

# Advances in paediatric gastroenterology

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Recent developments in paediatric gastrointestinal surgery have focused on minimally invasive surgery, the accumulation of high quality clinical evidence, and scientific research. The benefits of minimally invasive surgery for common disorders like appendicitis and hypertrophic pyloric stenosis are all supported by good clinical evidence. Although minimally invasive surgery has been extended to neonatal surgery, it is difficult to establish its role for neonatal disorders such as oesophageal atresia and biliary atresia through clinical trials because of the rarity of these disorders. Advances in treatments for biliary atresia and necrotising enterocolitis have been achieved through specialisation, multidisciplinary management, and multicentre collaboration in research; similarly robust clinical evidence for other rare gastrointestinal disorders is needed. As more neonates with gastrointestinal diseases survive into adulthood, their long-term sequelae will also need evidence-based multidisciplinary care. Identifying cures for long-term problems of a complex developmental anomaly such as Hirschsprung's disease will rely on unravelling its pathogenesis through genetics and the development of iPSC-based therapy.

## Introduction

Paediatric gastrointestinal surgery is used for a wide range of disorders that can be broadly classified into the common childhood diseases encountered on a daily

basis and the rare developmental malformations with long-term consequences. In this Series paper, we review recent advances and remaining challenges in the management of six disorders. In the most common paediatric gastrointestinal disorders such as appendicitis and hypertrophic pyloric stenosis, good clinical evidence has confirmed the benefits of minimally invasive surgery. In the more complex neonatal gastrointestinal disorders such as oesophageal atresia and biliary atresia, the advantages of minimally invasive surgery have yet to be determined, but their management by multidisciplinary teams has improved primary outcomes, including survival. Patients with developmental defects often have long-term functional sequelae that cannot be corrected by surgery alone, leaving them with lifelong medical burdens that need the services of a diverse team of adult health-care professionals.

Robust, evidence-based data to guide paediatric gastrointestinal surgical care is limited because of the rarity of most neonatal disorders, making multicentre collaboration necessary if strategic advances are to be made. Major advances in quality of care will likely rely on subspecialisation within the field. The benefit of specialisation is best illustrated in the case of biliary atresia, where the concentration of care in high-volume centres improves outcomes and allows the accumulation of evidence through combining multicentre data and randomised controlled trials. The benefit of multicentre studies is also well illustrated by some recent advances in treatment of necrotising enterocolitis, but many more of such studies are needed to fill the large gaps in high-quality clinical evidence. Hirschsprung's disease (congenital intestinal aganglionosis) is an example of a disease that cannot be cured by simple surgical correction because the physiological dysfunction persists; here the relevance of genetics and stem-cell therapies to supplement surgery is explored through multidisciplinary research in an attempt to find a cure.

## **Advances in minimally invasive surgery**

### **Appendicitis**

Appendicitis is a common surgical emergency in children. Unlike adults, young children have immature immunological defence and might not be able to explain the symptoms, hence increasing the likelihood of perforation and therefore morbidity. Although some clinical scoring systems have been developed to improve the diagnostic accuracy in up to 80-90% of cases, their predictive value is still debatable, particularly in certain subpopulations.<sup>1</sup> In adults, the problem of diagnostic uncertainty has led to the use of CT, which is very sensitive and specific—lowering the normal appendicectomy rate to 6%—and could reveal other important pathology, although the latter is of less concern in children.<sup>2</sup> Although protocols for low-dose and limited

CT scan protocols exist, this investigation has a less important role in the management of children because of the risks of radiation exposure.<sup>3</sup> Ultrasonography is a better diagnostic tool where experience exists.<sup>4</sup> Appendectomy has always been the standard treatment for appendicitis, and recently this has been predominantly done laparoscopically after the results of a systematic analysis<sup>5</sup> showed its superiority over open operation in reducing wound infection and length of stay in hospital. Initial non-operative management is an acceptable option for children with complicated disease, especially those who present with an abscess or palpable mass. Nonoperative management usually involves a period of intravenous broad-spectrum antibiotics with or without abscess drainage, followed by continued antibiotics after discharge and a scheduled appendectomy in 6-8 weeks. Findings from a meta-analysis<sup>6</sup> of complicated appendicitis in children and adults showed a reduction in complications and re-operation rates using non-operative management. Although results of a randomised controlled trial<sup>7</sup> showed that more than three-quarters of children can avoid interval appendectomy after successful non-operative management of an appendix mass, the risk factors for failure of non-operative management are yet to be fully determined.

The application of non-operative management has also been extended to uncomplicated cases, with good outcomes from a randomised controlled trial in paediatric patients.<sup>8</sup> Unlike for the complicated cases, antibiotic therapy is intended to be the definitive treatment for uncomplicated cases, with no interval appendectomy scheduled. The questions remaining are, what percentage of patients will have recurrent appendicitis, and is this rate low enough to justify non-operative management as the primary treatment? In a meta-analysis<sup>9</sup> of non-operative management for uncomplicated appendicitis, the overall failure rate was about 20%, and these data will serve as a baseline for several ongoing multicentre randomised trials.

### **Hypertrophic pyloric stenosis**

Although appendicitis is the most common gastrointestinal disorder in children, hypertrophic pyloric stenosis is the most common disorder in infants that needs surgery (at a rate 0.2%). Hypertrophic pyloric stenosis is an obstructive idiopathic hypertrophy of the pyloric muscle and typically occurs at 1 month, and rarely later than 3 months of age, and with a clear male preponderance. In several studies, the first-born child has been shown to have the greatest risk.<sup>10</sup> In other studies, an increased risk has been associated with caesarean delivery, bottle-feeding, and low gestational age at birth.<sup>10-12</sup> The presentation is non-bilious projectile vomiting in an infant who otherwise appears well and shows hunger after vomiting. Historically, diagnosis was confirmed by a palpable olive-shaped mass in the

epigastrium. Ultrasound is accurate, efficient, and inexpensive and has thus become the current standard diagnostic modality.

The protracted vomiting leads to acid loss and dehydration with renal compensation creating hypochloreaemic, hypokalaemic metabolic alkalosis with paradoxical aciduria. The electrolyte imbalance might represent a medical emergency, but never a surgical emergency; rehydration is therefore done until electrolyte correction and evidence of normal tissue perfusion with brisk capillary refill and moist mucous membranes. The operation rarely occurs on the day of admission to allow for rehydration.<sup>13</sup> The curative procedure is division of the circular and longitudinal muscle layers of the pylorus, leaving the mucosa and submucosa intact. The procedure has traditionally been performed with an upper abdominal incision but is now increasingly done by laparoscopy (figure 1), with support from evidence from several randomised trials.<sup>14</sup> In long-term follow-up, the appearance of the abdomen of patients who had laparoscopic pyloromyotomy is indistinguishable from those who have no operation.<sup>15</sup> Circumumbilical incision but open surgery is alternative approach with the same cosmetic benefit. Irrespective of access, the prognosis is excellent, with most patients resuming feeds and going home in 1-2 days after the operation.

The two complications of pyloromyotomy are mucosal perforation or incomplete myotomy. Traditional teaching was that perforations were more likely to occur from extending the myotomy too distal, whereas incomplete myotomies occur from not going proximal enough. Mucosal perforation occurs in about 0.5% of cases, and incomplete myotomy also usually occurs in less than 1% of cases.<sup>16</sup> However, in one study,<sup>17</sup> no incomplete myotomies occurred when the myotomy was at least 2 cm in length. Post-operatively, traditional reintroduction of feeds followed protocols that used a period of stomach rest followed by incremental increases in volume and concentration.<sup>18</sup> Randomised data show that feeds can be safely reintroduced at goal levels.<sup>19</sup> Feeds on demand are therefore becoming the preferred and more efficient means of post-operative feeding.

**Figure 1: Laparoscopic pyloromyotomy**

## Challenges for evidence-based surgery

### Oesophageal atresia

By contrast with common disorders in children that need surgery, neonatal gastrointestinal disorders are rare, complex, poorly understood, and sometimes lifethreatening. These patients need multidisciplinary management and frequently have long-term problems, despite initial life-saving surgery. Although surgical innovations to improve outcomes exist, the difficulty is to accumulate high-quality evidence to guide advances for these rare and heterogeneous diseases.

Oesophageal atresia with or without distal tracheoesophageal fistula is a congenital anomaly that affects 1/4000 newborn babies and represents the quintessence of neonatal gastrointestinal surgery. About 85% of patients with oesophageal atresia have a proximal blind-ending oesophagus just beyond the thoracic inlet and a distal oesophagus entering the trachea at or above the carina.<sup>20</sup> Other types include pure atresia (oesophageal atresia, no tracheo-oesophageal fistula; 7%), which is characterised by a long gap between the oesophageal ends, H-type tracheo-oesophageal fistula with no atresia (5%), double tracheo-oesophageal fistula with oesophageal atresia, and proximal tracheo-oesophageal fistula with oesophageal atresia. Oesophageal atresia was once a lethal disorder, but survival has markedly improved to more than 90% because of advances in multidisciplinary perioperative care in neonatal surgical centres and innovative surgical strategies for different anomalies.

Oesophageal atresia is associated with other congenital anomalies, often as part of the VACTERL complex: Vertebral (24%), Anorectal (14%), Cardiac (32%), Tracheoesophageal fistula (95%), Renal (17%), Limb/skeletal (16%), and others (11%).<sup>21</sup> Associated with a prenatal history of polyhydramnios, oesophageal atresia can be diagnosed by prenatal ultrasound with a sensitivity of only around 50%, although specificity is high, especially for pure oesophageal atresia.<sup>22</sup> However, prenatal diagnosis does not affect outcome. Diagnosis at birth is readily evident in children who are unable to swallow or when an orogastric tube cannot be inserted. X-ray will show a radio-opaque orogastric tube coiled up at the thoracic inlet and can reveal associated skeletal anomalies; pure oesophageal atresia shows the additional feature of a gasless abdomen.

About 60% of surgeons will use bronchoscopy to identify the location of the fistula and rule out a fistula from the proximal pouch.<sup>20, 23</sup> The operation can then be either open or thoracoscopic (figure 2). The first thoracoscopic repair was reported in 1999.<sup>24</sup> In a survey of surgeons interested in minimally invasive surgery, half of the respondents use the thoracoscopic approach.<sup>23</sup> In a study of 11 US medical centres, only 8% of the cases were treated thoracoscopically.<sup>20</sup> Randomised trials are scarce, but in a meta-analysis,<sup>25</sup> outcomes after open surgery versus thoracoscopic repair were comparable; thoracoscopy took the longest time, but was associated with the shortest time to extubation, feeding, and discharge. The long operating time could be of concern in view of results from a pilot randomised trial that revealed high arterial CO<sub>2</sub> and low pH during thoracoscopic repair, increasing the potential risk for brain damage due to hypoxia.<sup>26</sup> Perioperative management varies widely and includes the use of post-operative paralysis, antibiotics, acid suppression, and many other parameters.<sup>20, 23, 27</sup> As an

example, a multicentre series showed no benefit to antibiotics or acid suppression; however, some surgeons use protracted antibiotics and recommend acid suppression for life.<sup>20,28</sup> Traditional practice has also included trans-anastomotic stent placement, but this procedure is now associated with harm.<sup>28</sup> The variation in care is an opportunity for the paediatric surgical community to design studies of new methods to improve care for these infants.

The most challenging cases of oesophageal atresia are those with a long gap, defined as at least 3 cm or three vertebral bodies between the two ends of the oesophagus, which is usually associated with pure oesophageal atresia. In a recent position paper, the Working Group on long-gap oesophageal atresia<sup>29</sup> called for a new definition to include only pure atresia and for these cases be referred to high-volume centres. The two strategies for oesophageal preservation are anastomosis under tension and delayed anastomosis with

**Figure 2: Thoracoscopic repair of oesophageal atresia (oesophageal atresia) with distal trachea-oesophageal fistula**

(A) Thoracoscopic view (patient in prone position) showing tracheo-oesophageal fistula occlusion (Haemoclip used here, but ligation or suturing is preferred by some surgeons) and division; the diagram shows the distal tracheo-oesophageal fistula inserting into the back of the trachea just above the carina and blind proximal oesophageal pouch at the thoracic inlet (outside this thoracoscopic image). (B) Completed oesophageal anastomosis; the diagram shows anastomosis of proximal and distal oesophageal segments.

**A**  
**B**

staged operations to stretch the oesophagus by placing tension on the ends using traction sutures.<sup>27</sup> Although uncommon, these cases can also be approached thoracoscopically. The alternative to oesophageal preservation is replacement, with gastric, colonic, or jejunal replacement. The technically difficult method of jejunal interposition has been advocated.<sup>30</sup> The limited comparative data does not delineate a superior option.<sup>31</sup> The low quality of evidence on this topic will probably not change in the near future, given the rarity of the disorder. The best results are likely to be attained by surgeons who gain experience with one approach and build solid experience.

The main early postoperative complication is leakage of anastomosis, occurring in 20% of cases.<sup>20</sup> Most of these cases can be managed conservatively with drainage and usually heal in 1-2 weeks. Strictures, defined as the need for oesophageal dilatation, are the most common complication and occur in about 40% of cases.<sup>20</sup> Most cases of stricture can be managed with dilations alone. Vocal cord paralysis, presumably from recurrent laryngeal nerve injury, occurs in about 5% of routine cases<sup>32</sup> but can occur in more than 20% of patients with H-type fistulas.<sup>20</sup> Recurrent fistulas occur in about 5% of cases. A variety of techniques for endoscopically obliterating recurrent fistulas have been described,<sup>33</sup> but surgical repair has the highest success rate and requires fewest treatments.<sup>34</sup> Even after successful surgery, patients have severely impaired oesophageal motility, predisposing to gastrooesophageal reflux and associated complications.<sup>35</sup>

Intestinal metaplasia of the oesophageal mucosa occurs in 6-11% of young adults after oesophageal atresia repair.<sup>35,36</sup> The prevalence of metaplastic epithelial alterations of the oesophagus increases with age, whereas eight cases of oesophageal cancer have been reported in patients with oesophageal atresia younger than 50 years.<sup>37</sup> International multicentre studies are needed to define the risk of intestinal metaplasia and oesophageal cancer so as to guide evidence-based endoscopic follow-up protocols for patients after transition to adult care.

### **Biliary atresia**

Despite being the most common cause of obstructive jaundice in infancy, biliary atresia is a very rare neonatal gastrointestinal disorder for which high-quality clinical evidence is limited to a few studies from highly specialised centres. Biliary atresia is characterised by the obliteration of all or part of the extrahepatic bile duct, sometimes with absent parts. The incidence of biliary atresia varies between ethnic groups, being more common in Asian populations (about 1/5000 Asians vs 1/18000 Caucasians).<sup>38</sup> Biliary atresia is probably a final common pathway of a number of possible pathogenic mechanisms that could include genetic,<sup>39</sup> inflammatory, environmental, and developmental abnormalities (figure 3). One hypothesis suggests that an inflammatory cholangiopathy is triggered perinatally by viral exposure acting in a genetically predisposed patient.<sup>40</sup> Although biliary atresia appears to be an isolated disease in most cases, in about 10-20% of western series, it is associated with other visceral anomalies such as polysplenia, situs inversus, and a pre-duodenal portal vein (biliary atresia splenic malformation syndrome).<sup>41</sup> The possible association of a cytomegalovirus has been studied for many years, and cytomegalovirus-related biliary atresia could be a different form of biliary atresia with worse prognosis.<sup>42</sup> Further work is needed before a definitive conclusion can be made.

Biliary atresia should be managed by experienced surgeons in a specialised centre. Findings from national studies in England and Wales, Finland, and the Netherlands have shown that centralising resources improves the outcomes for those with biliary atresia.<sup>43-45</sup> Excision of the fibrous cord at a precise level at the porta

#### **Figure 3: Schematic illustration of proposed aetiology of biliary atresia**

Dotted lines indicate influence or contributory factors. Thin arrows indicate various inter-relations. Thick arrows indicate principal types of biliary atresia (isolated vs developmental).

Genetic  
(eg, *CFC*-\_)  
Cystic biliary atresia Genetic susceptibility  
(eg, *ADD*\_)  
Virus-initiated  
(eg, cytomegalovirus, reovirus)  
Epigenetic  
(eg, diabetes)  
Environmental  
(eg, bilatresone)  
Other syndromes

(eg, cat eye syndrome)  
Biliary atresia splenic  
malformation  
Inflammatory  
Developmental cholangiopathy  
Isolated

#### **Biliary atresia**

**First trimester Second trimester Third trimester Postnatal**

Mechanism

Clinical features

and portoenterostomy introduced by Kasai is regarded as the best hope for salvaging the native liver and restoring bile flow, although the outcome remains variable and unpredictable. Clearance of jaundice can be achieved in 60-70% of infants, with a 5 year native liver survival rate of around 50%, although even those children cannot be regarded as cured.<sup>43-45</sup> A direct correlation between the age at operation and the outcome can be difficult to discern even in large series, probably reflecting the variable underlying pathology.<sup>46,47</sup> It remains axiomatic that the fibrotic process within the liver is time-critical and ultimately irreversible.

The outcomes of a recent systematic review<sup>48</sup> of the laparoscopic technique of portoenterostomy, suggests inferiority compared with conventional surgery. Results of a small randomised study have since shown no advantage of the technically more challenging laparoscopic technique,<sup>49</sup> and in another small comparative study,<sup>50</sup> patients who had laparoscopic portoenterostomy had higher jaundice clearance but poorer biomarker values than patients who had open portoenterostomy, although these differences were statistically nonsignificant. The debate of open versus laparoscopic portoenterostomy is unlikely to be resolved without large randomised controlled trials involving multiple centres with relevant expertise and high volumes.

Adjuvant treatments to improve the success of surgery have been studied extensively, and steroids are one of the most popular drugs available. The effect of steroids on the outcomes of biliary atresia has been assessed in three meta-analyses, the most recent<sup>51</sup> of which showed a higher jaundice clearance rate in patients given a high-to-moderate dose of steroid, especially those operated on before day 70 of life. The proposed explanation is that steroids work better on the early inflammatory stage of disease when fibrosis is less prevalent.

Cholangitis has long been known as a post-operative risk factor that affects the outcome of biliary atresia. Recurrent cholangitis in particular is an important prognostic marker for predicting disease progression according to the results of one multivariate analysis.<sup>52</sup> Treating post-operative cholangitis aggressively is therefore essential to prevent disease progression. Although some surgeons have advocated prophylactic use of antibiotics, the evidence for this is scanty. Prolonged antibiotics might be justified when cholangitis becomes intractable. Home administration of intravenous antibiotics is a practical approach for those patients needing long-term treatment with a reduction in hospital stay.<sup>53</sup>

Despite a Kasai portoenterostomy, about 30-40% of patients will develop end-stage liver disease. The native liver survival rate decreases with time, with the 5 year, 10 year, and 20 year survival rates estimated in one study to be 63%, 54%, and 44%, respectively.<sup>54</sup> These long-term survivors with native livers slowly develop liver cirrhosis and its sequelae. A multicentre consortium reported high incidences of cholangitis (17%) and fractures (95%) and suboptimal health-related quality of life (47%) on long-term follow-up.<sup>55</sup> Conversely, normal school education was reported in most survivors with a native liver (88%).<sup>56</sup> Hence, lifelong postoperative care and surveillance should be continued, preferably in a dedicated centre.

For patients who deteriorate after Kasai portoenterostomy, liver transplantation is the only viable treatment option in most patients.<sup>57</sup> The overall longterm success rate of liver transplantation for patients with biliary atresia is 74-86%, and survivors can enjoy normal health-related quality of life despite the need for life-long immunosuppression.<sup>58-60</sup> However, access to donor organs is still a limitation in most places. Deceased donors are still the standard in Europe and North America, whereas living donor programmes<sup>61-62</sup> are paramount in China, Japan, and South Korea, essentially for cultural reasons.

### **Necrotising enterocolitis**

Although the diagnosis and surgery of oesophageal atresia and biliary atresia is quite standardised, this is not true of all diseases in newborn children. Necrotising enterocolitis, the most common surgical emergency in premature newborn babies, is an acquired inflammatory disorder affecting the gastrointestinal tract. Despite decades of research, the aetiology of necrotising enterocolitis remains elusive, and optimal treatment and preventive strategies are still not well defined. In the past few years, however, data from several clinical trials and population studies on aetiological factors have emerged. Of the many theories on the development of necrotising enterocolitis, an interesting possibility is the abnormal colonisation of the preterm infant's gastrointestinal tract with pathogenic Gram-negative organisms. Findings from prospective serial stool sample analysis by 16S rRNA sequencing<sup>63</sup> showed that, whereas the normal premature infant gut undergoes bacterial colonisation in a systematic fashion (from Bacilli to Gammaproteobacteria to Clostridia), necrotising enterocolitis is preceded by a relative abundance of Gammaproteobacteria (facultative Gram-negative bacilli) and a paucity of strict anaerobes.<sup>64, 65</sup> This suggests that identification and modulation of the microbiome could allow for early diagnosis and treatment of necrotising enterocolitis.

A generalised Gammaproteobacteria dysbiosis alone, however, does not fully explain the disease. With new technologies, the potential causative strains of bacteria can

be identified and insight can be gained into the role of the host-bacterial interaction in necrotising enterocolitis development. Results from a metagenomic sequencing study<sup>66</sup> has implicated colonisation with specific *Escherichia coli* subtypes as a risk factor for developing necrotising enterocolitis. The Gram-negative bacterial cell wall component lipopolysaccharide is the ligand for Tolllike receptor 4 (TLR-4). Compared with infants born to term, the enterocytes of preterm infants show excessive TLR-4 signalling in response to lipopolysaccharide, and TLR-4 could be part of a signalling pathway through which pathogenic bacteria induce necrotising enterocolitis. Thus, TLR-4 is a promising target for therapeutic intervention in necrotising enterocolitis,<sup>67</sup> and clinical trials are being developed.<sup>68</sup>

In view of the importance of the microbiota in stimulating the inflammatory cascade that leads to necrotising enterocolitis, numerous bacteria-derived and host-derived biomarkers have been investigated. Faecal calprotectin,<sup>69</sup> urine alanine,<sup>70</sup> and urine intestinal fatty acid-binding protein<sup>71</sup> are examples of promising biomarkers; however, they have yet to be validated. Biomarkers could become the best opportunity to achieve early diagnosis, which would be a paradigm shift in necrotising enterocolitis.

Prevention of necrotising enterocolitis is ideal. Human breast milk decreases the risk of necrotising enterocolitis, presumably by reducing colonisation with pathogenic bacteria, while promoting the growth of commensal organisms and blunting the inflammatory response. Putative protective factors in breast milk include secretory IgA, epidermal growth factor,<sup>72</sup> and human milk oligosaccharides.<sup>73</sup> These factors might exert their protective effects, in part, by modulating bacterial-epithelial interaction via TLR-4.<sup>66</sup> However, necrotising enterocolitis can occur despite the use of breast milk, and no single component of human breast milk given as a formula additive can decrease the incidence of human necrotising enterocolitis. There may be additional, unknown components in breast milk, or perhaps it is the combination of factors that lead to its beneficial effects. Another preventive strategy is to modulate the microbiota using probiotics. Results of a Cochrane review<sup>74</sup> showed that enteral probiotics significantly reduced the incidence of advanced necrotising enterocolitis and decreased mortality, with no reported systemic infections. In a more recent meta-analysis,<sup>75</sup> probiotics were also found to decrease the risk of necrotising enterocolitis and death. Data from a randomised trial of 1315 preterm infants comparing *Bifidobacterium breve* and placebo showed no difference in the incidence of necrotising enterocolitis, sepsis, or death in either treatment group.<sup>76</sup> The use of a new probiotic strain here might explain the negative finding. The concern for potential risk of bacteraemia, although unproven, has impeded universal acceptance of

probiotics. This highlights the difficulty of studying necrotising enterocolitis, where thousands of patients must be enrolled to show a change in incidence. Since a myriad of organisms and doses could be tested in trials of probiotics, it is no surprise that the role of probiotics in necrotising enterocolitis prevention is still undefined. Overall, surgical intervention is still necessary in up to 50% of necrotising enterocolitis cases for intestinal necrosis or perforation (figure 4).<sup>77</sup> Findings from large, population-based studies in the UK and the USA show a substantial increase in mortality to at least 30% of operative necrotising enterocolitis cases.<sup>78,79</sup> Peritoneal drainage, in lieu of laparotomy, has been used to minimise operative risk in critically ill, premature infants. Studies surrounding this concept are the best example of clinical trials guiding the treatment of necrotising enterocolitis. In a North American randomised controlled trial<sup>80</sup> of 117 patients weighing less than 1500 g with perforated necrotising enterocolitis from 15 centres, no difference was found in mortality, total parenteral nutrition dependence, or length of hospital stay in neonates treated with drain or laparotomy. In a European randomised controlled trial<sup>81</sup> of 69 patients weighing less than 1000 g from 31 centres, although no difference was seen in outcomes (6 month survival was 51·4% with drain and 63·6% with primary laparotomy;  $p=0·3$ ), 74 % of patients who had peritoneal drainage needed rescue laparotomy, and thus laparotomy was superior. The long-term effects of either treatment were not examined. Results of the Neonatal Research Network prospective cohort study<sup>82</sup> suggest that long-term outcome (>18 months) might be better with laparotomy than with peritoneal drainage. Additional insight on this issue is anticipated from the ongoing Necrotizing Enterocolitis Surgery Trial (NCT01029353). These and other similar studies are crucial for improving the acute management of the disease, which has long-term implications for children living with short gut, dysmotility, and neurological sequelae as a result of necrotising enterocolitis.

### **Surgery meets science: Hirschsprung's disease**

Among the rare neonatal gastrointestinal disorders, Hirschsprung's disease (congenital intestinal aganglionosis) exemplifies a developmental anomaly for which

**Figure 4: Plain abdominal X-ray of an infant with perforated necrotising enterocolitis showing pneumoperitoneum**

clinical trials alone are unlikely to alter the unsatisfactory outcome. Current operative techniques are inherently imperfect: they relieve neonatal intestinal obstruction but are not able to eradicate lifelong sequelae related to bowel dysfunction, because to preserve faecal continence they invariably leave behind the aganglionic internal sphincter. Here the surgery-meets-science scenario is being used in an attempt to find a cure through multidisciplinary research.<sup>83</sup>

Defective cranial-caudal migration of vagal neural-crest cells along the intestine during early embryonic development is known to result in Hirschsprung's disease.<sup>92</sup> Despite an incidence of only 1/5000 births, Hirschsprung's disease is the most common congenital enteric neuropathy. Disease severity varies between patients: the aganglionosis is limited to the rectum and the rectosigmoid colon in 80% of cases, but extends more proximally to various lengths of the intestine in the remaining cases. Delayed passage of meconium, constipation, bilious vomiting, and abdominal distension are clinical hallmarks of Hirschsprung's disease. Hirschsprung's-associated enterocolitis, typically manifested by fever, diarrhoea, and increased abdominal distension, is a life-threatening complication in 6-60% of patients that requires early decompression (rectal washouts) and antibiotics.<sup>84</sup> Although the exact cause of Hirschsprung's-associated enterocolitis is unknown, research findings suggests that changes in the intestinal barrier, abnormal intestinal mucosal immunity, and dysbiosis of the gut flora could contribute to its development.

The gold standard of diagnosis for Hirschsprung's disease is rectal biopsy showing an absence of ganglia. Diagnostic accuracy can be improved with ancillary tests such as acetylcholinesterase histochemistry (showing thickened nerve fibres) and the more reliable calretinin immunohistochemistry (showing an absence of calretinin immunoreactivity).<sup>85</sup>

After diagnosis, the traditional treatment was temporary bowel decompression with a stoma formation, followed by an open pull-through operation at a later age and closure of the stoma. Transanal rectosigmoidectomy with or without laparoscopic assistance has become the surgical standard for short-segment Hirschsprung's disease after its introduction in the late 1990s.<sup>86</sup> Singlestage pull-through has been increasingly done in neonates with Hirschsprung's disease without evidence from prospective comparative trials;<sup>87</sup> findings from a retrospective, multi-institutional cohort<sup>88</sup> suggest that although single-stage pull-through has some clinical advantages (reduced rates of wound infection, postoperative enterocolitis, and adhesive bowel obstruction and reduced duration of hospital stay), multistage pullthrough might be necessary for the subgroup of severely ill infants with Hirschsprung's disease.

Surgery is effective in the short term, but functional issues remain. Collective data from earlier studies suggested that bowel dysfunction, including constipation, soiling, and Hirschsprung's-associated enterocolitis, persisted in 14% of patients who had pull-through.<sup>89</sup> Assessment of long-term functional outcomes in patients reaching adulthood has improved recently.<sup>90,91</sup> According to population-based and multicentre follow-up studies,<sup>90,91</sup> 75% of the patients are socially continent in cross-section,

but imperfections in faecal control diminish with age, allowing quality of life comparable to controls. However, the emotional and sexual deficiencies that might prevail in adulthood and lead to social limitations should be investigated further.<sup>90</sup> High-quality clinical evidence could guide empirical treatment and improve outcomes, but bowel resection alone cannot cure an abnormal enteric nervous system. In the past decade, developmental biologists, geneticists, stem-cell biologists, pathologists, gastroenterologists, and surgeons have been collaborating in multidisciplinary research to better understand the disease mechanisms of Hirschsprung's disease and design new management strategies involving precision and regenerative medicine.

The enteric nervous system is complex and contains as many neurons as the spinal cord. Until recently, neither the cause of the defect nor the heterogeneity of Hirschsprung's disease were well understood. Hirschsprung's disease is now understood to be an oligogenic disorder with variable clinical subtypes. The rare subtype with long-segment aganglionosis or familial Hirschsprung's disease (4-8%) and associated syndromes (Down syndrome, Shah-Waardenburg syndrome, Haddad syndrome, Mowat-Wilson syndrome) have dominant or recessive inheritance with incomplete penetrance. The common, sporadic short-segment subtype of Hirschsprung's disease follows a complex non-Mendelian inheritance pattern.<sup>93,94</sup> The most important gene associated with Hirschsprung's disease is *RET*, which encodes the RET receptor tyrosine kinase protein, a cell-surface signal transducer. Deleterious coding mutations in *RET* are found in 50% of familial cases but only in 20% of the more common sporadic cases; other Hirschsprung's disease genes have been identified, but these account for only an additional 7% of sporadic cases (figure 5).<sup>93</sup> In most patients with sporadic Hirschsprung's disease, the aetiology is complex and might involve several disease susceptibility factors, including non-coding (regulatory) mutations.<sup>94</sup> Current technology allows the whole genome to be searched for genes involved in Hirschsprung's disease; for example, the Neuregulin1 gene (*NRG1*) was implicated in Hirschsprung's disease in a genome-wide association study,<sup>95</sup> and several more candidate genes are being identified by whole-exome sequencing.<sup>96</sup> Genotype-phenotype correlations in large patient populations will provide the basis of precision medicine, which could allow treatment options to be tailored according to genetic classification of patient subtypes.

Since the primary pathology of Hirschsprung's disease is an absence of enteric ganglion cells, an attractive alternative to resection of aganglionic bowel tissue is cell replacement therapy. General advances in stem-cell biology and breakthroughs in induced human pluripotent stem-cell (iPSC) research in particular (avoiding ethical

and immunological obstacles), have raised hope of regenerative medicine as a treatment option for patients with Hirschsprung's disease. The successful production of functional enteric neurons from human iPSCs has provided proof-of-concept evidence for this approach.<sup>97</sup> Human iPSCs have also been used to recapitulate Hirschsprung's disease development under cell-culture conditions, allowing correction of Hirschsprung's disease-associated mutations by genome editing and identifying possible drug targets for disease treatment.<sup>98</sup> As an alternative to a pure cell-based approach, human iPSC-derived tissue with a functional enteric nervous system can now be engineered for transplantation into patients with short bowel syndrome associated with ultra-long-segment Hirschsprung's disease.<sup>99</sup> These studies have prompted a multidisciplinary group of 30 experts to conclude that although many obstacles remain to be overcome, clinical application of enteric neural stem cells and first-in-human trials for Hirschsprung's disease is now a real prospect.<sup>100</sup>

## Discussion

In the next 5-10 years, paediatric gastrointestinal surgeons should focus efforts on generating robust clinical evidence, which is particularly deficient in rare neonatal gastrointestinal disorders. The popularisation of minimally invasive surgery in the management of common childhood disorders exemplifies the importance of well designed randomised controlled trials in the justification of new treatment modalities. The outcome of such trials for non-operative treatment of appendicitis is now eagerly awaited (NCT02687464, NCT02271932, NCT02795793). As findings from several studies of biliary atresia and necrotising enterocolitis have shown, the patient volume needed to achieve high-quality clinical evidence for neonatal gastrointestinal disorders is achievable through specialisation and international research consortiums. The evidence for minimally invasive surgery in oesophageal atresia and biliary atresia is still scarce: the paediatric surgical community needs not only to show leadership in designing multicentre, randomised controlled trials to resolve these issues in an objective manner but also to set the best standards of paediatric surgical care.

The results of novel microbiome studies in necrotising enterocolitis open up the possibility of prevention through early diagnosis with new biomarkers and by modifying microbiota-host interactions with relevant probiotics and early intervention by targeting the TLR-4 signalling pathway; the latter represents pathophysiologybased management. The final clinical value of these translational achievements can only be confirmed with large, multicentre patient cohorts managed by multidisciplinary teams. The multidisciplinary teams will have the responsibilities of not only enhancing short-term results but also, more importantly, improving

long-term outcomes, involving adult practitioners and specialists for transition care, and promoting follow-up studies beyond childhood.

When available measures alone are insufficient to eradicate the long-term sequelae, as in the case of Hirschsprung's disease, genetic studies and iPSC research have the potential to provide better treatment, either by enabling pathophysiology-based selection of patient subgroups for surgery, or, more radically, by providing iPSC-based therapy as an alternative or adjunct to existing surgery.

#### Contributors

SSP wrote the section about oesophageal atresia. CG and HF wrote the section about necrotising enterocolitis. PT, PC, and MP wrote the section about Hirschsprung's disease. PC, KW, and MD wrote the section about biliary atresia. SSP wrote the section about hypertrophic pyloric stenosis. SSP and PC wrote the section about appendicitis. GT advised and did additional literature search. PT critically reviewed and redrafted the whole manuscript. All authors approved the final version for publication.

#### Declaration of interests

We declare no competing interests.

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#### Figure 5: Genetics of Hirschsprung's disease: coding mutations

\*20% of patients have the sporadic form of Hirschsprung's disease, and 50% of patients have the familial form of Hirschsprung's disease. †Genes associated with Hirschsprung's disease include *EDNRB* (3–7% of patients), *NRG1* (<5% of patients), *GDNF*, *GFRα1*, *NTN*, *PSPN*, *EDN3*, *SEMA3C/D*, and *GLI3* (<3% of patients), and *ECE-1*, *SOX10*, *PHOX2B*, *ZFHX1B*, *L1CAM*, *KIAA1279*, *NRG3*, *GLI1*, *GLI2*, *DENND3*, *NCLN*, *NUP98*, *TBATA* (<1% of patients).

?

*RET*

(20%)

Others†

(7%)

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