is associated with a range of complications including postoperative complications such as oesophageal anastomotic leak or stricture, musculoskeletal deformities following thoracotomy, recurrence of diaphragmatic hernia, adhesional intestinal obstruction following laparotomy, pain, scarring, and infection, as well as intraoperative complications including bleeding, cardiorespiratory compromise, hypercapnia and acidosis. Minimally invasive surgery for these techniques has been introduced in the hope of reducing some of these complications, but brings complications of its own (Chapter 7).

With regard to the latter, I have conducted and reported a prospective randomised controlled trial investigating the effects of minimally invasive surgery for OA/TOF and CDH on intraoperative arterial blood gases (Chapter 7). I found that neonatal thoracoscopy was associated with more severe hypercapnia and acidosis compared to open surgery, particularly in patients with CDH. This calls into question the safety of thoracoscopic repair of CDH and highlights a need for further studies examining the neurodevelopmental outcomes of infants undergoing neonatal thoracoscopy. Further and larger (i.e. multicentre) randomised studies should be performed to determine the role of neonatal thoracoscopy in repair of OA/TOF and CDH, including evaluating different ventilatory techniques (such as intraoperative percussive ventilation or high frequency

oscillatory ventilation) to reduce hypercapnia and acidosis, comparing rates of complications such as recurrence of CDH and leakage or stricture at oesophageal anastomosis, and evaluating long-term neurodevelopmental outcome. In addition, data on neonatal thoracoscopy for these conditions should be gathered prospectively in international registries.

Studying infants receiving parenteral nutrition after abdominal surgery, I showed that the introduction of chlorhexidine antisepsis when accessing central venous catheters (CVCs) was associated with significantly decreased infection rates in surgical infants, halving the rate of septicaemia (Chapter 2). This effect was largely due to a decrease in coagulase-negative staphylococcal septicaemia. However, the persistently high rate of infections and the failure to match the rate of infection reduction seen in other patients with CVCs suggest that routes of infection other than CVC-related are important in this group of patients. This is borne out to some extent by the persistent level of enteroccocal infections after the introduction of chlorhexidine; while there was a significant reduction in overall septicaemia, and in coagulase-negative staphylococcal septicaemia, I found there was no corresponding decrease in the rate of enterococcal infection, which may be more likely to originate from the gastrointestinal tract. It therefore seems likely that endogenous routes, such as translocation from the gastro-intestinal tract, play a significant role in causing infection in these infants with gastrointestinal anomalies, as well as the exogenous route of infection via CVCs.

In Chapter 3, I showed that among surgical infants receiving PN for gastrointestinal disease, septicaemia due to enterococci or Gram-negative bacilli occurs later in the course of PN (after at least three weeks in this study) than septicaemia due to coagulase-negative

staphylococci. This suggests that these infants become more vulnerable to translocation of enteric micro-organisms after a longer period of parenteral nutrition.

In Chapter 4, I examined the outcomes of managing congenital duodenal obstruction with or without parenteral nutrition. I demonstrated that infants with congenital duodenal obstruction can often be managed with gastric feeds without PN or a transanastomotic tube (an approach which is not widely reported in the modern literature). However, while avoidance of initial PN was successful for almost two thirds of cases in which it was attempted, the remaining third went on to require PN, and this group showed poorer growth than children who commenced PN soon after surgery; a fact which must be balanced against the advantages conferred upon the majority. This illustrates the difficulties of trying to weigh up the advantages and disadvantages of parenteral nutrition in babies where the indication is not always clear, and the postoperative course is unpredictable.

In Chapter 5, I found that one third of surgical infants with intestinal failure (defined as receiving PN for at least 28 days) develop intestinal failure associated liver disease (IFALD), and a remarkable 61% developed septicaemia. Surprisingly, I found no association between septicaemia and the development of IFALD in these infants. I also unexpectedly found an association between female sex and more severe IFALD, which warrants further investigation.

In Chapter 6, I conducted and reported a prospective randomised controlled trial investigating the effects of parenteral and enteral glutamine supplementation on microbial invasion. Overall, the study showed no effect of glutamine supplementation on microbial

invasion in surgical infants. There was a high incidence of microbial invasion in these patients, with 60% showing evidence of microbial invasion at some point. Only half of this was detectable by conventional blood culture. To my knowledge, this is the first study to apply these techniques in this group of patients.

Planned secondary outcome analysis showed that glutamine supplementation did have a significant effect on monocyte HLA-DR expression, which is reduced post operatively, and rises thereafter. This suggests that glutamine helps the recovery of immune function after surgery. Further analysis shows that low levels of monocyte HLA-DR expression were both predictive of and associated with clinical sepsis in this study. I surmised that this could be used to identify a high-risk category of infants who may particularly benefit from interventions to prevent microbial invasion. Analysis confirms that among patients who had a low monocyte HLA-DR expression at enrolment, glutamine does appear to be protective against microbial invasion.

Study in this group of patients has always been challenging due to the relative rarity of the individual conditions, leading to studies such as those reported here, which are limited by a significant degree of heterogeneity. Application of the laboratory techniques used here can allow the selection of a subgroup of patients who may be similar in terms of their immune status and vulnerability to infection/microbial invasion, allowing the demonstration of effects which were not apparent in the larger group. This opens the possibility of such parameters being used in future to tailor nutritional support to the immune status of the individual infant in order to prevent infections. This should be explored further with a trial of glutamine supplementation in selected infants with immunoparesis (infants selected based on low monocyte HLA-DR expression). This

could work by recruiting patients according to the same criteria as I used in the MIGS trial, but explaining that immune function will be tested at enrolment and only patients found to be more vulnerable to infection will continue in the study (those who do not meet the criteria for immunoparesis would be excluded). Standardised techniques exist which would allow these measurements to be used in a multicentre study. Ultimately this line of investigation could lead to biomarker-guided tailored immunomodulation (with glutamine and/or cytokine therapies such as GM-CSF) to prevent infections in these infants. Another promising avenue for further study is to apply new high-throughput molecular techniques targeting 16S rDNA to characterise and analyse the gut microbiome in this group of patients and look for differences in those patients who experience bloodstream infections. This could then guide and inform interventions such as the use of selective bowel decontamination or probiotics.

This thesis has focussed on complications of two major innovations in neonatal surgical practice: postoperative parenteral nutrition (introduced and disseminated 40-50 years ago) and neonatal thoracoscopy (introduced and taken up over the last 10-20 years). Parenteral nutrition has transformed outcomes for neonates with certain severe gastrointestinal diseases, while neonatal thoracoscopy has the potential to reduce complications and speed recovery from surgery to correct life-threatening congenital anomalies. However, this thesis has shown that both innovations have entailed significant challenges and complications: infections, liver disease, and poor growth with parenteral nutrition, and intraoperative hypercapnia and acidosis in neonatal thoracoscopy. This work has applied a wide range of innovative techniques to learn more about these complications, and the findings can guide the development of strategies to prevent these complications, most

notably by suggesting that a selected subgroup of patients with more severe immunoparesis can benefit from glutamine supplementation to enhance the recovery of immune function and prevent microbial invasion.

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10. Appendices

1. MIGS Trial Protocol
2. MIGS Trial ethical approval
3. MIGS Trial CONSORT checklist
4. CO_2 Trial Protocol
5. CO_2 Trial ethical approval
6. CO_2 Trial CONSORT checklist
7. Publications

MIGS:
Microbial Invasion and Glutamine Supplementation Study

Protocol Full Title: *Microbial invasion during parenteral nutrition in surgical infants receiving glutamine*

Sponsor's Protocol Number: *07 SG 10*

Version 2.0 11/05/2009

Sponsor: Great Ormond Street Hospital, Great Ormond Street London WC1N 3EH

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Contents

1. Brief Summary

The rate of infection in surgical infants requiring parenteral nutrition is very high. In a multicentre randomised controlled trial in surgical infants we demonstrated that glutamine supplementation reduces the risk of developing clinically evident sepsis during total parenteral nutrition but not during the subsequent period of partial parenteral with partial enteral feeding. It is not clear if the effect of glutamine is related to a reduction in bacterial invasion, or whether this effect could be extended to the period of partial parenteral/enteral feeding.

We propose a double blind randomised controlled trial comparing surgical infants receiving glutamine supplementation (study group n=30) with a control group receiving isonitrogenous amino acids (control group n=30). The supplementation will be performed during both total and partial parenteral/enteral nutrition. The primary end point will be evidence of microbial invasion as demonstrated by: positive blood cultures, detection of bacterial DNA by both conventional single-target and broad-range 16S PCR, elevated levels of plasma endotoxin or plasma lipopolysaccharide binding protein.

This trial is highly relevant to a large number of surgical infants in the UK and abroad.

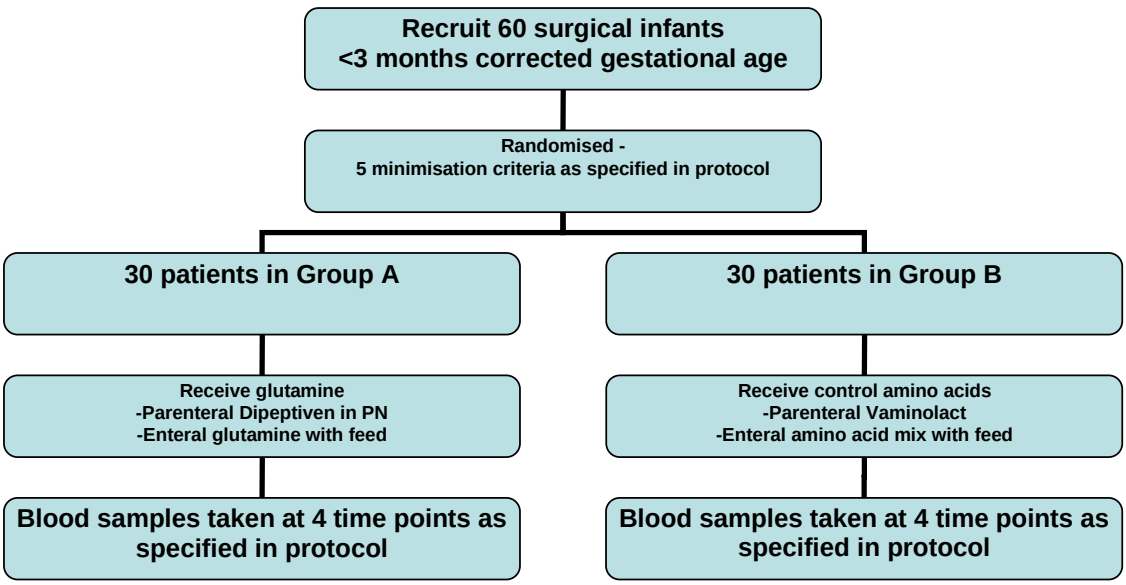

Figure 1. Flowchart of trial design

2. Background

Newborn infants with congenital or acquired gastrointestinal anomalies commonly require parenteral nutrition postoperatively because they are unable to tolerate enteral feeds. Up to one third of these infants may develop sepsis after surgery while receiving parenteral nutrition (PN) (1;2), leading to impaired liver function (2;3), critical illness and removal of central venous catheters.

Microbial invasion
It has always been assumed that the central venous catheter is the major portal of entry for micro-organisms causing septicaemia in these patients (4). However, previous research from our group and others (5) indicates that the gut is another major route for invasion by micro-organisms. The gastrointestinal tract is the principal source of bacteria or microbial products entering the blood because of its massive bacterial load compared to other anatomical sites (6).We have reported that almost half the surgical infants with congenital or acquired gastro-intestinal dysfunction develop abnormal flora and that all cases of septicaemia were preceded by gut colonisation with abnormal flora (2;3). We have also described the migration of micro-organisms from the intestinal lumen to the systemic circulation in surgical neonates with intestinal overgrowth (2). Major difficulties with the investigation of microbial infection in neonates are the small volumes of blood available and the high use of antibiotics. Therefore blood cultures are an insensitive means of detecting microbial invasion. In the last few years, molecular techniques for detecting bacterial DNA and sensitive methods to measure bacterial invasion have been developed. These provide new opportunities for investigating infections in neonates.

Glutamine supplementation
Infants receiving PN may have an increase in gut permeability (7;8) and eventually lose gut mucosal integrity (9). These alterations may lead to increased bacterial translocation (10) and sepsis and are directly related to the duration of parenteral nutrition. These complications may be due to a deficiency in nutritional components which might normally be derived from human breast milk and other enteral feeds.

Glutamine is the most abundant free amino acid in the human body and is found in large quantities in breast milk. It plays key roles in many metabolic pathways, is a preferential substrate for enterocyte (11) and leukocyte metabolism (12), and is a major interorgan nitrogen transporter (13). Endogenous production in organs such as the liver and kidneys is normally sufficient for metabolic demand. However, in conditions of physiological stress such as surgery, critical illness and sepsis, endogenous glutamine stores may dwindle as consumption exceeds production. Thus, it has been considered to be a conditionally essential amino acid (14-16). In surgical neonates who are at risk of glutamine deficiency, this

relative deficiency may be exacerbated due to the lack of enteral feeding as currently PN does not routinely contain glutamine.

Previous Trial
We have completed a prospective double-blind multi-centre randomised controlled trial (SIGN Trial – submitted for publication) in surgical neonates less than 3 months old who required surgery and PN because of gastro-intestinal dysfunction. Infants received either 0.4 g/kg/day glutamine (treatment group) or isonitrogenous isocaloric PN (placebo group) until full enteral feeding was achieved. 174 patients were randomized of which 164 completed the trial and were analysed (glutamine n=82, placebo n=82). The incidence of clinically evident sepsis (43-51%) and proven septicaemia (22-33%) were both very high in this trial. Glutamine had no effect on overall incidence of clinically evident sepsis (51% glutamine vs. 43% control (p=0.27)) or septicaemia (32% vs. 22% (p=0.16)).

However, during total PN, i.e. before the first enteral feed was introduced, glutamine significantly reduced the risk of developing clinically evident sepsis by 68% (relative risk 0.32 [95% confidence intervals 0.15-0.69] p=0.004) but did not appear to have any effect on the risk of having a positive blood culture. In addition, in those patients who did have clinical evidence of sepsis, the time to the first episode of sepsis was significantly longer (12 days) in the glutamine group compared to 4 days in the placebo group (p<0.0001). In this trial we did not supplement glutamine enterally and we did not directly investigate whether the glutamine effect on sepsis during total PN was related to increased microbial invasion.

3. Trial Objectives

The aim of the study is to ascertain whether the addition of glutamine supplementation to both parenteral nutrition and enteral feeds in surgical newborn infants leads to a reduction in bacterial invasion.

Primary end-point: The focus of this trial is to investigate bacterial invasion in surgical infants receiving PN. Evidence of bacterial invasion will be sought by detecting any of the following

(i) positive blood cultures

(ii) presence of bacterial DNA in the blood by both conventional single-target and broad-range 16S PCR (20;21)

(iii) elevated levels of endotoxin (defined as greater than 50 pg/ml (22))

(iv) elevated plasma lipopolysaccharide binding protein (LBP) (defined as greater than 13 µg/mL (23)).

Based on the timing of sepsis in our previous trial (the SIGN Trial), evidence of microbial invasion (primary endpoint) will be sought at 5 days after starting PN. Blood samples will also be taken at the beginning of the trial, at the introduction of first enteral feeding and when full enteral feeding is achieved.

Secondary end-points:

Other parameter indicative of microbial invasion: We will also measure the EndoCAb (also termed endotoxin-core antibodies) which in conditions of acute microbial translocation such as sepsis, bind and clear the LPS from the circulation (24).

Monocyte HLA-DR expression: Decreased monocyte HLA-DR expression is predictive of post-operative infection (25) and is related to outcome and survival. We have recently shown in a small double-blind randomised controlled trial that glutamine administration (in the form of alanyl-glutamine, Dipeptiven) to septic infants and children prevents the fall in monocyte HLA-DR.

Serum amino acid profile (including glutamine level) will also be measured.

Infection*:* Episodes of sepsis and septicaemia, timing of sepsis and septicaemia (days from start of PN). Sepsis is defined (26) as the clinical state of generalised inflammation manifested by at least 3 of the following clinical signs: fever, hyperthermia (>38°C) and/or hypothermia (<36°C), lethargy, poor perfusion, age-related tachycardia and tachypnoea, and hypotension.

At the time of suspected sepsis, blood samples will be taken for culture and tested for other evidence of bacterial invasion as described above. Septicaemia is defined as sepsis combined with a positive blood culture drawn through the catheter and/or at a peripheral site (2;3;27).

Intestinal flora: surveillance samples will be obtained to investigate the status of intestinal flora (2;3). Surveillance cultures of the oropharynx and gut will be obtained at the beginning of the study and thereafter twice each week. These cultures will be processed for all microorganisms in a semiquantitative manner to detect overgrowth (2;3).

Intestinal permeability: this will be measured by 3-*O*-methyl glucose, mannitol and lactulose uptake and recovery in urine (28). This will be undertaken at the same time points illustrated for primary end point.

Intestinal function*:* Time to full enteral feeding and time on PN (days). Time to full enteral feeding is defined as the time (days) required to reach adequate calorie intake orally and /or enterally (when at least 75% of expected average requirement (29) is given enterally).

Survival/mortality will also be noted.

4. Trial Design

This study will be a double blind randomised controlled trial, carried out at Great Ormond Street Hospital for Children NHS Trust. Infants (corrected gestational age <3 months) requiring parenteral nutrition (PN) and surgery for congenital or acquired gastrointestinal anomalies will be eligible. These would include infants with gastroschisis, necrotising enterocolitis, bowel atresia or intestinal surgery for other reasons.

These infants will initially receive a period of exclusive intravenous feeding (total PN), followed by a gradual increase in enteral feeding and a decrease in PN (mixed PN and enteral) until full enteral feeding is achieved.

The decrease in PN and concomitant increase in enteral feeding will be carried out as per the usual clinical practice. Parenteral feeding will be stopped when more than 75% of the patient's full nutritional requirement is tolerated enterally for a minimum of 24 hours. Those with inborn errors of metabolism, immune deficiency, renal failure, PN already given for more than 5 days or enrolled in another trial at the same time will be excluded.

Group allocation: Infants will be randomised to receive glutamine supplementation (study group) or isonitrogenous amino acid supplementation (control group).

Group A will receive glutamine supplementation using parenteral Dipeptiven® and enteral glutamine.
Group B will receive isonitrogenous amino acids, using parenteral Vaminolact® and amino acid mix as enteral supplement.

Allocation to the groups will be made using minimisation(17) based on the following 5 criteria:

i) length of functional small bowel (no intestinal resection [i.e. normal small bowel length] / remaining small bowel length ≥30 cm / remaining small bowel length <30 cm);
ii) diagnosis (congenital intestinal obstruction / congenital defect of the abdominal wall / necrotizing enterocolitis / other);
iii) gestational age (derived from last menstrual period) at the time of enrolment in the study (<30 weeks/ 30 - 36 weeks / >36 weeks);
iv) ileo-caecal valve in continuity (yes/no);
v) weight at the time of enrolment in the study (<1 kg / 1-2kg / >2 kg).

The clinical team will be blind to the child's allocation but the hospital pharmacist who prepares the PN solution and the dietician preparing the enteral glutamine supplementation will be unblinded. Written informed

consent for randomisation will be obtained from the parents at the time of enrolment. Parents will have at least 24 hours to make an informed decision.

Intervention: Parenteral glutamine will be given as a chemically stable dipeptide solution (Dipeptiven®, Fresenius-Kabi, Runcorn, Cheshire, UK; L-alanyl-L-glutamine 200 mg/ml) in a dose of 0.4 g/kg/day glutamine equivalent to 0.6 g/kg/day Dipeptiven® which ensures that the nitrogen intake of the intervention and control infants is equal and that no more than 35% of the total nitrogen intake will be provided by Dipeptiven®. The dose proposed is equal to that used in our previous randomised controlled trial which demonstrated that there were no negative effects from administration of glutamine and no abnormal levels of serum ammonia, urea nitrogen and glucose. This level is also based on our published research which has confirmed beneficial effects on enteral mucosa at this dose (18).

There are no reported adverse effects of glutamine intravenous administration. The amount of glutamine that we intend to administer in the intervention group has been safely administered in infants and children (18) and higher amounts have been administered to low birth weight infants with no adverse effects (19).

The control group will receive isonitrogenous Vaminolact® (Fresenius-Kabi, Runcorn, Cheshire, UK; this contains no glutamine). PN solutions will be prepared by the hospital pharmacy and enclosed in identically coloured external bags. Prescriptions and labels will specify the amount of nitrogen and not its source. Supplementation of PN with glutamine will continue for the entire duration of PN.

The doses of both solutions used will provide the same amount of nitrogen equivalent to 0.116g of nitrogen/kg/day. The PN provided for the duration of the study for both groups will have the appropriate amount of nitrogen reduced in their PN for that day.

It is difficult to maintain parenteral glutamine supplementation during the period of partial enteral feeding, as the intake of other parenteral amino acids would have to be reduced, and stability issues could also become a problem. During the period of partial enteral feeding, in which the parenteral intake of glutamine/placebo is reducing, we will supplement the enteral diet with the balance which is no longer being given parenterally. This glutamine will be given as Adamin-G® (SHS International Ltd, Liverpool, UK). The control group will receive Complete Amino Acid Mix (SHS International Ltd, Liverpool, UK; contains 0.7% glutamine).

We will restrict the enteral diet to: breast milk, SMA Gold, SMA LBW Gold, Pepti-Junior, Neocate and modular feeds, and we will measure the glutamine content of each of these.

5. Randomisation

Patients will be randomised using the Simin software. This will be carried out by Dr Simon Eaton (or other trained persons), thereby leaving the investigators fully blinded. This software has been validated as a randomisation program following its use in three large randomised international studies. The patient allocation will be recorded, placed in a sealed envelope, and given to pharmacy for preparation of parenteral solutions. The patient allocation will thus be recorded in pharmacy.

Procedure for breaking randomisation codes – It would be unlikely that breaking randomisation would be needed out of hours, given that there is no restriction on medical management of patients and that there are no known adverse effects of the supplements used. In any event, in the case of emergency, the PI would be contacted via hospital switchboard and he will contact Resident Pharmacist to break the code when necessary.

6. Sampling

Blood samples will be taken at the start of the study, at day 5, once enteral feeds commence and at the completion of the study for the primary endpoint. These samples will be taken from the indwelling central line at the same time as the line is accessed for change of parenteral nutrition.

Intestinal permeability will be assessed using a stable isotope method at the start of enteral feeds and once the study is finished. This will be done by giving 1 mL/kg via feeding tube of 30 mg/mL 3-*O*-methyl glucose, 20 mg/mL mannitol and 30 mg/mL lactulose (osmolarity 352). Urine will be collected for 8 hours from the nappy of patients enrolled.

Follow-up samples and data may be collected at University College London Hospitals NHS Trust if patients are transferred there before enteral feeding is established.

Samples will be stored in the freezers at the Paediatric Surgery Department /Department of Infectious Diseases and Microbiology at the Institute of Child Health which comply with the Human Tissue Act 2006.

7. Data Monitoring and Trial Steering Committees

A Data Monitoring and Ethics Committee will be convened. This will be independent of both the trial organisers and those providing therapy. This

committee will perform interim analyses to: a) review assumptions underlying sample size considerations; b) modify or close intake to trial.

The membership of the Committee will be:

1) Consultant Surgeon not involved in trial (Chairman)
2) Consultant Paediatrician not involved in trial
3) Statistician

The first interim analysis will be done after 30 patients are recruited. The criteria for stopping the trial will be:

1) A significant difference between the two arms of the study in the primary endpoint $p<0.01$
2) Excess episodes of sepsis or mortality in one arm of the trial

A Trial Steering Committee will also be convened, whose members include:

1) The trial co-ordinators (A Pierro, S Eaton, N Klein, M Bishay)
2) A representative of the Data Monitoring and Ethics Committee.

The role of this Committee is to provide overall supervision of the trial and ensure that the trial is conducted to rigorous scientific, clinical and ethical standards. It will particularly concentrate on progress of the trial, adherence to the protocol, data collection and maximise the chances of completion within the agreed timetable. This committee will meet six monthly or more frequently if required by either the Trial co-ordinator or the Data Monitoring Committee representative.

8. Data management

All patient data will be recorded onto an electronic record and we will use direct transcription to Case Report Forms.

Trial Subjects will be coded for anonymity to comply with the data protection act. We will use our Sponsor Protocol number followed by a logical sequence of numbering e.g. 07SG10:01, 07SG10:02 etc.

All clinical observations and laboratory results will be recorded on a secure database, to be stored on secure ICH computers. The Principal Investigator shall be responsible for recording and entering all data in this study.

The investigators as previously detailed will permit regular data review by the Data Monitoring Committee. The data will be freely available for audit, and it is planned that regular reports will be filed with the local REC.

9. Selection and Withdrawal of Subjects

Identification of possible candidates for the study will be made when admitted as inpatients to Great Ormond Street Hospital. Parents or legal guardians will be approached and offered informed consent.

Inclusion criteria: Infants (corrected gestational age <3 months) requiring PN and surgery for congenital or acquired gastrointestinal anomalies will be eligible. These would include infants with gastroschisis, necrotising enterocolitis, bowel atresia or intestinal surgery for other reasons.

Exclusion criteria: Those with inborn errors of metabolism, immune deficiency, renal failure, PN already given for more than 5 days or enrolled in another trial at the same time will be excluded.

Withdrawal criteria: patients will be withdrawn if there are thought to be adverse effects directly related to the study. Patients may also be withdrawn by parental request. Laboratory data would still be gathered if the withdrawn patients still required parenteral fluids. Subjects would be replaced to ensure statistical power.

10. Statistics

In our previous trial (the SIGN Trial), 50% of infants had at least one episode of clinical sepsis, and during total parenteral nutrition, glutamine decreased the risk of sepsis by 68% using a Cox proportional hazards model. Assuming that 50% of surgical infants have some evidence of microbial invasion at 5 days, using the primary endpoints described above, and that glutamine decreases this risk by 68%, 30 infants in each arm would be required to detect this difference at 80% power, alpha = 0.05. We are confident that we will recruit 60 patients in the time frame of the study.

30 patients in each group are to be recruited over 30 months.

Termination of the trial will occur once 60 patients have been recruited or if the stopping criteria are reached at interim analysis as described above (page 10).

Any deviations from the initial statistical plan will be documented in a revised version of this protocol and distributed to REC, Research and development, and all other involved groups.

Patients will be allocated to the two study groups using minimisation to ensure that the groups are comparable with respect to the 5 criteria outlined in Trial Design above (page 7).

Glutamine and isonitrogenous solutions will be labelled identically and there will be no means by which the investigators or clinical carers can know to which group the patients will be allocated. Comparisons will be made between patients in Group A and B before and after glutamine or isonitrogenous amino acids administration. SPSS software will be used for the statistical analysis.

11. Ethics

The trial will be conducted in compliance with the ethical principles enunciated in the declaration of Helsinki 1996, and in accordance with Good Clinical Practice.

12. Financing and insurance

This study is supported by a grant from the children's charity SPARKS. All financial aspects of the grant are managed through the R&D department of GOSH/ICH.

Negligent fault is covered by the NHS CNS. Indemnity and insurance has been applied for under the No Fault Compensation Policy Held by UCL.

13. Publication Policy

Data will be published in medical journals and at relevant conferences. All of the applicants on the grant application are likely to appear as co-authors of work, along with any number of surgical consultants involved in the routine care of children enrolled. Patients and their GPs will have access to a summary of the results of this trial. A full report will be submitted to the REC, and R&D department at ICH.

14. References relevant to and providing background for the trial

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Signature of Chief Investigator …….
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Nuffield Professor of Paediatric Surgery

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30 April 2008

Prof Agostino Pierro
Nuffield Professor of Paediatric Surgery
Institute of Child Health
30 Guilford St
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Dear Prof Pierro

Full title of study: **Microbial invasion during parenteral nutrition in surgical infants receiving glutamine**
REC reference number: **08/H0713/31**

The Research Ethics Committee reviewed the above application at the meeting held on 29 April 2008.

Ethical opinion

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission at NHS sites ("R&D approval") should be obtained from the relevant care organisation(s) in accordance with NHS research governance arrangements. Guidance on applying for NHS permission is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Application	1	18 March 2008
Investigator CV	1	
Protocol	1	18 March 2008
Covering Letter	1	
Letter from Sponsor	1	06 September 2007
Participant Information Sheet	1	18 February 2008
Participant Consent Form	1	18 February 2008
Response to Request for Further Information	1	23 April 2008

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Website: After Review

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nres.npsa.nhs.uk.

08/H0713/31	Please quote this number on all correspondence

With the Committee's best wishes for the success of this project

Yours sincerely

[redacted]

Dr Victor Larcher
Chair

Email: a.gordon@ich.ucl.ac.uk

Enclosures: *"After ethical review – guidance for researchers"*
Site approval form (SF1)

Copy to: *Dr Traci Assari*
[R&D office]

CONSORT 2010 checklist for MIGS trial – Chapter 6

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	136
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	137
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	139
	2b	Specific objectives or hypotheses	141
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	143
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	157, 159
Participants	4a	Eligibility criteria for participants	143
	4b	Settings and locations where the data were collected	143
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	145
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	146
	6b	Any changes to trial outcomes after the trial commenced, with reasons	157
Sample size	7a	How sample size was determined	143
	7b	When applicable, explanation of any interim analyses and stopping guidelines	161
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	144
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	144
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	144,146
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	145
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	146

	11b	If relevant, description of the similarity of interventions	145
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	158
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	158
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	160
	13b	For each group, losses and exclusions after randomisation, together with reasons	159-161
Recruitment	14a	Dates defining the periods of recruitment and follow-up	159
	14b	Why the trial ended or was stopped	161
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	162
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	163ff
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	163ff
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	163ff
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	174
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	161
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	182ff, esp 186ff
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	188
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	182-188
Other information			
Registration	23	Registration number and name of trial registry	144
Protocol	24	Where the full trial protocol can be accessed, if available	143 (App 1)
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	144

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

CO_2 Trial Protocol
Version 1.0
Date: 07/01/2009

The CO_2 TRIAL

Hypercapnia during thoracoscopy or open surgery for repair of oesophageal atresia with tracheoesophageal fistula or congenital diaphragmatic hernia in neonates

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1. Background

After the success of minimally invasive surgical techniques in adults, application in paediatric patients was a logical next step. The use of these technique in young children spread slowly, however, because the surgical instruments had to be downsized, the learning curve was relatively long, and safe and reliable anaesthetic procedures had to be developed to ensure good tolerance of pneumoperitoneum and pneumothorax.

Recently, progress has accelerated and the number of procedures that are being performed in children is rising rapidly.[1]
Increasingly younger patients now benefit from these techniques, with laparoscopy and thoracoscopy in neonates among the most recent applications[7,8].
Nevertheless, the potential impact of carbon dioxide pneumothorax on an immature neonatal cardiopulmonary system is a matter of great concern.[2]

Relatively few studies reporting on the cardiorespiratory consequences have been published, and most of those that support the feasibility and the safety of these methods in the first month of life are case reports or short clinical series.[3]
The advent of this new surgical procedure in such young children, given their cardiovascular, pulmonary, and thermoregulatory specificities, nevertheless requires a thorough evaluation of its tolerance.[4,5]

Carbon dioxide is commonly used for pneumothorax in children, as it is non-combustible, inexpensive and least likely to cause embolism[12].
Thoracoscopy can result in haemodynamic changes and may lead to adverse effects on the cardiovascular system requiring increasing minute ventilation to prevent hypercapnia. It has been demonstrated that small children eliminate relatively more carbon dioxide during laparoscopy and thus required scrupulous anaesthetic management.[6,10,11,13,15]

There has not been a prospective evaluation of carbon dioxide absorption in infants undergoing thoracoscopy.[9]

2. Aims

The aim of this study is to evaluate the tolerance of thoracoscopic surgery in newborn and infants and to determine the risk factors of perioperative and postoperative complications particularly due to carbon dioxide insufflations and brain oxygen saturation.

3. Research Objectives

To test our hypothesis, we aim to achieve the following *research objectives* in infants and newborns with oesophageal atresia and tracheoesophageal fistula, or congenital diaphragmatic hernia

1. to compare the changes in arterial blood gases during thoracoscopic surgery with those occurring during open surgery

2. to measure absorption of exogenous carbon dioxide in expired breath during thoracoscopy in infants using a isotope-ratio mass spectrometry method[14,16]
3. establish the hemodynamic, metabolic and thermic effects of thoracoscopy in infants and newborn
4. to determine differences between oxygenation of the brain (measured by a non-invasive technique, near infra-red spectroscopy)[18], arterial carbon dioxide levels (measured transcutaneously using a probe placed on the skin)[17]

4. Methods

A single centre pilot randomised trial will be conducted in 20 infants and newborns who will undergo surgical repair of oesophageal atresia with tracheoesophageal fistula [OA+TOF] or congenital diaphragmatic hernia [CDH] with thoracoscopic or open surgical approach at Great Ormond Street Hospital for Children, London, UK

OA + TOF can be repaired by two approaches (i) open surgery, requiring a thoracotomy, or (ii) minimally invasive surgery (via thoracoscopy).

CDH can be repaired by two approaches (i) open surgery, which is best performed via a laparotomy, or (ii) minimally invasive surgery, which is best performed via the chest and not through the abdomen. The reason why, in minimally invasive surgery, the operation for CDH is best performed through the chest is due to the fact that insufflation of the chest reduces the hernia and allows an easier closure of the defect in the diaphragm. Conversely, in open surgery, where insufflation cannot be given, if operation for CDH is performed through the chest, the hernia cannot be reduced manually and the defect is more difficult to close. For this reason, if open surgery is performed, the CDH is best repaired from the abdomen where the hernia is reduced manually and the defect closed more easily.

The aim of this pilot study is to compare CO_2 levels during open surgery [thoracotomy for OA+TOF][laparotomy for CDH] with minimally invasive surgery [thoracoscopy] in infants undergoing oesophageal atresia or congenital diaphragmatic hernia repair.

Before, during and after surgery, infants will undergo the following measurements:
(i) arterial blood samples will be taken for measurement of blood gases every 30 minutes during the operation (these samples are normally taken during an operation to check that the patient is being adequately ventilated)
(ii) breath samples will be taken every 15 minutes to measure the amount of carbon dioxide that originates from the carbon dioxide that is used to inflate the chest.
(iii) oxygenation of the brain will be measured continuously via a non-invasive technique called near-infra red spectroscopy. This involves placing a probe on the head. Measurement will be done preoperatively, during operation, 2 hours post operation and at 24 hours after the end of operation.
(iv) the amount of CO_2 in the blood will be measured continuously by a probe placed on the skin (transcutaneous CO_2 monitoring) Measurement will be done preoperatively, during operation, 2 hours post operation and at 24 hours after the end of operation[19]

In addition, other clinical parameters which are routinely measured, such as end-tidal carbon dioxide levels, heart rate, minute ventilation, body temperature will be recorded and compared between the groups.

We will monitor brain oxygen saturation with an In Vivo Optical Spectroscopy (INVOS). [The device is already use in this hospital]
The INVOS system is designed to noninvasively, directly and continuously measure and monitor changes in the approximate regional haemoglobin oxygen saturation in the blood in the brain individual. Since the haemoglobin in the sensor's field is made up of approximately 75% venous blood, 20% arterial blood and 5% capillary blood, the clinical interpretation of the readings is consistent with that of a venous measurement.
The measurement takes place in real time, providing an immediate indication of a change in the critical balance of regional oxygen delivery and oxygen consumption.
The device is intended for use of any patients for whom there is concern regarding the potential for cerebral oxygen imbalances, including infants and newborns undergoing carbon dioxide exogenous insufflations. There are two kinds of disposable non-invasive sensor, one paediatric (<40 Kg) and one neonatal (<5 Kg). There are no contraindications for use INVOS oximeter.

Sometimes, infants undergoing minimally invasive procedures can no longer be managed without converting the operation to an open procedure. This can happen for surgical reasons (e.g. inadequate view, excessive bleeding) or anaesthetic reasons (e.g. persistent acidosis on a blood gas measurement, inability to maintain adequate oxygenation). Where the anaesthetist is concerned that carbon dioxide levels are increasing, on the basis of transcutaneous measurements, a blood gas measurement will be obtained to confirm definitively that there is a problematically high carbon dioxide level and severe acidosis before recommending that the operation is converted to an open procedure.

The ***inclusion criteria*** for the trial are:

1. Infants or newborn with diagnosis of oesophageal atresia with trachea-oesophageal fistula or congenital diaphragmatic hernia
2. >1.6 Kg
3. Conventional ventilation (no high frequency ventilation or iNO) for at least 24 hours.
4. FiO_2 <0.4
5. No requirement for inotropes for at least 24 hours

The ***exclusion criteria*** for the trial are:

1. Late diagnosis (after 1 month of age)
2. Major congenital heart defects or pulmonary hypertension
3. Bilateral grade IV intraventricular haemorrhage
4. Previous ECMO
5. $FiO_2 \geq 0.4$

The ***primary end point*** will be arterial blood carbon dioxide levels. Blood samples are normally taken clinically during repair of these conditions, to allow monitoring of carbon dioxide, oxygen and other gases. In this study, these samples will be taken at defined timepoints to allow comparison between the groups.

The ***secondary end points*** of the study will be:

What are the effects on:
(a) oxygenation of the brain (measured by a non-invasive technique, near infra-red spectroscopy)
(b) arterial carbon dioxide levels measured transcutaneously (non-invasively using a probe placed on the skin)
(c) the percentage of exhaled carbon dioxide that comes from the carbon dioxide used to inflate the chest. This will be accomplished non-invasively by taking breath samples from patients during the operation.
(d) other parameters obtained from the blood gas analysis (e.g. pH, base deficit, pO_2
(e) length of hospital stay
(f) length of time on a ventilator
(g) pause of operation or conversion to open surgery
(h) duration and number of pauses
(i) intraoperative and postoperative complications

Although transcutaneous carbon dioxide levels (b above) provide a similar measurement to the primary end-point, transcutaneous measurement provides a continuous readout, whereas blood samples for blood gas measurements can only be made at a few discrete timepoints to limit the amount of blood taken from patients. Transcutaneous measurements are, however, not as reliable at blood gas measurements, which still provide the "gold standard". Hence, when there is concern about CO_2 levels on the basis of the continuous transcutaneous measurement, this will be verified by an arterial blood gas analysis before interrupting or converting the procedure.

5. Randomisation

We aim to recruit 10 infants undergoing oesophageal atresia with tracheoesophageal fistula repair and 10 undergoing congenital diaphragmatic hernia repair. Parents of eligible infants will be approached and given information about the study, and given the opportunity to ask any questions. If parents give informed consent, these infants will be randomly assigned to either thoracoscopy or open surgery. We will use a randomisation process called minimization to try to make sure that the groups are as similar as possible. [Table 1]

Minimisation criteria	Definition
Birth Weight	[Grams]
Postnatal age	[days]
Diagnosis	[OA+TOF] [CDH]

Table 1 Minimisation Criteria

6. Statistics and Sample Size Estimation

The primary comparison will be carbon dioxide levels in arterial blood in patients undergoing thoracoscopy compared to those undergoing open surgery. This is a pilot study as we do not have any data on which to perform a power calculation. The primary and secondary endpoints will be compared by appropriate parametric and non-parametric tests

7. Treatment Schedules

Surgery will be performed by consultant surgeons.
The only exception to this will be in the case when the trainee surgeon has completed more than 5 thoracotomy (OA+TOF) or laparotomy (CDH) or thoracoscopic repair of OA with tracheoesophageal fistula or CDH.

The two standardised operations compared in the trial are:

- *Thoracoscopy for the repair of OA and TOF or CDH:*

  Verress needle access is inserted through the 3rd or 4th intercostal space in the posterior axillary line. The chest is insufflated to 3 to 5 mmHg using carbon dioxide at a flow rate of 0.5-1-0 L/min. The surgical interventions will be performed with the aid of 2-3 trocars.[20]

- *Open surgery:*

  1. Thoracotomy for oesophageal atresia and closure of tracheoesophageal fistula[21]

  2. Laparotomy for repair of congenital diaphragmatic hernia[22]

8. Studies Proposed

Stage 1 – Enrolment

i) Patient is identified as being eligible
ii) Consent obtained from parent or guardian
iii) Demographics recorded and treatment randomised

Stage 2 – Day of Surgery

i) details of the operative procedure (duration, scoring of the difficulty, surgeon performing the operation)
ii) studies described above as primary and secondary endpoints

Stage 3 – Patient post operative follow up

i) 2 and 24h post-operative measurement of primary and secondary endpoints
ii) clinical data collected for the rest of hospital stay

9. Data Monitoring and Interim Analysis

Participants will be allocated a unique study number, and all study data will be stored with this number as the identifier. Identifiers will be held in a separate database. This separation will happen at the time of transcribing the data
Data will be analysed at the Institute of Child Health and will be compared by appropriate parametric or non-parametric analyses. We will convene a Data Monitoring and Ethics Committee who will review the data when 10 patients have been recruited.

10. Compliance

In order to maximise the compliance of participating consultants in the trial there will be a link surgeon who will liaise with consultants of GOSH. Confidentiality of data will be ensured.

11. Timetable

0-1 months: establish trial, complete Research Ethics Committee approvals, develop data management systems and databases; *2-34 months*: recruitment, randomisation and follow-up; *34-36 months:* Complete analyses, write final report for peer review publication.

12. Relevance

This trial addresses a fundamental question in paediatric surgery concerning the safety and feasibility of thoracoscopy in newborn infants with oesophageal atresia with tracheoesophageal fistula or congenital diaphragmatic hernia.
It will provide data on the feasibility and safety of a new minimal access technique for OA/TOF or CDH repair.
This pilot study will provide novel data for a power calculation aimed to a larger multicentre randomized controlled trial.
We will seek consent from parents of participants in the trial to record the NHS number of the infants. This may be used to trace and contact the participants in the future to obtain further longer term follow up data on the effects of surgery. The participants will be re-consented at this future date for any further follow-up study.

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NHS

National Research Ethics Service

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09/S904

09 February 2009

Professor Pierro Agostino
Nuffield Professor of Paediatric Surgery
Institute of Child Health
30 Guilford Street
London WC1N

Dear Professor Agostino

Full title of study: **A pilot study of blood and breath carbon dioxide during thoracoscopy or open surgery in infants with oesophageal atresia and tracheo-oesophageal fistula or congenital diaphragmatic hernia**

REC reference number: **09/H0714/2**

The Research Ethics Committee reviewed the above application at the meeting held on 29 January 2009.

We were grateful to yourself, and your colleagues, Simon Eaton, Luca Giacomelle, and Jo Brierley for coming to discuss this study.

Ethical opinion

All the surgeons involved are equally skilful in open and laparascopic methods. The committee felt that the random allocation of the babies to one or other type of surgery was ethical. The committee questioned whether the study could be done with just one anaesthetist, however you replied that this would mean the study would take too long. The anaesthetists were equally skilled in the procedure (of CO_2 monitoring) but this trial would simply formalize the time events during surgery.

There is confusion about whether there is to be a data monitoring committee and there are different comments on this in different parts of the application.

The committee noted that there were no curricula vitae for the surgeons and anaesthetists involved. However this is not deemed necessary

We recommended minor changes to the PIL which were handed to you at the meeting. Any modified PIL should be sent to Ms Mittu for the file.

We considered the research question to be very important and were happy to give the study a favourable opinion

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Ethical review of research sites

The Committee has not yet been notified of the outcome of any site-specific assessment (SSA) for the research site(s) taking part in this study. The favourable opinion does not therefore apply to any site at present. I will write to you again as soon as one Research Ethics Committee has notified the outcome of a SSA. In the meantime no study procedures should be initiated at sites requiring SSA.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission at NHS sites ("R&D approval") should be obtained from the relevant care organisation(s) in accordance with NHS research governance arrangements. Guidance on applying for NHS permission is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Participant Consent Form: Hypercapnia during thoracoscopy or open surgery for repair of oesophageal atresia with tracheoesophageal fistual in neonates	1	07 January 2009
Participant Consent Form: Hypercapnia during thoracoscopy or open surgery for repair of congenital diaphragmatic hernia in neonates	1	07 January 2009
Participant Information Sheet: Congenital Diaphragmatic Hernia	1	07 January 2009
Participant Information Sheet: Oesophageal Atresia with Tracheoesophageal fistula	1	07 January 2009
Protocol	1	07 January 2009
Investigator CV	Professor Agostino Pierro	
Application		07 January 2009

Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Website > After Review

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nres.npsa.nhs.uk.

09/H0714/2	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project

Yours sincerely

[redacted]

Dr Geoff Scott
Chair

Email: a.mittu@ich.ucl.ac.uk

Enclosures: *List of names and professions of members who were present at the meeting and those who submitted written comments*
"After ethical review – guidance for researchers" [SL-AR1 for CTIMPs, SL-AR2 for other studies]

Copy to: *Dr Tracy Assari*
[R&D office for NHS care organisation at lead site]

CONSORT 2010 checklist for CO_2 trial – Chapter 7

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	189
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	190
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	192
	2b	Specific objectives or hypotheses	193
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	194
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	N/A
Participants	4a	Eligibility criteria for participants	194
	4b	Settings and locations where the data were collected	194
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	195
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	197-**201**
	6b	Any changes to trial outcomes after the trial commenced, with reasons	N/A
Sample size	7a	How sample size was determined	194
	7b	When applicable, explanation of any interim analyses and stopping guidelines	231
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	194
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	194
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	194
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	194
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	194 (No blinding)

	11b	If relevant, description of the similarity of interventions	195
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	201
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	201
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	204
	13b	For each group, losses and exclusions after randomisation, together with reasons	N/A
Recruitment	14a	Dates defining the periods of recruitment and follow-up	203
	14b	Why the trial ended or was stopped	203
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	205
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	204
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	207ff
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	N/A
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	203, 214
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	209-212
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	222ff, esp 230ff
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	231
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	222ff
Other information			
Registration	23	Registration number and name of trial registry	201
Protocol	24	Where the full trial protocol can be accessed, if available	194 (App 3)
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	201

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

This is a non-final version of an article published in final form in:
Bishay M, Giacomello L, Retrosi G, Thyoka M, Garriboli M, Brierley J, Harding L, Scuplak S, Cross KM, Curry JI, Kiely EM, De Coppi P, Eaton S, Pierro A. Hypercapnia and Acidosis during Open and Thoracoscopic Repair of Congenital Diaphragmatic Hernia and Esophageal Atresia: Results of a Pilot Randomized Controlled Trial. *Ann Surgery* 2013 Dec; 258(6):895-900.
www.annalsofsurgery.com

Hypercapnia and Acidosis during Open and Thoracoscopic Repair of Congenital Diaphragmatic Hernia and Esophageal Atresia: Results of a Pilot Randomized Controlled Trial

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Conflicts of Interest and Source of Funding: There are no conflicts of interest. We are grateful to the Mittal Paediatric Surgery Research Fund for supporting this work. SE and PDC are supported by Great Ormond Street Hospital Children's Charity. No sponsors played any role in study design; in the collection, analysis, or interpretation of the data; in the writing of the report; or in the decision to submit the paper for publication.

Running Head: *Hypercapnia and Neonatal Thoracoscopy*

Mini-abstract: This pilot randomized controlled trial shows that neonatal thoracoscopy, particularly thoracoscopic repair of congenital diaphragmatic hernia, is associated with prolonged and severe intraoperative hypercapnia and acidosis, compared to open surgery. This calls into question the safety of this practice.

Structured Abstract:

Objective: We aimed to evaluate the effect of thoracoscopy in neonates on intra-operative arterial blood gases, compared to open surgery.

Summary Background Data: Congenital diaphragmatic hernia (CDH) and esophageal atresia with tracheo-esophageal fistula (EA/TEF) can be repaired thoracoscopically, but this may cause hypercapnia and acidosis which are potentially harmful.

Methods: This was a pilot randomized controlled trial. The target number of 20 neonates (weight >1·6 kg) were randomized to either open (5 CDH, 5 EA/TEF) or thoracoscopic (5 CDH, 5 EA/TEF) repair. Arterial blood gases were measured every 30 minutes intra-operatively, and compared by multilevel modelling, presented as mean and difference (95% confidence interval) from these predictions. ClinicalTrials.gov Identifier: NCT01467245

Results: Overall the intraoperative $PaCO_2$ was 61 mmHg in open and 83 mmHg (difference 22 mmHg [2, 42]; p=0·036) in thoracoscopy and the pH was 7·24 in open and 7·13 (difference -0·11 [-0·20, -0·01]; p=0·025) in thoracoscopy. Duration of hypercapnia and acidosis was longer in thoracoscopy compared to open. For patients with CDH, thoracoscopy was associated with a significant increase in intra-operative hypercapnia (open 68 mmHg; thoracoscopy 96 mmHg; difference 28 mmHg [8, 48]; p=0·008) and severe acidosis (open 7·21; thoracoscopy 7·08; difference -0.13 [-0·24, -0·02]; p=0·018). No significant difference in $PaCO_2$, pH or PaO_2 was observed in patients undergoing thoracoscopic repair of EA/TEF.

Conclusions: This pilot randomized controlled trial shows that thoracoscopic repair of CDH is associated with prolonged and severe intraoperative hypercapnia and acidosis, compared to open surgery. These findings do not support the use of thoracoscopy with CO_2 insufflation and conventional ventilation for the repair of CDH, calling into question the safety of this practice. The effect of thoracoscopy on blood gases during repair of EA/TEF in neonates requires further evaluation.

Keywords: Thoracoscopy, congenital diaphragmatic hernia, esophageal atresia, hypercapnea, acidemia, hypercarbia

Introduction

The use of minimally invasive surgical techniques has been increasingly applied in infants and children leading to the use of thoracoscopy in neonates[1,2] for congenital anomalies such as congenital diaphragmatic hernia (CDH)[3] and esophageal atresia with distal tracheo-esophageal fistula (EA/TEF)[4].

During our early experience of thoracoscopic procedures in neonates, we noticed a degree of intraoperative hypercapnia and acidosis – presumably due to carbon dioxide (CO_2) insufflation into the pleural cavity and collapse/reduced ventilation of the ipsilateral lung in an immature and often pathological neonatal cardiopulmonary system. This was naturally a matter of great concern to us as surgeons, anesthesiologists, and neonatal intensivists. Relatively few studies reporting on the cardiorespiratory consequences of thoracoscopy in neonates have been published[5-9] and these studies have relied on retrospective review of anesthetic charts.

Because of our concerns about acidosis during the thoracoscopic repair of both CDH and EA/TEF, we performed a prospective pilot randomized controlled trial to evaluate the effect of thoracoscopy with CO_2 insufflation on (i) arterial blood gases, and (ii) absorption of insufflated CO_2. The main aim was to determine whether thoracoscopic repair of CDH and EA/TEF is associated with hypercapnia and acidosis, compared to open surgery. The primary outcome measure was intra-operative arterial blood carbon dioxide level.

Methods

Participants, randomization, masking and sample size

This was a pilot randomized controlled trial to establish the safety of thoracoscopy and to plan a larger trial if necessary (for the full trial protocol, see Supplemental Digital Content 1). The target number of twenty patients was chosen based on feasibility. Ethical approval

(09/H0714/2) for this study was obtained and written informed consent was obtained from parents or guardians of all participants. Eligible patients were neonates (i.e. in the first month of life), diagnosed with CDH or EA/TEF, and not requiring high frequency oscillatory ventilation, inhaled nitric oxide or inotropic support for at least 24 hours. Patients weighing less than 1·6 kg, or requiring more than 40% oxygen were excluded, as were neonates who had required extra-corporeal membrane oxygenation (ECMO), or had major congenital heart defects, pulmonary hypertension, or bilateral grade IV intraventricular haemorrhage. Subjects were randomly allocated to open or thoracoscopic surgery in a 1:1 ratio using computerised balanced minimisation[10] with the following criteria: i) diagnosis (congenital diaphragmatic hernia / esophageal atresia with trachea-esophageal fistula); ii) weight at the time of enrolment in the study (<2·5 kg / >2·5 kg); iii) age at time of enrolment (less than seven days / seven days or older). There was no blinding of clinicians, parents, or investigators. Enrolment and randomization were performed by members of the research team (MB, LG, GR, MG, MT), who then informed the clinical team of the allocation. This trial is registered with ClinicalTrials.gov, number NCT01467245.

Surgery

Thoracoscopy was performed with both lungs ventilated. Patients were positioned semi-prone. A 3- or 5-mm Hasson cannula was inserted through the third or fourth intercostal space in the posterior axillary line and the chest was insufflated using CO_2 and if possible the initial pressure reduced after reduction of hernia content (CDH) or after collapse of the lung (EA/TEF). If necessary to maintain visualization, the insufflation pressure was increased for a transient period. Surgery was performed with the aid of 2 or 3 working ports. For thoracoscopic repair of CDH, the hernia contents were reduced into the abdomen, and if a hernia sac was present, this was not resected. The defect was closed with interrupted non-absorbable stitches. If necessary, a polyester patch (Bard Sauvage Filamentous Fabric; Bard,

Billerica, MA, USA) was used to close the defect. When necessary, the posterolateral stitches were ligated extra-corporeally using small skin incisions. For the thoracoscopic repair of EA/TEF the azygos vein was divided and the tracheo-esophageal fistula was transfixed and ligated close to its entry into the trachea with a fine non-absorbable suture. The esophageal anastomosis was performed with interrupted non-absorbable sutures, with minimal handling of the esophageal ends.

For patients randomized to open surgery, CDH was repaired via an upper transverse laparotomy, and the defect was closed using non-absorbable sutures, with a polyester patch if necessary. EA/TEF was repaired via right posterolateral thoracotomy in the third or fourth intercostal space, using an extrapleural approach when possible. The TOF was divided and the trachea closed with interrupted stitches. The esophageal anastomosis was performed using interrupted stitches.

The trial protocol specified that if ventilation was felt to be a significant concern during thoracoscopy, insufflation of CO_2 would be temporarily paused to allow improvement in ventilation. If this was not successful, the operation would be converted to open surgery.

Data Collection and sample analysis

Arterial blood gases were measured in all patients, pre-, intra-, and post-operatively, at predetermined intervals (every 30 minutes intraoperatively, and at twelve and twenty four hours post operation). Breath samples were collected at 15-minute intervals using a 10 mL syringe connected to a 3-way valve at the sampling line for measurement of end tidal CO_2. The air was aspirated into a 10 mL syringe and immediately transferred into 10 ml vacuum test tubes (Labco Limited, High Wycombe, United Kingdom) for analysis. Samples were collected before the start of the operation, and every 15 minutes during the operation. In addition, samples of medical CO_2 used for insufflation were obtained for each thoracoscopic operation. These breath and medical gas samples were analyzed for $^{13}CO_2/^{12}CO_2$ enrichment

by isotope ratio mass spectrometry as previously described[11]. Using the $^{13}CO_2/^{12}CO_2$ of the medical CO_2 used for insufflation to represent 100% of exhaled CO_2 originating from insufflation and baseline breath $^{13}CO_2/^{12}CO_2$ at the start of operation to represent 0%, the percentage of exhaled CO_2 originating from insufflation at each time point was calculated as previously described[11].

End points and monitoring

The primary end point of the trial was intraoperative arterial CO_2 level. The secondary end points included acidosis (arterial pH), arterial oxygenation, percentage of exhaled CO_2 derived from the medical CO_2 used to inflate the chest in thoracoscopy, type and duration of postoperative ventilation, postoperative analgesia, pause of operation or conversion to open surgery, intraoperative and postoperative complications. The trial was monitored by the Data Monitoring and Ethics Committee (DMEC) comprising paediatric specialists not involved in the trial: anesthesiologist, surgeon, intensivist, and neonatologist. The DMEC met 3 times during the trial to evaluate progress and review interim analysis, and reported recommendations to the Trial Steering Committee (AP, SE, JB, MB). The DMEC recommended (following interim analysis) that investigators should calculate the amount of time each patient had pH below 7.2, 7.1 and 7.0, or $PaCO_2$ greater than 10, 12, and 14 kPa (75, 90 and 105 mmHg respectively).

Statistical analysis

Data are presented as mean ± SEM, unless stated otherwise. Normally distributed data were analyzed using a t test, and non-parametric data were analyzed using a Mann-Whitney test. Proportions were compared using a Fisher's exact test. The effects of operation type (MIS/open; CDH/OA-TOF) on intraoperative $PaCO_2$, pH and PaO_2 were compared by multilevel regression modelling of each arterial blood gas measurement using MLWin v2.11. A P value $\leq 0{\cdot}05$ was considered statistically significant.

Results

The target number of twenty neonates underwent surgery between August 2009 and February 2011. Patients were randomized to either open (5 CDH, 5 EA/TEF) or thoracoscopic (5 CDH, 5 EA/TEF) repair (Figure 1). There was no significant difference between the open and thoracoscopic groups in demographics, pre-operative ventilation, or duration of operation (Table 1). In thoracoscopy, initial insufflation pressure was 7·1±0·5 mmHg; this was reduced or maintained in all except one neonate undergoing repair of CDH where an increase from 6 to 9 mmHg was necessary to achieve visualization. Using multilevel regression modelling to compare intraoperative blood gases, thoracoscopy was found to be associated with a significant increase in intraoperative hypercapnia (the primary outcome) and acidosis, while oxygenation was no different (Table 2).

Duration of hypercapnia and acidosis

As advised by the trial DMEC, we compared the duration of different levels of hypercapnia and acidosis between the two groups. The thoracoscopic patients experienced a markedly longer duration of the most extreme levels of hypercapnia and acidosis, compared to the open group (Table 3). In the thoracoscopic group, four out of ten patients experienced pH <7, up to a maximum duration of 135 minutes. No patients in the open group experienced pH <7. With regard to hypercapnia, six out of ten patients in the thoracoscopic group experienced hypercapnia with $PaCO_2$ >105 mmHg (14 kPa; maximum duration 225 minutes), compared to one out of ten patients in the open group (duration 30 minutes).

Congenital Diaphragmatic Hernia

There were no cases of thoracoscopy converted to open surgery. There was one case where the anesthesiologist requested a pause of insufflation (7 minutes) during thoracoscopy due to

concerns regarding hypercapnia (which improved after hand ventilation/aspiration of secretions).

Figure 2 illustrates the highest $PaCO_2$ and lowest pH reached for each patient with CDH. While there is evidence of hypercapnia and acidosis in both groups, more extreme values were attained in the thoracoscopic group, with four patients out of five attaining a $PaCO_2$ >105 mmHg (14 kPa; compared to one out of five in the open group), and two patients experiencing extreme acidosis with pH <7.0 (compared to none in the open group). Using multilevel regression modelling, thoracoscopy was found to be associated with a significant increase in intra-operative hypercapnia and acidosis for patients with CDH, while oxygenation was no different (Table 2). There was no difference between the two groups in $PaCO_2$, pH or PaO_2 observed at 12 or 24 hours post operation. Post-operative outcomes are given in Table 1: thoracoscopy was associated with significantly shorter duration of postoperative intravenous opiate analgesia, and lower peak inspiratory ventilatory pressure at 24 hours post operation. There were no surgical complications in the open group. In the thoracoscopic group, one patient had an intestinal perforation, while another had a recurrence of diaphragmatic hernia. Median follow up was 56 weeks (range 12 – 92).

Esophageal Atresia with Tracheo-Esophageal Fistula

There were no cases of thoracoscopy converted to open surgery due to ventilatory concerns. In one patient, thoracoscopic EA/TEF repair was converted to thoracotomy for technical reasons (lack of proper visualization) unrelated to hypercapnia or acidosis. There was one case where the anesthesiologist requested a pause of insufflation during thoracoscopy (6 minutes) due to concerns regarding hypercapnia (which improved after hand ventilation/aspiration of secretions).

Figure 2 illustrates the highest $PaCO_2$ and lowest pH reached for each patient. Again there is evidence of hypercapnia and acidosis in both groups, with more extreme values attained in

the thoracoscopic group: two patients out of five attained a $PaCO_2$ >105 mmHg (14 kPa; compared to none out of five in the open group), and two patients experienced extreme acidosis with pH <7 (compared to none in the open group). Using multilevel regression modelling, no significant change in $PaCO_2$, pH or PaO_2 was observed in patients undergoing thoracoscopic repair of EA/TEF (Table 2). There was no difference between the two groups in $PaCO_2$, pH or PaO_2 observed at 12 or 24 hours post operation.

There was no significant difference in postoperative outcome between the two groups (Table 1), using intention to treat analysis (one patient in the thoracoscopic group was converted to open surgery). In the open group, one patient developed esophageal stricture requiring two dilatations of the anastomosis. In the thoracoscopic group, three patients developed strictures requiring at least one dilatation (range 1-3). One of these patients also developed an anastomotic leak repaired thoracoscopically on postoperative day 4 and was subsequently diagnosed as having an upper pouch tracheo-esophageal fistula which required ligation via cervical approach (postoperative day 34). Median follow up was 80 weeks (range 31 – 113).

Analysis of Breath Samples

Analysis of $^{13}CO_2/^{12}CO_2$ enrichment in breath samples taken intra-operatively during thoracoscopy showed a progressive decrease in ppm $^{13}CO_2$ during operation, suggesting absorption of medical CO_2. No such change was observed in patients undergoing open surgery. We calculated the percentage of exhaled CO_2 originating from the insufflated medical gas, using baseline $^{13}CO_2/^{12}CO_2$ to represent zero, and the $^{13}CO_2/^{12}CO_2$ of the insufflation gas to represent 100%. Up to 39±3% (range 32-59) of exhaled CO_2 was derived from insufflated gas rather than being of metabolic origin (Figure 3; Data are presented for 9 of the 10 thoracoscopic patients: samples from one case could not be analyzed for technical reasons). There was no significant difference in absorption of insufflated CO_2 between CDH and EA/TEF.

Discussion

This pilot randomized controlled trial has demonstrated that thoracoscopic repair of congenital diaphragmatic hernia is associated with significantly increased hypercapnia and acidosis, compared to open surgery. Previous retrospective studies have reported hypercapnia and acidosis during thoracoscopic CDH repair[5-7,9], but this is the first randomized trial to address this issue, and demonstrates a significantly more severe hypercapnia and acidosis in thoracoscopic repair compared to open surgery. In the UK, National Institute of Health and Clinical Excellence (NICE) guidelines on the use of thoracoscopy for the repair of CDH in neonates support the use of this procedure, but do not address safety concerns related to hypercapnia and acidosis[12]. However, in this report, NICE "encourages collection of data and publication of results" on this procedure. Although hypercapnia and acidosis are associated with adverse outcome in neonates with hypoxia, these neonates undergoing thoracoscopy did not show changes in arterial oxygenation, so the effects of these physiological changes on future brain development are unknown. Our study did not demonstrate any significant effects of thoracoscopy on blood gases in neonates undergoing repair of EA/TEF, but this needs further evaluation with a larger study, given the extreme levels of hypercapnia and acidosis observed in 40% of thoracoscopic operations.

This prospective study provides evidence that absorption of CO_2 from thoracic insufflation in neonates is much greater than we have previously reported from peritoneal insufflation during paediatric laparoscopy: reaching an average of 39% compared to 19% during laparoscopy[13].

This pilot study was not powered to detect a significant difference in surgical complications, but we did observe more surgical complications requiring re-operation in patients undergoing thoracoscopic repair compared to those undergoing open repair. This has been shown in

previous retrospective studies[7,9,14,15], a meta-analysis of retrospective comparative studies[16], and data from an international CDH registry[17]: all demonstrate that thoracoscopic repair of CDH has a higher recurrence rate than open repair. It is difficult to ascertain how much of this is due to the effect of the learning curve.

However, this study also confirms that thoracoscopic repair of CDH may have some advantages, such as less postoperative requirement for narcotic analgesia and ventilation, as observed in a previous retrospective study[7]. Therefore if strategies can be found to minimise hypercapnia and acidosis, there may be advantages to the minimally invasive technique. One such strategy which has been reported is the use of high-frequency oscillatory ventilation during thoracoscopic repair[18,19]. Another possibility may be to use an alternative insufflation gas such as helium, argon, nitrogen or air for insufflations, but none of these dissolve as rapidly as carbon dioxide, resulting in a potential risk of embolization, as well as issues of cost[20,21]. Nitrous oxide has been used successfully in prospective studies[22,23], despite earlier anecdotal reports of intra-peritoneal explosions[24,25].

In patients with EA/TEF, thoracoscopy has a significant potential benefit in avoiding long term musculoskeletal sequelae of open thoracotomy: open repair of EA/TEF has been reported to be associated with subsequent scoliosis in up to 56% of cases[26], while thoracoscopy has been shown to be associated with a lower incidence of musculoskeletal sequelae (including scoliosis) compared to open surgery, in infants and children requiring thoracic surgery for various indications[27].

The main limitation of this study is its small size, being a pilot study of relatively uncommon anomalies requiring lifesaving neonatal surgery. Nonetheless, it is a prospective randomized trial and we believe that the significant findings – of increased hypercapnia and acidosis associated with thoracoscopic repair of CDH – are valid and relevant to other institutions performing neonatal thoracoscopy.

This pilot study shows that thoracoscopic repair of CDH is associated with profound and prolonged hypercapnia and acidosis compared to open surgery. The levels of hypercapnia and acidosis were of such concern that the trial's DMEC have advised that thoracoscopic repair of CDH should no longer be performed with this type of conventional insufflation and ventilation. The trial steering committee accepted these recommendations and the thoracoscopic repair of CDH is no longer performed in this way in our institution.

Authors' Contributions

LH, JB, SE, and AP conceived the study. LG, AP, SE, LH, SS, KMC, JIC, EMK, and PDC designed the study. AP and SE obtained funding. MB, GR, MT, MG, LG, KMC, JIC, EMK, and PDC participated in the enrolment of patients and acquisition of data. MB and SE performed statistical analysis. SE performed mass spectrometry. MB, SE, and AP wrote the draft, with critical revision from all other authors. All authors have seen and approved the final version.

Acknowledgements: We are grateful to the following for their assistance: Shireen Nah, Sonia Basson, and Stavros Loukogeorgakis for assistance with data collection, and the Data Monitoring and Ethics Committee for their valuable input: Allan Goldman, Ann Black, Imran Mushtaq, and Nikki Robertson.

Funding Sources: We are grateful to the Mittal Paediatric Surgery Research Fund for supporting this work. SE and PDC are supported by Great Ormond Street Hospital Children's Charity. No sponsors played any role in study design; in the collection, analysis, or interpretation of the data; in the writing of the report; or in the decision to submit the paper for publication.

Conflicts of Interest: None.

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Legends for illustrations:

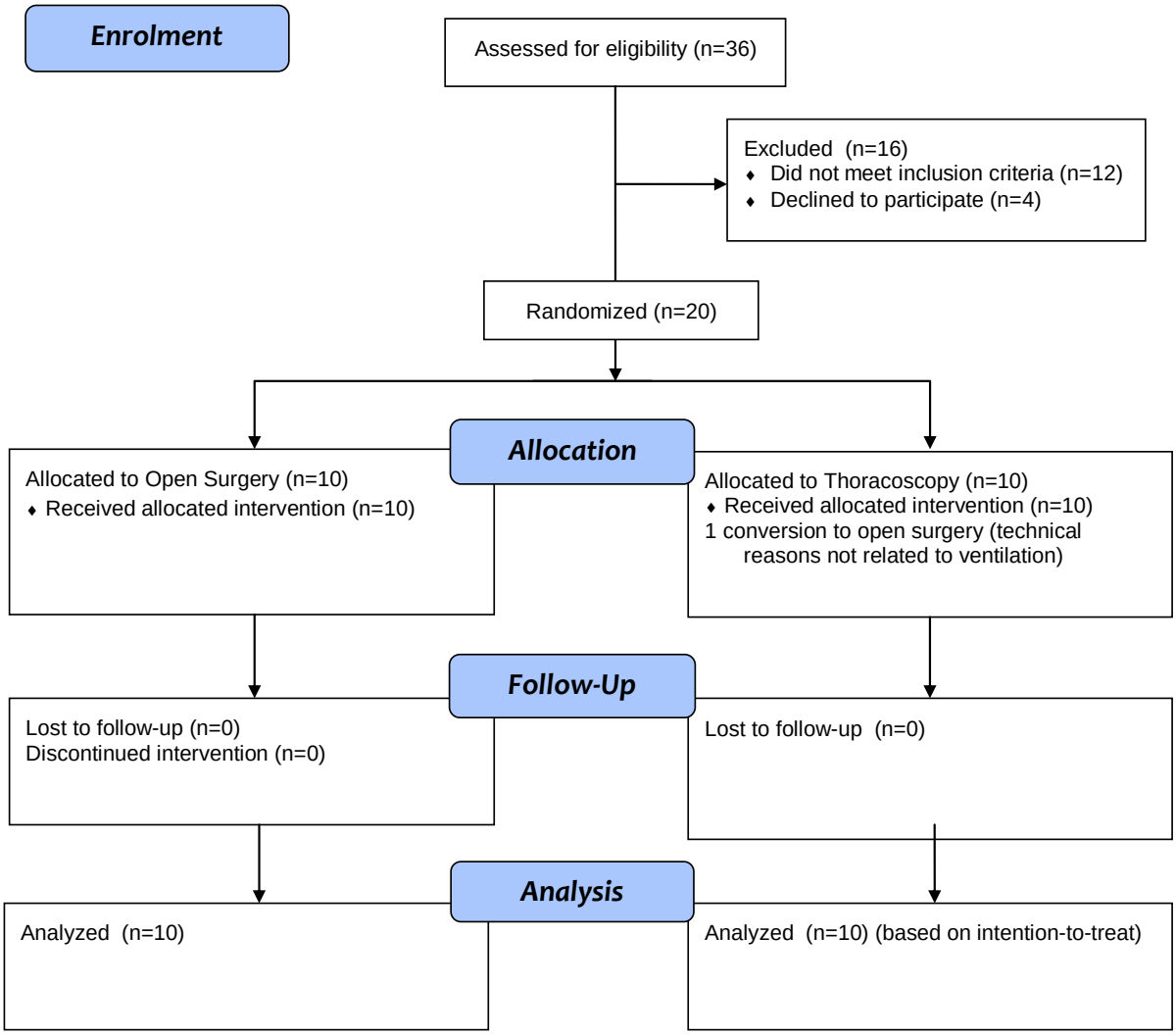
Figure 1. Trial profile

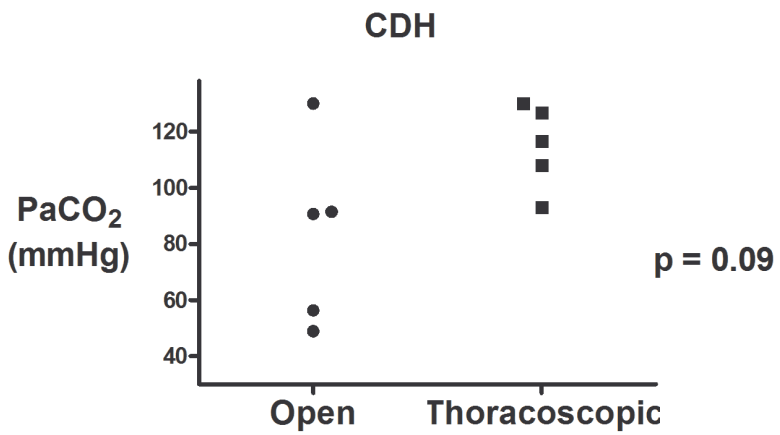
Figure 2. Intra-operative blood gases: Peak intra-operative $PaCO_2$ and nadir intra-operative pH. Data compared using unpaired t test.

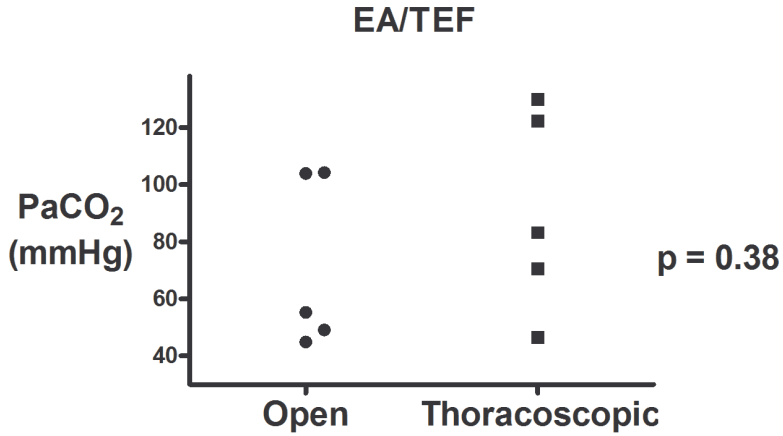
Figure 3. Percentage exhaled CO_2 originating from insufflated gas in patients undergoing thoracoscopic repair.

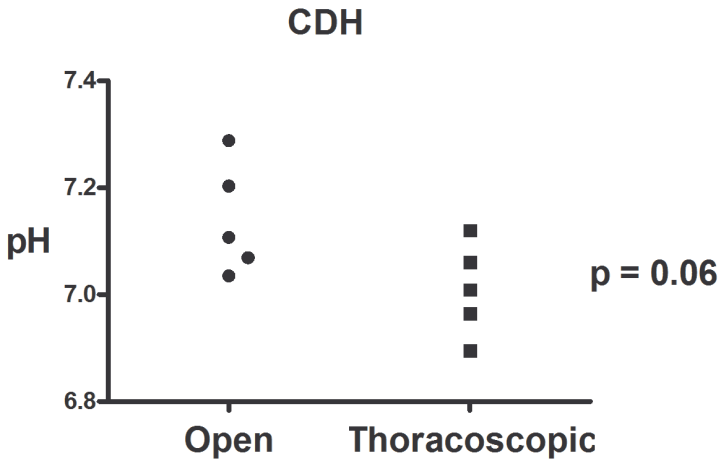
Supplemental Data File (Trial Protocol):
CO2 Trial Protocol1.0.pdf

Figure 1. Trial profile




CDH
PaCO2 (mmHg)
120
100
80
60
40
Open
Thoracoscopic
p = 0.09


EA/TEF
PaCO2 (mmHg)
120
100
80
60
40
Open
Thoracoscopic
p = 0.38


CDH
pH
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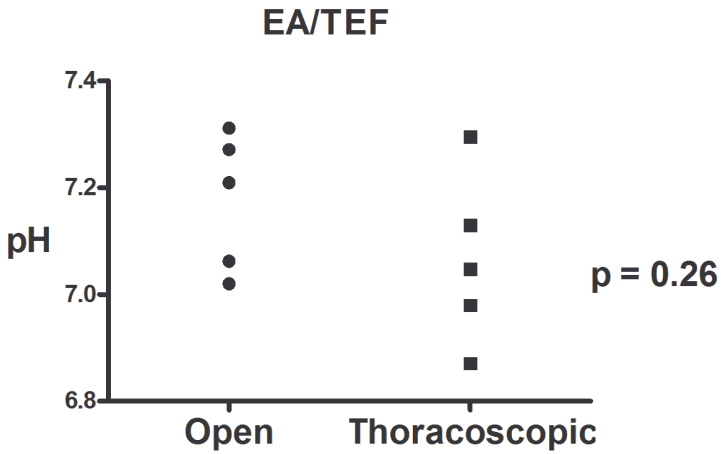
7.2
7.0
6.8
Open
Thoracoscopic
p = 0.06


EA/TEF
pH
7.4
7.2
7.0
6.8
Open
Thoracoscopic
p = 0.26

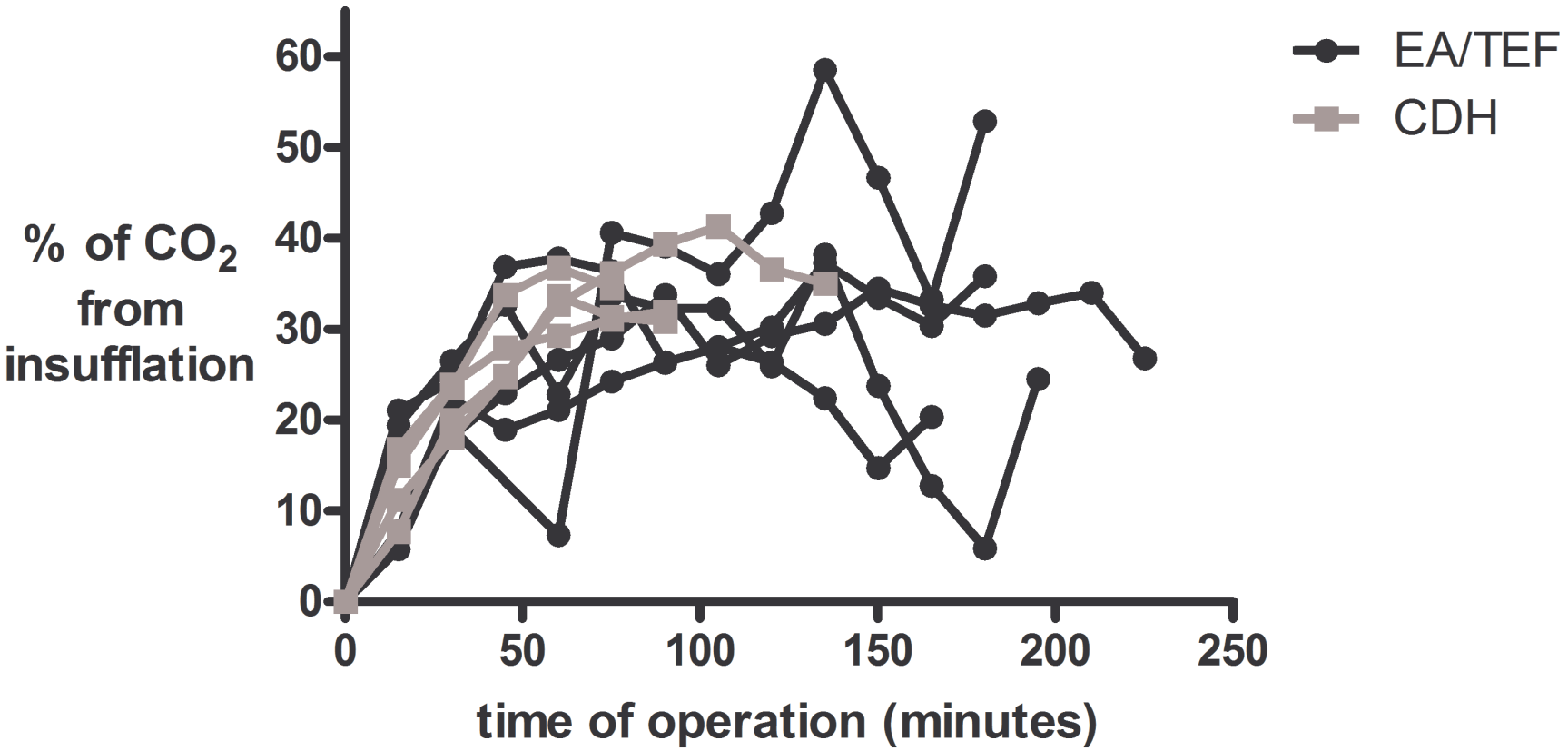

% of CO_2 from insufflation
60
50
40
30
20
10
0
0
50
100
150
200
250
time of operation (minutes)
EA/TEF
CDH

Table 1 Patient demographics, preoperative ventilation, duration of operation and postoperative outcomes. Data as median (range); compared by Mann Whitney Test. HFOV = High-frequency oscillatory ventilation (used at any time prior to operation, but stopped at least 24 hours before operation). iNO = inhaled nitric oxide (used at any time prior to operation, but stopped at least 24 hours before operation). PIP = Peak inspiratory pressure supplied at 24 hours post operation. HFOV = High-frequency oscillatory ventilation. ICU = Intensive Care Unit. IV = intravenous.

	Open	**Thoracoscopic**	**P-value**
Congenital Diaphragmatic Hernia Patients			
Weight (kg)	3·0 (2·7 - 3·5)	3·4 (2·5 – 4·0)	0·69
Age (days)	5 (2 - 25)	4 (1 – 6)	0·53
Gestational Age (weeks)	38 (36 - 41)	39 (37 – 42)	0·40
Sex (M/F)	3/2	4/1	N/A
Duration of preoperative ventilation (days)	3 (1 - 11)	2 (1 – 6)	0·67
Preoperative Oxygenation Index	0·31 (0·19 – 0·47)	0·38 (0·16 – 0·85)	1·0
Preoperative HFOV	2/5	2/5	N/A
Preoperative iNO	1/5	1/5	N/A
Operating time (minutes)	79 (45-130)	91 (78-148)	0·56
PIP (cmH_2O)	22 (16 - 25)	15 (0 – 16)	0·02
Postoperative HFOV	2/5	0/5	N/A

ICU stay (days)	5 (3 - 13)	2 (2 – 6)	0·12
IV morphine (days)	3 (2 - 11)	1 (1 – 2)	0·02
Esophageal Atresia Patients			
Weight (kg)	3·3 (2·6 - 3·5)	3·3 (2·9 – 3·7)	0·60
Age (days)	1 (1 - 2)	1 (1 – 5)	0·92
Gestational Age (weeks)	40 (38 - 41)	40 (39 – 41)	0·40
Sex (M/F)	4/1	3/2	N/A
Operating time (minutes)	150 (95-213)	180 (143-225)	0·41
PIP (cmH_2O)	16 (0 - 20)	0 (0 – 18)	0·12
Extubated within 24h	1/5	3/5	N/A
ICU stay (days)	5 (1 - 8)	3 (1 – 13)	0·92
IV morphine (days)	5 (1 - 10)	2 (1 – 11)	1·0

Table 2. Intra-operative arterial blood gases.

Data were compared by multilevel modelling; average intraoperative blood gas values and the mean difference with 95% confidence intervals are reported .

	Open	Thoracoscopic	Difference (95% CI)	P-value
All Patients				
$PaCO_2$ (mmHg)	61	83	22 (2, 42)	0·036
pH	7·24	7·13	-0·11 (-0·20, -0·01)	0·025
PaO_2 (mmHg)	182	151	-32 (-82, 18)	0·21
CDH				
$PaCO_2$ (mmHg)	68	96	28 (8, 48)	0·008
pH	7·21	7·08	-0·13 (-0·24, -0·02)	0·018
PaO_2 (mmHg)	214	185	-29 (-100, 43)	0·43
EA/TEF				
$PaCO_2$ (mmHg)	56	70	14 (-14, 44)	0·33
pH	7·26	7·18	-0·08 (-0·22, 0·05)	0·22
PaO_2 (mmHg)	158	127	-31 (-85, 23)	0·26

Table 3. Duration of hypercapnia and acidosis in all patients, in minutes.

	Open	**Thoracoscopic**
$PaCO_2$		
>105 mmHg	0,0,0,0,0,0,0,0,0,30	0,0,0,0,30,45,107,120,125,225
>90 mmHg	0,0,0,0,0,15,30,30,30,60	0,0,0,23,30,105,107,120,125,225
>75 mmHg	0,0,0,0,0,15,60,60,60,198	0,0,30,53,111,120,125,183,218,225
pH		
<7·0	0,0,0,0,0,0,0,0,0,0	0,0,0,0,0,0,0,22,30,125,135
<7·1	0,0,0,0,0,0,15,30,30,198	0,0,0,30,30,53,111,125,135,225
<7·2	0,0,0,0,0,45,60,60,162,298	0,30,61,107,111,120,120,241,248,255