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Hirschsprung´s Disease & Gastroesophageal Reflux. Aspects on Two Gastrointestinal Motility Disorders in Childhood

Gunnarsdottir, Anna

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Gastroesophageal Reflux

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by

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UNIVERSITY
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Neurogastroenterology

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Men love to wonder, and that
is the seed of science

Ralph Waldo Emerson

To my children

Gunnar Húni, Kolbrún and Lilja

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II. **Gunnarsdóttir A,** Sandblom G, Arnbjörnsson E, Larsson LT. Quality of life in adults operated for Hirschsprung´s disease. *J Pediatr Gastroenterol Nutr,* 2009 *(In press)*

III. **Gunnarsdóttir A,** Larsson LT, Arnbjörnsson E. Transanal Endorectal vs. Duhamel Pull-Through for Hirschsprung´s disease. *Eur J Pediatr Surg* 2010; 20(x):1-5 *(In press)*

IV. **Gunnarsdóttir A,** Stenström P, Arnbjörnsson E. Wireless Esophageal pH Monitoring in Children. *J Laparoendosc Adv Surg Tech* 2008 Jun; 18(3):443-7.

V. **Gunnarsdóttir A,** Stenström P, Arnbjörnsson E. 48-Hour Wireless Oesophageal pH-Monitoring in Children: Are Two Days Better than One? *Eur J Pediatr Surg* 2007 Dec; 17(6):378-81.

ABBREVIATIONS

Ach	Acetylcholine
ATP	Adenosine triphosphate
BP	Bodily Pain
CART	Cocaine- and amphetamine- regulated transcript
CNS	Central nervous system
CRF	Corticotrophin-releasing factor
D-group	Duhamel operated group of patients
ENS	Enteric nervous system
ER	Emotional role
ESPGHAN	European Society for Pediatric Gastroenterology, Hepatology, and Nutrition
GER	Gastroesophageal reflux
GERD	Gastroesophageal reflux disease
GH	General Health
GIT	Gastrointestinal tract
GIQLI	Gastro-intestinal Quality of Life
HD	Hirschsprung´s disease
ICC	Interstitial cells of Cajal
IR	Immunoreactivity/immunoreactive
LBF	Large bowel function
LES	Lower esophageal sphincter
MCS	Summary measure of Mental Health
ME	Meteorism
MH	Mental Health
MII	Multiple Intraluminal Impedance
MIS	Minimal invasive surgery
MMC	Migrating myoelectric complex
NANC	non-adrenergic/non-cholinergic
NASPGHAN	North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition
NO	Nitric oxide
NOS	Nitric oxide synthase
NOTES	Natural orifice translumenal endoscopic surgery
PACAP	Pituitary adenylate cyclase activating peptide
PCS	Summary measure of Physical Health

PF	Physical functioning
PPI	Protein pump inhibitors
PR	Physical role
QoL	Quality of Life
RE	Role-Emotional
RI	Reflux index
RP	Role-Physical
SF	Social Functioning
SF-36	Short-Form 36 questions health status survey
T-group	TERPT-operated group of patients
TCA	Total colonic aganglionosis
TERPT	Transanal endorectal pull-through
TLESR	Transient lower esophageal sphincter relaxation
UGI	Upper gastrointestinal tract function
VIP	Vasoactive intestinal peptide
VT	Vitality

INTRODUCTION

Gastrointestinal motility

Embryology and anatomy of the gastrointestinal tract

During embryonic development, the tubular gastrointestinal tract (GIT), about the same length as the crown to heel length of the fetus, appears in approximately the 4th week of gestation. The GIT is divided in four sections: 1) the pharyngeal gut, which gives rise mainly to the pharynx and related glands, 2) the foregut, that gives rise to the esophagus, trachea and lung buds, the stomach and the proximal duodenum, 3) the midgut, that gives rise to the distal part of duodenum and extends to the colon transversum (about 2/3 of the colon), and 4) the hindgut, that gives rise to the distal one third of the colon to the upper part of the anal canal, whereas the distal part of the anal canal originates from the ectodermal anal pit, see review in (Sadler 1985). The GIT undergoes an elongation and a rotation of the intestinal tract so that the small intestine will reach a length of about four times the crown to heel length at birth (Reiquam et al. 1965; Tomomasa and Kuroume 1994), the stomach rotates about 90° clockwise around its longitudinal axis and the midgut rotates about 270° in a counter clockwise direction so that the transverse colon passes in front of the duodenum. This rotation occurs during the herniation as well as during the withdrawal of the intestinal loops into the abdominal cavity which starts at the end of the third month of embryonic development (Sadler 1985).

The esophagus is about 8-10 cm in length at birth and reaches approximately 25 cm in adults (Vandenplas and Heymans 1992b). The greater part of the esophagus is intrathoracic in adults but in the 8-week-old fetus the abdominal esophagus is as long as the stomach, it shortens to only a few millimetres at birth but then elongates over a few years until it reaches its final abdominal length of about 3-6 cm (Vandenplas and Heymans 1992b). This is important for understanding the pathophysiology of gastroesophageal reflux (GER) in young infants. The muscular layer in the esophagus differ from the rest of the GI-tract in that the outer and inner layers of the muscularis propria of the upper third of the esophagus are composed of striated muscle derived from the caudal bronchial arches. In the middle third of the esophagus there is a mixture of striated and smooth muscle and

in the lower third there is smooth muscle that is derived from the splanchnic mesoderm. It has a more traditional arrangement of outer longitudinal and inner circular layers with a myenteric plexus in between them (see Figure 1) (Vandenplas and Heymans 1992b; Mittal and Bhalla 2004). The lower end of the esophagus comprises the lower esophageal sphincter (LES) which provides an important antireflux defense mechanism. The LES is made up of two sphincters, an intrinsic smooth muscle and a striated extrinsic muscle which is formed by the crura of the diaphragm (see Figure 1) (Mittal and Balaban 1997). The LES is about 2-4 cm in length and obtains a constant tone with a basal LES pressure of 10-45 mmHg (Mittal and Bhalla 2004). In normal swallowing, a reduction in LES pressure, causing a relaxation in the sphincter, is necessary for the ingested bolus to reach the stomach, and a complete pressure fall is seen to a level of <8 mmHg above gastric pressure (Spechler and Castell 2001; Mittal and Bhalla 2004). The neuronal control of the LES is a complex mechanism where cholinergic nerves have an excitatory effect and nitric oxide (NO) and vasoactive intestinal peptide (VIP) are two of the major inhibitory neurotransmitter that induces relaxation of the LES (see review in (Mittal and Bhalla 2004)).

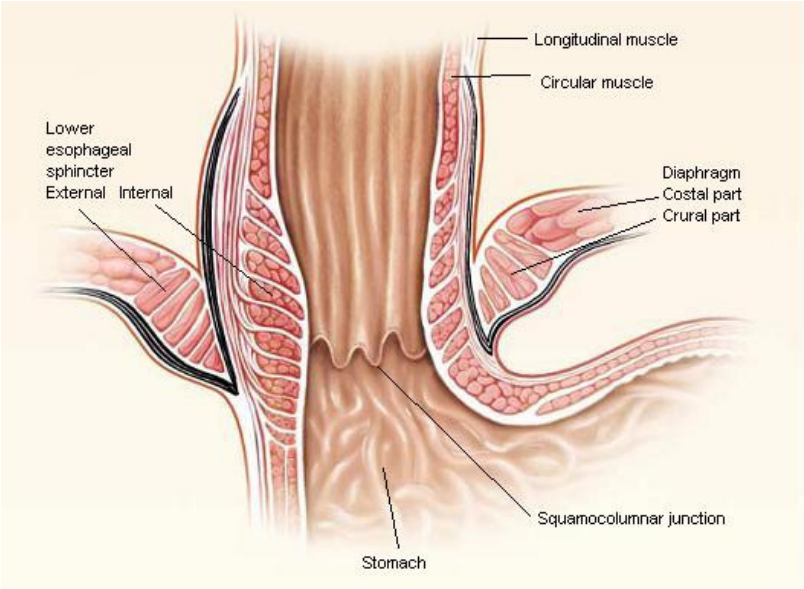


Figure 1. Anatomy of the esophagogastric junction (from Mittal and Balaban 1997).

Enteric nervous system

The enteric nervous system (ENS) has been reported to be the most complex part of the peripheral nervous system and belongs to the autonomic nervous system. It has been estimated that it contains about 200-600 million enteric neurons, about the same number as in the spinal cord (Furness and Costa 1980; Gershon 1993). The enteric neurons originate in the neural crest *in utero.* In the 4^{th} -14^{th} week of fetal development, vagal neural crest cells enter the gastrointestinal tract at the level of the foregut to migrate within the intestinal wall in a cranio-caudal direction to colonize the lengthening gut (Okamoto and Ueda 1967; Okamoto et al. 1982; Burns 2005; Wallace and Burns 2005), for review see (Furness 2006). During the same time an extensive migration, proliferation and differentiation takes place to form a functional ENS (see review in (Burns et al. 2009)).

Anatomically, the intestinal wall consists of four layers; 1) the mucosa nearest to the intestinal lumen, which is subdivided into epithelium, lamina propria, and the muscularis mucosa, 2) the submucosa, 3) the muscular layer containing the circular and the longitudinal muscle and 4) the serosa in the outer layer (see Figure 2) (Feichter et al. 2009). The ENS is made up of numerous interconnected networks (plexuses) of neurons and glia cells, for review see (Furness 2006). The enteric plexuses are two; the myenteric plexus (Auerbach´s plexus) which lies between the inner circular and outer longitudinal muscle layers which it mainly innervates, and the submucosal plexus (Meissner´s plexus) which is found in the submucosa of the small and large intestines and to some extent in the stomach and esophagus, innervating the mucosa, the submucosa and, to some extent, also the circular smooth muscle (Furness 2006) (see Figure 2).

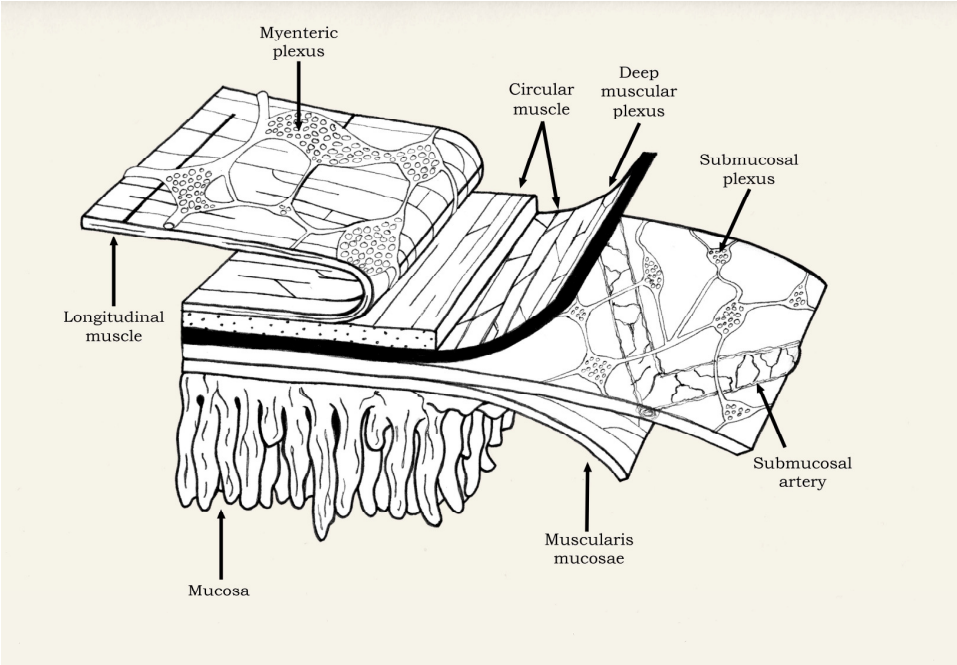


Figure 2. Anatomy showing the different layers of the intestinal wall (from Furness 2006 , modified by Erla Maria)

Interstitial cells of Cajal (ICC) are found adjacent to the muscularis plexus in the intestine and control gastrointestinal motility by generating slow waves in the intestinal muscle; they have therefore been called the pacemaker cells of the intestine (Ward et al. 1994; Burns et al. 1997). The motility of the gut varies in different parts of the digestive tract. In the esophagus the peristaltic movements in the proximal striated muscle during swallowing is mostly by way of vago-vagal pathways, whereas the distal smooth muscle and the lower esophageal sphincter are innervated by motor neurons with cell bodies in the myenteric ganglia and can exhibit peristaltic contraction indicating an intrinsic activity (Lu and Bieger 1998). However the central nervous system (CNS), through the vagus nerve, does appear to exert command over the distal esophagus as well (Furness 2006). In the stomach, the main motility function is to mix the food with gastric juices and then to push the liquefied product using peristaltic movements towards the duodenum, this is achieved with a combined intrinsic and external (vago-vagal) activity, for review see (Furness 2006). In the small intestine there are three main complex motor patterns which have different roles in intestinal motility (Burns et al. 2009), 1) segmentation, causing mixing of intestinal content to the digestive enzymes, 2)

peristalsis causing movement of intestinal content in the caudoanal direction, these two patterns are named collectively the fed state, and 3) the migrating myoelectric complex (MMC) during the fasted state, which is divided into four phases causing periodic activity which initiates peristaltic contractions with phase III as the active component (Tomomasa and Kuroume 1994; Furness 2006; Burns et al. 2009). The human colon displays no MMC but the smooth muscle of the colon exhibits slow wave activity causing mixing movements, especially in the right colon, and occasional colonic peristaltic movement propelling part of the colonic content in an anal direction (Christensen et al. 1969; Mann and Hardcastle 1970; Hasler 2003). Thus the CNS can influence the motility of the gut, especially in the esophagus and in the stomach but the ENS in the small intestine and the colon usually controls motility movements independent of the brain (Furness et al. 1995), for review see (Furness 2006; Burns et al. 2009).

The ENS consists of three functional categories of neurons; sensory, inter- and secreto-motor neurons, required to manifest local reflex behavior and is central to the regulation of both motor and secretory activities and independent of the CNS, for review see (Ekblad 2006). The complexity of the ENS is further illustrated by its great number of neurotransmitters. The neurotransmitters in motor neurons can be excitatory such as acetylcholine (ACh) and the tachykinins, or inhibitory as the NO, VIP, PACAP and adenosine triphosphate (ATP), for review see (Furness 2006). VIP and ACh are also the primary transmitters of secretomotor neurons that control fluid secretion in the small and large intestines (Furness 2006).

CART peptide

Origin and function

The abbreviation CART stands for cocaine- and amphetamine-regulated transcript peptides and is a group of putative neurotransmitters, for review see (Douglass et al. 1995). CART peptide fragment was first identified in the rat striatum after acute administration of cocaine and/or amphetamine (Douglass et al. 1995). It was later identified in other parts of the central nervous system (Kuhar et al. 2000), for review see (Koylu et al. 1998). CART peptide has also been found in the peripheral nervous system including the GIT of rat and guinea pig (Couceyro et al. 1998; Ekblad et al. 2003; Ellis and Mawe 2003). In rats, CART is also expressed in endocrine cells such as the adrenal glands, the pancreas, the stomach antral mucosa and the epididymis (Couceyro et al. 1997; Jensen et al. 1999; Dun et al. 2000; Ekblad et al. 2003; Wierup et al. 2006b).

Two protein products have been found in the rat, a short amino acid sequence of 116 amino acids and a long one of 129 amino acids (Douglass et al. 1995). Only

the short amino acid sequence is found in humans and the human CART gene has been localized to chromosome 5 (Douglass and Daoud 1996).

CART is involved in feeding, reward and reinforcement, development, sensory processing, endocrine regulation and stress (Kuhar and Dall Vechia 1999). In rats, intracerebroventricular injections of CART cause anorexogenic as well as gastrointestinal effects. Rapid inhibition of feeding has been noted in addition to reduced gastric emptying and prolonged colonic transit time (Kristensen et al. 1998; Lambert et al. 1998; Larsen et al. 2000; Okumura et al. 2000; Smedh and Moran 2003; Tebbe et al. 2004). It also decreases the circulating levels of corticosterone and ACTH (Smith et al. 2004). These effects are reversed by the corticotrophin-releasing factor (CRF) receptor antagonist suggesting a central regulation via CRF dependent mechanisms (Okumura et al. 2000; Smith et al. 2004; Tebbe et al. 2004): for review see Ekblad (Ekblad 2006).

CART peptide in the enteric nervous system

In rat gastrointestinal tract, CART peptide and CART mRNA have been located in enteric, particular myenteric neurons, and CART immunoreactive fibres are abundant in the myenteric plexus while few in the mucosa (Ekblad et al. 2003). CART was found within a subpopulation of neurons displaying immunoreactivity for choline acetyltransferase (ChAT), presumably cholinergic neurons (Couceyro et al. 1998). In addition CART was found to be co-localised with nitric oxide synthase (NOS) and VIP (Ekblad et al. 2003). By immunohistochemistry CART was also identified in the GIT of guinea pigs and pigs (Tornoe 1999; Ellis and Mawe 2003). The knowledge of the presence and functional role of CART peptide in the human intestine is very limited. The localization of CART immunoreactivity in the myenteric neurons and nerve fibres in the smooth muscle layers in these animals suggests that CART peptide has a functional role in gastrointestinal motility, possibly as a neurotransmitter or a neuromodulator (Couceyro et al. 1998; Ekblad et al. 2003; Ellis and Mawe 2003).

Some reports have suggested a neurotrophic effect of CART peptide *in vitro* with increased survival of central motor neurons as well as for myenteric neurons in the ENS (Louis 1996; Ekblad et al. 2003; Ekblad 2006). Based on the findings of an early and transiently high expression of CART-peptide in the central nervous system of rat embryo, an ontogenetic role for CART has been suggested (Risold et al. 2006). Also in the periphery an ontogenetic role of CART-peptide is indicated, i.e., in endocrine pancreas and urinary bladder (Zvarova and Vizzard 2005; Wierup and Sundler 2006a). If CART peptide is shown to possess neurotrophic effects, use of this peptide as a neuroprotective agent could be possible.

Gastrointestinal motility disorders

Gastrointestinal motility disorders are common in childhood. Malfunction or dysfunction of the normal motility or secretion of the gastrointestinal wall can cause various motility disorders such as Hirschsprung´s disease, hypertrophic pyloric stenosis, intestinal pseudo-obstruction, gastroesophageal reflux disease (GERD), irritable bowel syndrome, chronic constipation, chronic diarrhea, diabetes-related motility disorders etc. Many of them have the same name as in adults although the clinical presentations, epidemiology and response to treatment vary, such as, for example in GERD and intestinal pseudo-obstruction.

In this thesis we have studied various aspects on two gastrointestinal motility disorders, i.e., Hirschsprung´s disease and gastroesophageal reflux (GER). A more detailed description of these disorders follows.

Hirschsprung´s disease

Definition, history and etiology

Hirschsprung's disease (HD), also called congenital megacolon, is a congenital gastrointestinal motility disorder affecting the ENS. Structurally, it is characterized by an absence of neuronal cell bodies in both myenteric (Auerbach´s) and submucous (Meissner´s) plexuses in the intestinal wall (Dasgupta and Langer 2004). This aganglionosis is most commonly limited to the rectosigmoid segment of the colon and rectum (80-85%). The aganglionic segment always starts distally to the internal anal sphincter extending proximally in variable lengths, in most extreme cases involving the entire large intestine (5-8%). A total aganglionosis of the entire GIT is extremely rare. Functionally, this aganglionosis results in a sustained contraction of the aganglionic segment causing obstructive symptoms. In the aganglionic bowel, transmural nerve stimulation fails to evoke a non-adrenergic, non-cholinergic (NANC) smooth muscle relaxation revealing the deficiency in inhibiting intrinsic neurons (Wright and Shepherd 1965; Menezes et al. 2006a). Despite the lack of intramural ganglia, the aganglionic segment is densely innervated by adrenergic and acetylcholinesterase-positive, presumably cholinergic nerve fibres (Kamijo et al. 1953; Bennett et al. 1968).

HD is named after Harald Hirschsprung, a pathologist at Queen Louise Children´s Hospital in Copenhagen, Denmark who, in 1887, described 2 cases of children with megacolon (Hirschprung 1887). It was not until the beginning of the twentieth century that Tittel noted the absence of ganglion cells in the distal colon of a child with HD (Tittel 1901). Whitehouse and Kernohan, in 1948, presented their own series of 11 children that documented that the aganglionosis within the

distal colorectum was the cause of the functional obstruction (Whitehouse and Kernohan 1948): for review see (Dasgupta and Langer 2004). HD occurs in about 1/5000 live born babies and is more common in boys than girls (4:1) (Spouge and Baird 1985).

The etiology of HD is heterogeneous and not fully understood. Under embryonic development, the neural crest cells migrate caudally where they differentiate amongst other cell types to ganglionic cells of the enteric nervous system. Studies of mice suggest that there is a delay or arrest in this migration, which results in the neural crest cells failing to reach their correct positions in the distal intestine (Okamoto and Ueda 1967; Webster 1973), for review see (Puri et al. 1998; Dasgupta and Langer 2004). Other studies have indicated that neural crest cells fail to survive, proliferate, or differentiate due to abnormalities within their microenvironment after the migration has occurred (Gaillard et al. 1982; Tosney et al. 1986; Clavel et al. 1988; Langer et al. 1994; Hoehner et al. 1996), for review see (Puri et al. 1998; Dasgupta and Langer 2004).

The fact that about 30% of patients with HD have other abnormalities/defects and increased familial incidence suggests a genetic etiology for HD (Passarge 1967; Spouge and Baird 1985). Genetic studies have showed a genetic defect located on chromosome 10 (Angrist et al. 1993; Lyonnet et al. 1993). Different animal and human studies have identified at least 6 gene defects in HD. The most common one is the RET proto-oncogene, which accounts for approximately 20% of sporadic cases of HD and in about 50% of familial cases (Romeo et al. 1994; Yin et al. 1994; Kusafuka and Puri 1997; Robertson et al. 1997). The other genes that seem to be involved in only 1-5% of cases of HD are EDN3, EDNRB, GDNF, ECE-1, SOX10, for review see (Martucciello et al. 2000; Dasgupta and Langer 2004).

Diagnosis

The patients with HD are most often diagnosed in the neonatal period (Singh et al. 2003). The clinical presentation is distended abdomen, delay in meconium passage and vomiting. Older children present with chronic obstipation, which often starts after the breastfeeding period. Most children who present later have short-segment aganglionosis. Approximately 10% of patients with HD present with enterocolitis, with fever, abdominal distension and pain, and sepsis. This may be a life-threatening condition, for review see (Dasgupta and Langer 2004).

Contrast enema shows a transition zone between the normal (often dilated) and the narrow aganglionic bowel in about 70-90% of the cases (Rosenfield et al. 1984; Laurin and Larsson 1990; Smith and Cass 1991). For definite diagnosis a rectal biopsy is needed for histological evaluation. Pathognomonic for HD is the absence

of ganglionic cells in the myenteric and submucous plexuses and the finding of hypertrophic nerve trunks. This is attained traditionally with a full-thickness biopsy or more commonly nowadays, with a suction-biopsy. Anorectal manometry may aid in the diagnosis where the rectoanal inhibitory reflex is absent in children with HD (Tobon et al. 1968).

Surgical methods

Surgical management for HD aims at removing the aganglionic bowel and reconstructing the intestinal tract by bringing the normally innervated bowel down to the anus while preserving normal sphincter function (Dasgupta and Langer 2004). Swenson and Bill were the first to describe an operation for HD by removing the aganglionic bowel with a pull-through in 1948 (Swenson and Bill 1948). Traditionally, this was done in a three-stage manner with diverting colostomy prior to the reconstructive surgery, where the Swenson-, Duhamel- and Soave pull-through were the most common ones used (Swenson and Bill 1948; Duhamel 1960; Soave 1964; Rescorla et al. 1992; Martucciello et al. 2005) (see Figure 2).

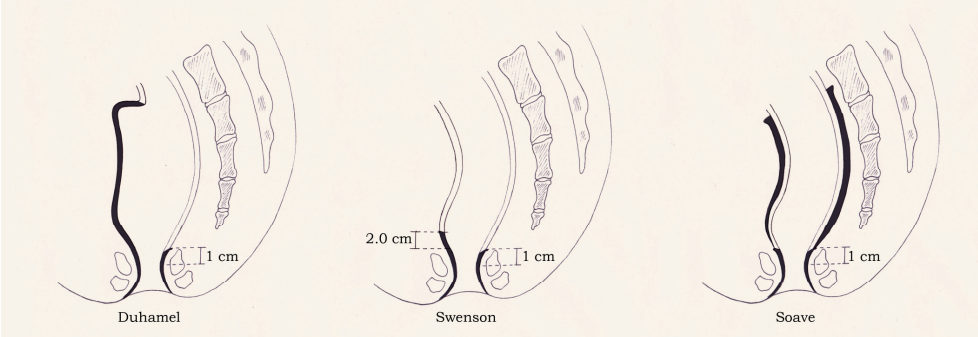


Figure 2. A schematic figure of the three most common pull-through procedures performed previously, Duhamel, Swenson and Soave pull-through. The dark lines of the colon and rectum represent the distal aganglionotic segment left behind whereas the white lines represent the healthy ganglionic colon that is "pulled down" (drawn by Erla Maria).

The surgical approach has gradually changed from a three-stage to a one-stage pull-through without colostomy in the 1980s. This has turned out to be as favourable as the multistage procedures with benefits for the patients and reduction in health care costs due to shorter hospital stay (Langer et al. 1996; Hackam et al. 1997; Pierro et al. 1997).

At our center at the Pediatric Surgical Department in Lund University Hospital the Duhamel pull-through has been used since 1969. The Duhamel pull-through for

HD was introduced by Duhamel in 1960 (Duhamel 1960). In the original description, the native aganglionic rectum was left in place and the normal, ganglionic colon brought down behind the rectum in the presacral place using two crushing Kocker clamps for the end-to-side anastomosis (Duhamel 1960). There have been several modifications of the original description over the years (Martin and Caudill 1967; Vrsansky et al. 1998). Most surgeons use linear staplers for the anastomosis today. For the patients with total colonic aganglionosis (TCA) a Duhamel pull-through with the Martin modification has been used at our center (Martin and Altemeier 1962).

TERPT

During the last decade, the surgical management of HD has changed tremendously with the introduction of minimal invasive approaches using laparoscopy in 1995 (Georgeson et al. 1995; Jona et al. 1998) and with total transanal endorectal pull-through (TERPT), without laparotomy in 1998 (De la Torre-Mondragon and Ortega-Salgado 1998; Albanese et al. 1999; Langer et al. 1999). In May 2005 we started using the TERPT method in our clinic at Lund University Hospital, with a short unsplit muscular cuff described by Rintala et al (Rintala 2003).

The original description of the TERPT method included leaving a long muscular cuff for about 6 cm and some report an even longer cuff for about 10-15 cm, adding a longitudinal myectomy in the posterior wall (De la Torre-Mondragon and Ortega-Salgado 1998; Albanese et al. 1999; Elhalaby et al. 2004). During the last years, reports of TERPT operations using a shorter muscular cuff without the myectomy have been shown to be just as beneficial and this was therefore used in our center (Rintala 2003; Wester and Rintala 2004).

The main advantage of the TERPT technique is that it avoids the laparotomy thereby minimizing certain possible complications i.e., risk of adhesions. It usually includes faster recovery time postoperatively and patients can be discharged from the hospital 2-3 days after surgery. TERPT results in excellent cosmetic appearance of the abdominal wall with no compromise on bowel function (De la Torre-Mondragon and Ortega-Salgado 1998; Albanese et al. 1999; Langer et al. 1999; De la Torre and Ortega 2000; Langer et al. 2003; Rintala 2003; Elhalaby et al. 2004; Wester and Rintala 2004; Zhang et al. 2005). The TERPT method has, however, its limitations and is mostly recommended for patients with aganglionosis limited to the rectosigmoid colon. With a longer or undefined aganglionic segment, a combination of a laparoscopic assisted pull-through or an umbilical mini-laparotomy for segmental biopsy is recommended (Georgeson et al. 1999; Langer et al. 2000).

Quality of life

Early complications after the pull-through operation in children with HD are well-known and most commonly include: constipation, incontinence and enterocolitis (Rescorla et al. 1992; Yanchar and Soucy 1999; Dasgupta and Langer 2004). Most long-term follow-up studies for HD are limited to 5-15 years postoperatively and indicate an improvement in bowel symptoms by the time the children reach adolescence (Livaditis 1981; Rescorla et al. 1992; Bjornland et al. 1998; Yanchar and Soucy 1999; Athanasakos et al. 2004; Menezes et al. 2006a; Niramis et al. 2008). This improvement up to adolescence has also been noted in the general quality of life (QoL) of patients with HD (Hartman et al. 2007; Mills et al. 2008). Whether or not this improvement is maintained in adults with HD has not been confirmed (Hartman et al. 2006). It appears that the psychosocial function has the most important impact on QoL of adult patients with HD, while fecal incontinence and constipation has almost no impact (Hartman et al. 2004). In children with HD, Mills et al found that fecal continence was an important predictor of overall QoL (Mills et al. 2008). In 1977, a study by Puri et al, of long-term results in adults after Swenson's pull-through showed that 99% of the patients had normal bowel control and were in good health 18-27 years after the operation (Puri and Nixon 1977). A study by Heikkinen et al in 1995 also showed good bowel function in adults operated on for HD, without any limitations in occupation, social or physical activities (Heikkinen et al. 1995). This study also indicated that in spite of the high incidence of bowel symptoms during the first years after pull-through, bowel movements had more or less normalized on reaching adulthood.

Gastroesophageal reflux disease and wireless esophageal pH-monitoring in children

Gastroesophageal reflux disease (GERD)

Gastro-esophageal reflux (GER) is very common in infants with a peak of 67% in 4-month-olds where physiological vomiting is common in the first months of life (Nelson et al. 1997). About 5% of children after the age of 1 year have symptoms of GER (Nelson et al. 1997). Recently, pediatric gastroenterologists from both sides of the Atlantic, i.e., the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) came together and published a joint recommendation for pediatric gastroesophageal reflux clinical practical guidelines (Vandenplas et al. 2009). According to the guidelines GER "*is the passage of gastric contents into the esophagus with or without regurgitation and vomiting. GER is a normal physiologic process occurring several times per day in healthy infants, children, and adult*s" (Vandenplas et al. 2009). In contrast, gastro-esophageal reflux *disease* (GERD) is present "*when the reflux of gastric contents causes troublesome symptoms and/or complications*" (Vandenplas et al. 2009). It is important to differentiate between GER and GERD; the main difference is when the reflux is causing clinical symptoms, then we can name it a disease. GERD is one of the most common GI motility disorders in childhood.

Gastroesophageal reflux is due to a defective mechanism at the esophagogastric junction, most commonly due to weakness in the LES or in the crural diaphragm or both (Mittal and Balaban 1997). Inappropriate transient lower esophageal sphincter relaxation (TLESR) is characterized by simultaneous relaxation of the LES and crural diaphragm in the absence of swallowing (Holloway et al. 1995). It is believed that TLESR plays a sustantial role in GER both in healthy people and in GERD patients, although more common in the latter group (Orenstein 1994; Mittal and Balaban 1997). TLESR is a long period of relaxation lasting 10-60 seconds, is more common postprandially and gastric distension and pharyngeal stimulation are two mechanisms strongly suspected to cause TLESR (Orenstein 1994; Mittal and Balaban 1997).

In adults, the symptoms of GER are typically heartburn and/or acid regurgitation. The clinical picture of GER is more complex in childhood and depending of age. The infantile reflux with vomiting usually presents early and resolves in at least 80% of the children before 18 months of age (Shepherd et al. 1987; Vandenplas et

al. 2009). Subjective descriptions of symptoms are unreliable in infants and children younger than 8-12 years of age and many symptoms are non-specific. Various symptoms can occur and those are summarized in Table 1, for review see (Vandenplas et al. 2009). In the older age group symptoms of GER are more similar to those of adults.

Symptoms	Signs
Recurrent regurgitation/vomiting	Esophagitis
Weight loss or poor weight gain	Esophageal stricture
Irritability in infants	Barrett esophagus
Ruminative behavior	Laryngeal/pharyngeal inflammation
Heartburn or chest pain	Recurrent pneumonia
Hematemesis	Anemia
Dysphagia, odynophagia	Dental erosion
Wheezing	Feeding refusal
Stridor	Dystonic neck posturing
Cough	Apnea spells
Hoarseness	Apparent life-threatening events (ALTE)

Table 1. Symptoms and signs that may be associated with gastroesophageal reflux (Vandenplas et al 2009)

In the diagnosis of GERD in children, the history and physical examination are of utter importance holding in mind the symptoms and signs given in Table 1, which are often non-specific. Various parent- or patient- reported questionnaires have been developed with variable sensitivity and specificity for GERD in different studies: for review see (Vandenplas et al. 2009).

Esophageal manometry has been widely used in studying the physiology of the esophagus by measuring upper and lower esophageal sphincter pressure and peristalsis during swallowing. This can be of value in the diagnosis of achalasia and other motor disorders. However, it has no place in the diagnosis of GERD (Orenstein 1994; Vandenplas et al. 2009).

Upper GI endoscopy is of great value in the diagnosis of GERD and includes a valuable direct vision of the mucosa in the esophagus, stomach and the duodenum. It is of special value in estimating esophagitis, mucosal erosions, strictures, ulcerations and the existence of Barrett´s esophagus and hiatus hernia. It also adds the possibility to take mucosal biopsies for further valuing the existence of inflammation and possible metaplasia. However, normal endoscopy does not

exclude the existence of GERD as the majority of patients with GER have a normal endoscopy (Venables et al. 1997; Vandenplas et al. 2009). Reflux esophagitis has recently been defined by global consensus as visible breaks in the esophageal mucosa at, or immediately above, the gastroesophageal junction (Vakil et al. 2006). However, for the pediatric population a consensus was not reached for this statement because some participants felt that esophagitis should also be defined by histology outlining further the diversity in the diagnosis of GERD (Sherman et al. 2009; Vandenplas et al. 2009). The Los Angeles (LA) classification is the most widely used for grading the severity of esophagitis according to endoscopic findings, dividing the severity of mucosal changes into four stages (A-D), and can be used in both adults and children. Another classification, also used for the pediatric population, is the Hetzel-Dent classification (Hetzel et al. 1988; Armstrong et al. 1996; Lundell et al. 1999).

For the diagnosis of GERD, barium contrast radiography is not justified due to its low sensitivity and specificity. It is, however, often valuable to detect anatomic abnormalities such as stricture/obstruction, hiatus hernia, achalasia or intestinal malrotation which are often the differential diagnosis in the vomiting child (Simanovsky et al. 2002; Vandenplas et al. 2009).

Nuclear scintigraphy, in which food, labelled with ^{99}technetium, is ingested and Bernstein test which is an acid perfusion test to mimic heartburn symptoms are also examples of tests that lack sensitivity and specificity and are not commonly used nowadays (Wenner 2007a; Vandenplas et al. 2009).

The empiric trials of acid suppression medication with protein pump inhibitors (PPI) for patients with symptoms indicating GER are common in general practice in both adults as well as in children. The sensitivity and specificity of such PPI-trial for the diagnosis of GERD are, however, variable and the doses and length of time recommended are yet to be determed, see review in (Vandenplas et al. 2009). According to the NASPGHAN/ESPGHAN guidelines, an empiric PPI treatment is justified for up to 4 weeks in an older child or adolescent but there is no evidence to date supports such a PPI-trial in infants and young children as a diagnostic test of GERD (Vandenplas et al. 2009).

Esophageal multichannel intraluminal impedence (MII) measures the movement of fluids, solids and air in the esophagus and was introduced in 1991 (Silny 1991). It has multiple sites with electrodes located along the catheter and measures the impedence (i.e., resistance) between electrodes and thereby the amount and the direction of boluses travelling the esophagus. It can also be combined with a pH sensor (MII-pH) (Silny 1991; Vandenplas et al. 2009). MII is superior to the traditional pH-monitoring in that it also detects non-acid refluxes and various studies have shown that non-acid reflux (pH>4) varies from about 40% to 90% of total reflux events during 24 hour monitoring, see summary in (Vandenplas et al.

2007). However, the clinical significance of non-acid reflux in GERD is unclear as it is the acid reflux causing the mucosal injury in the pathogenesis of esophagitis (Oberg et al. 1998). Many children and infants have respiratory symptoms such as chronic bronchitis, cough, infant apnea, and asthma-like weezing, believed to be caused by GERD. MII are most likely to be of better value in detecting a relationship between reflux and respiratory symptoms showing stronger association beween respiratory symptoms with non-acid reflux than with acid reflux (Wenzl et al. 1999; Wenzl et al. 2001; Rosen and Nurko 2004). Other studies have, however, not been able to confirm this (Mousa et al. 2005). Therefore, up to now, data confirming that MII offers a clear benefit in the diagnosis of GERD of clinical value for the pediatric group is still not conclusive.

Esophageal pH-monitoring

The intraluminal esophageal pH-monitoring system has been widely used in clinical practice for the diagnosis of GERD since the 1980s. It was, however, first published in 1969 and a scoring system was introduced in 1974 (Spencer 1969; Johnson and Demeester 1974), see review in (Wenner 2007a).

A 24-hour pH-monitoring has been the "gold-standard" in the diagnosis of GERD. The normal pH value in the esophagus is about 5-7 but 1-2 in the stomach (Bremner et al. 1995). The definition of a reflux episode has traditionally been a pH drop below 4.0 as a cut-off limit, and that it lasts for at least 15 seconds (Vandenplas et al. 1992a; Vandenplas et al. 2009). The reflux index (RI), i.e., the percentage of measured time that pH is less than 4 during the pH-monitoring, is the most commonly used summary score (Vandenplas et al. 2009). It is, however, difficult to determine a pathological cut-off limit for RI in children to discriminate between physiologically normal GER in infants/children and GERD. In 1991, Vandenplas and colleagues found in a study of 509 healthy infants that the normal range for the RI during the first 12 months was about 10%, decreasing from 13% at birth to 8% at 12 months (Vandenplas et al. 1991). According to recent NASPGHAN/ESPGHAN guidelines there is consensus for considering RI>7% abnormal, RI<3% is considered normal and RI between 3-7% is indeterminate (Vandenplas et al. 2009). Other scoring systems have also been used such as the DeMeester score, where several different measurements result in a final score, see Table 2 (Johnson and DeMeester 1986).

The positioning of the pH-probe is a crucial methodological aspect in pH-monitoring. Traditionally, pH-monitoring has been performed by using a nasal catheter with a pH-electrode, either glass or antimony, placed fluoroscopically above the diaphragm at the width of two vertebra bodies according to ESPGAN criteria in children (Vandenplas et al. 1993) or 5 cm above the upper border of the LES in adults (Jamieson et al. 1992; Kahrilas and Quigley 1996). When using the

catheter-based technique the electrode is not fixed to the esophageal mucosa, any movement induced by changes in body positioning or swallowing may displace the pH-probe (Mones et al. 2001; Aksglaede et al. 2003). Furthermore, the nasal-esophageal catheter can cause discomfort and is not well-tolerated by all children. It can lead to a modified life style with a reduction in reflux-provoking activities, which may conceal the presence of GERD and influence the reliability of the test (Fass et al. 1999).

DeMeester score
Reflex index, % time pH<4.0
% time pH<4.0, upright position
% time pH<4.0, supine position
Total reflux episodes
Total reflux episodes >5 min
Time for the longest reflux episode

Table 2. DeMeester combines six different parameters from the pH-monitoring results into a composite pH DeMeester score with a normal (>95%) value of <14.72.

With the introduction of a new wireless technique, a radio-transmitted capsule for esophageal pH-monitoring, the Bravo™ pH-system (Medtronic, Shoreview, Minn, USA), the use of an indwelling catheter can be avoided. The wireless pH-system consists of a capsule attached to the end of a catheter delivery system and a pager-sized receiver (see Figures 4 and 5). The capsule is 6 x 5.5 x 25 mm in size, has an antimony pH and reference electrode located on the distal tip and contains a battery and a transmitter. The capsule is attached to the esophageal mucosa and can be placed either transnasally or transorally as defined by the results of a manometry, endoscopy or fluoroscopy. The pH-data are transmitted using radio telemetry and recorded via a portable receiver. The Bravo™ pH-system is known to cause less discomfort, is well-tolerated by children and enables measurements over 48 hours (Bothwell et al. 2004; Wenner et al. 2005; Croffie et al. 2007).

At our institution, we routinely perform upper GI endoscopy and the placement of the Bravo capsule in general anesthesia. It might therefore be suggested that the first 24 hours during pH-monitoring do not particularly represent a day in the life of a child because during that day he/she is recovering after the anesthesia. That makes the possibility of 48-hour monitoring an interesting alternative.

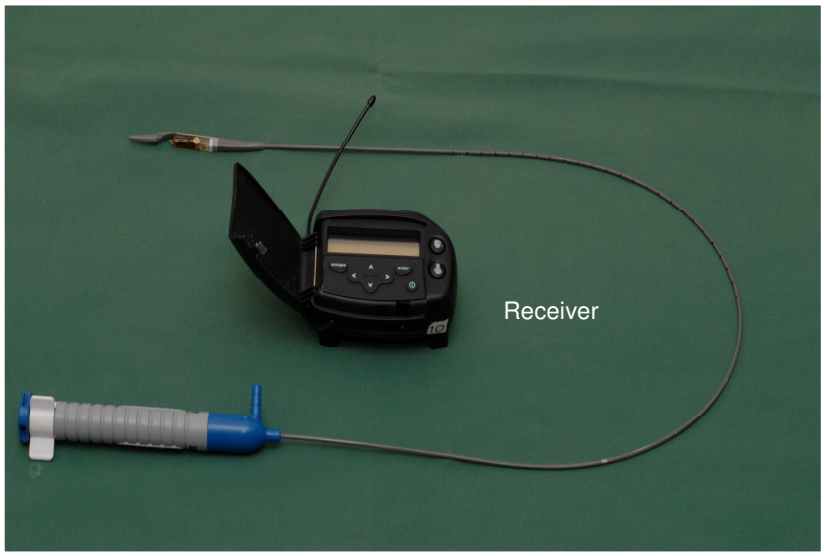


Figure 4. Wireless pH capsule attached to the, delivery system and portable receiver (photo Lars Bodin, (reproduced with permission from Dr. Jörgen Wenner (Wenner 2007a))

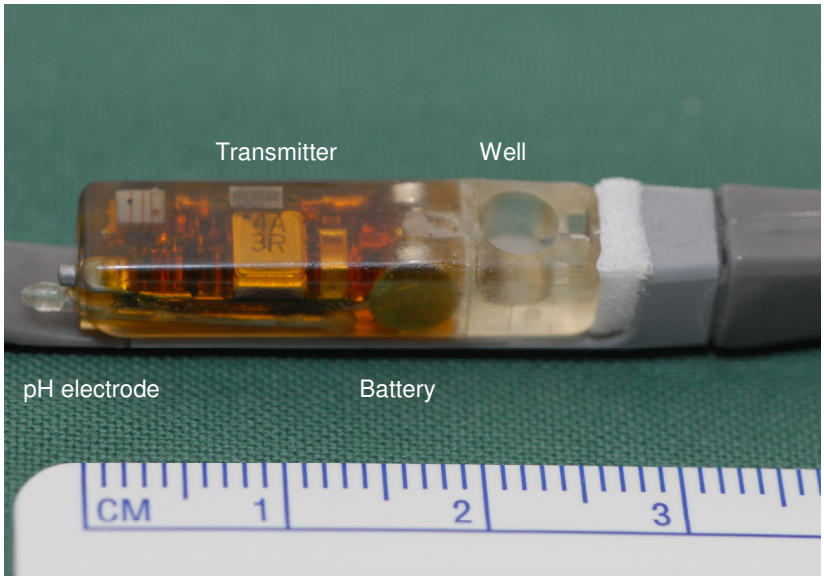


Figure 5. Wireless pH capsule attached to the catheter delivery system (photo Lars Bodin, reproduced with permission from Dr. Jörgen Wenner (Wenner 2007a)).

AIMS OF THE THESIS

To study the long-term quality of life for adults operated for Hirschsprung´s disease in childhood.

To compare short term operative and functional results of transanal endorectal pull-through (TERPT) vs. Duhamel pull-through for children with rectosigmoid Hirschsprung´s disease.

To examine the presence and topographic distribution of CART peptide containing nerves in the small and large intestine in both normal and aganglionic human intestine.

To study the possible colocalization of CART with other known neurotransmitters in the gut, i.e., NO and VIP.

To study and evaluate the use of a wireless pH-monitoring system in children

To access if 48-hour pH-monitoring is of additional value in the diagnosis of GERD in children compared to the traditional 24-hour pH-monitoring

To evaluate the discriminatory power of various parameters of esophageal acid exposure to differentiate children with esophagitis from those without esophagitis.

SUBJECTS AND METHODS USED

Patients in studies I-V

Paper	Motility disorder	Number of patients	Mean age ± SD	Range	♂/♀
I	HD	9	9m ± 12m	2m-41m	8/1
II	HD	42	29y ± 7y	18y-45y	27/15
III	HD	29	29m ± 6m	25m-48m	22/7
IV	GERD	58	8y ± 4y	9m-15y	37/21
V	GERD	23	8y ± 4y	9m-15y	15/8

Table 3. All participants in the different studied listed. Number and age of the patients at the time of the study and the sex distribution are presented. In paper III the age given is the one at the last clinical follow-up.
HD: Hirschsprung´s disease, GERD: Gastroesophageal reflux disease, m=months, y=years, ♂: males and ♀: females

Paper I

Immunohistochemistry

Between 1992 and 1999, rectal and colonic specimens were sampled, during surgery from nine patients with HD and included biopsies from the ganglionic and aganglionic part of the intestine, including the terminal ileum in one patient.

The specimens were fixed overnight at 4^{o}C in Stefanini´s solution. Three to five sections were cut from each specimen and processed for the immunohistochemical demonstration of the CART peptide. The sections were exposed to the primary antiserum overnight and incubated with fluorescence isothiocyanate (FITC) or Texas Red labelled secondary antibodies. The coexistence of NOS and CART, VIP and CART, and NOS and VIP in nerve fibers in the muscle layer and in the nerve cell bodies in the myenteric and submocosal ganglia was studied by means of double immunostaining. The results are given in percentage of overlapping between the different neuropeptides studied (VIP, NOS and CART).

To study the presence of CART in neurons we used a polyclonal antiserum raised in rabbit against the CART peptide fragment 106-129 (dilution 1:1280, Cocalico Corp., Reamstown, PA, USA) (Koylu et al. 1997; Ekblad et al. 2003). For double–immunolabeling, the CART antiserum was used in combination with either monoclonal antibodies against VIP (dilution 1:640, code MaVIP; East Acres Biologicals, Southbridge, MA, USA) (Ekblad et al. 2003) or an antiserum against rat neuronal NOS sequence 1409-1429 raised in sheep (dilution 1:2560, code AB1529; Chemicon International, Ltd, Harrow, UK, (Ekblad et al. 2003). For double-immunolabelling of NOS and VIP, monoclonal VIP antibodies were used in combination with a polyclonal antiserum against rat cerebellar NOS raised in rabbit (dilution 1:2500, code 9223, Euro Diagnostica, Malmö, Sweden) (Ekblad et al. 1998).

The site of the antigen-antibody reaction was visualized by FITC- or Texas Red-conjugated antibodies to rabbit immunoglobulin G (IgG) raised in pigs (DAKO, Copenhagen, Denmark), affinity purified FITC-conjugated antibodies to mouse IgG raised in goat (Jackson Immuno Research Laboratories Inc., West Grove, PA, USA), FITC-conjugated antibodies to sheep IgG raised in donkey or FITC-conjugated antibodies to guinea-pig IgG raised in goat (Sigma, St Louis, MO, USA) (Ekblad et al. 2003). Absorption controls (addition of 10-100 μg of synthetic peptide/ml diluted antiserum) were run on control sections to exclude non-specific staining.

Paper II

During the years 1969-1989, 51 children underwent a pull-through operation for HD at our center. 47 children were alive in the year 2007 at the time of the study and 42 of these participated in the study with an 89% response rate: see Table 3 for age and gender of the patients. Patient records were assembled retrospectively for gestational age and birth weight, associated anomalies, age at presentation, clinical presentation, level of aganglionosis, surgical procedures, number of operations performed by each surgeon, complications (immediate and late), reoperations, occurrence of enterocolitis and bowel function during clinical follow-up time.

For long-term follow-up into adulthood, two QoL questionnaires were distributed to all patients in the year 2007, i.e. 18-37 years after surgery.

SF-36 Health survey

The general health-related SF-36 QoL questionnaire is a generic validated survey for health-related quality of life, and age-adjusted reference values for the Swedish population are available (Sullivan and Karlsson 1998 and 2002). It yields 8 scales and 2 summary measures: physical functioning (PF), role-physical (RP), bodily pain (BP) and general health (GH) make up the summary measure of physical health (PCS); and vitality (VT), social functioning (SF), role-emotional (RE) and mental health (MH) make up the summary measure of mental health (MCS). The theoretical maximum score is 100 for each scale (Ware 2000) (see Table 4).

Summary measure of physical health	**PCS**	**Definition**
Physical functioning	PF	Limitation in performing all physical activities
Role-physical	RP	Interference with work or other daily activities due to physical problems
Bodily pain	BP	Limitation due to pain
General health	GH	Personal evaluation of health
Summary measure of mental health	**MCS**	**Definition**
Vitality	VT	Personal feeling of energy
Social functioning	SF	Limitation of social activities due to physical and emotional problems
Role-emotional	RE	Interference with work or other daily activities due to emotional problems
Mental health	MH	Personal evaluation of mental status

Table 4. SF-36 QoL questionnaire yields 8 subscales and 2 summary measures (PCS and MCS) as shown here.

GIQLI

Gastrointestinal Quality of Life Index (GIQLI) is a 36-item instrument concerning gastrointestinal disease-related symptoms, physical status and psychosocial dysfunction. Recently, a validated Swedish translation of the GIQLI questionnaire became available and was used in this study (see Appendix 1) (Sandblom et al. 2009). In a factor analysis of the Swedish version of GIQLI (Sandblom et al. 2009), the questions were divided into five main outcomes: physical role (PR); large bowel function (LBF); emotional role (ER); upper gastrointestinal tract function (UGI); and meteorism (ME). The highest theoretical score is 144 points. Other studies have shown that scores between 95 and 105 occur in clearly symptomatic individuals (Eypasch et al. 1995; Slim et al. 1999). A GIQLI score lower than 105 indicates that the individual has constant ongoing gastrointestinal symptoms and has been used as a cut-off point in previous studies on long-term QoL for other congenital malformations; this was also chosen for the present study (Koivusalo et al. 2005 (a and b)). The GIQLI questionnaire was also distributed to a group of 627 individuals in Sweden, randomly selected from the general population in order to determine expected GIQLI scores in a healthy population. From this sample, a control group of 42 individuals was identified, matched for age and gender.

Statistical analysis

The mean value from different scales in the SF-36 questionnaire was compared with the age-matched norms for the general Swedish population, and male and female subgroups using the non-parametric Wilcoxon Signed Ranks Test. Similarly, the observed GIQLI scoring was compared with the age- and gender-matched control group using the Wilcoxon Signed Ranks Test. Statistical significance was $P<0.05$. One way ANOVA with post hoc Tukey HSD was used for multiple comparisons between groups with different aganglionic segment length and GIQLI score.

Univariate and multivariate linear regression analysis with gender, age at surgery, level of agangliosis (limited to sigmoid colon/above sigmoid colon), operating surgeon and number of surgical procedures as covariates was performed in order to identify factors predicting total GIQLI score.

Difference in outcome between patients with and without persisting bowel symptoms at the last clinical control was tested with Student´s t-test.

Paper III

The study includes all consecutive patients operated on for HD defined to the rectosigmoid colon at our center in 2000-2007 (n=29): see patient age and gender in Table 3. Eleven patients were operated on with the TERPT method (T-group) during 2005-2007 and were prospectively followed up for 24 months post-operatively. For comparison, the medical records of 18 patients operated on with the Duhamel pull-through (D-group) during 2000-2006 were retrospectively viewed. The number of patients was chosen after power analysis.

At our center, the transanal pull-through is carried out with a circumferential incision in the rectal mucosa about 5 mm above the dentate line, to establish a submucosal plane. We use a short undivided muscular cuff about 2-3 cm after which the rectal muscle is divided circumferentially and the full thickness of rectum mobilized out through the anus. Full thickness biopsy is taken from macroscopically normal ganglionic colon for frozen section to determine the resection level of the colon before doing the final anastomosis. If the patient has a colostomy prior to the pull-through procedure the colostomy is closed in the same operation with a mini-laparotomy around the stoma opening. All patients in the T-group were seen at our outpatient clinic two weeks after the operation for calibration of the anal canal with Hegar dilators and most of them continued with daily anal dilatations/calibrations by the parents in the home.

The standard way of performing Duhamel pull-through at our clinic has been as described by Duhamel (Duhamel 1960), through a vertical paramedian incision in the left lower abdomen and around the colostomy if present. The aganglionic colon is identified and resected distally to a point above the peritoneal reflection. A retrorectal end-to-side colorectal anastomosis is performed, with the level of anastomosis above the dentate line, using a stapling device for the colorectal anastomosis. Those patients with colostomy have their ganglionic level verified from previous biopsies from the colostomy site. In the other patients, levelling biopsies are taken during the pull-through operation to establish ganglionic level before the anastomosis is completed.

Data recorded from patient records included patient demographics, type of presentation and diagnostic work-up, need for colostomy, age at diagnosis and operation, operative time, operative bleeding, time of oral feeding and bowel movement postoperatively. Early and late postoperative complications and additional surgical interventions (re-operations) were also listed. Need for analgesics, antibiotics and blood transfusion postoperatively and the length of hospital stay, were also noted. During follow-up, episodes of enterocolitis, dilatations required, other operations or hospitalizations were noted. Stooling habits including continence and frequency and need for laxatives or other

medications were registrated during follow-up. The functional status at last clinical control was also recorded.

The follow-up time was limited to 24 months for early outcome so that both groups could be compared. Follow-up data was missing for one patient in the D-group so data from this patient was only included in the operative results but excluded from the follow-up calculations.

Statistical analysis

The Mann-Whitney test was used for comparison between the T- and the D-groups with significant *p-value* of <0.05. The 2-sided Fisher´s Exact Test was used to compare different demographics and incidence of different variables in the T- and D-groups during follow-up.

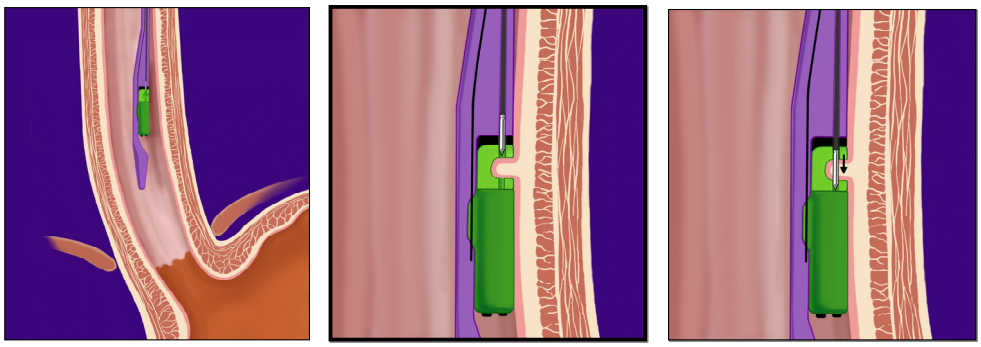
Papers IV & V

A total of 62 pH-measurements with the wireless Bravo™ capsule were made in 58 consecutive children who had been admitted to our clinic due to symptoms of GERD. The study time period was from 2005-2007. Of these childen, 23 were included in a separate study during the period of September 2006-April 2007 for comparing the result of 24-hour with 48-hour pH-monitoring. See Table 3 for age and gender distribution of the patients.

After six hours of fasting and a week without PPI or H2-antagonist medication, an upper gastrointestinal endoscopy under general anesthesia was performed, using a 9.3 or 6.2 mm endoscope (Olympus™, Sweden). A complete examination of the esophagus, stomach and proximal duodenum was performed and biopsies taken if indicated. The presence of esophagitis, hiatus hernia or strictures was noted.

Before placement, the wireless Bravo™ capsule was calibrated in buffer solutions of pH 1.0 and 7.0 and activated by a magnetic switch according to manufactures instructions.

The Bravo™ capsule was placed distally in the esophagus, at the width of two vertebra bodies above the diaphragm, as visualized by fluoroscope (Vandenplas et al. 1993). External vacuum pump suction with at least 500 mmHg pressure was applied for at least 60 seconds, after which the pH-capsule was fixed to the mucosa with a stainless steel pin according to instructions, see Figure 6.



A B C

Figure 6. Application of the wireless pH capsule. The capsule at the end of the delivery system is positioned with the pH electrode at the desired level in the esophagus **(A).** Suction is applied to capture the esophageal mucosa within the well of the capsule **(B),** and the esophageal mucosa is secured within the capsule by a stainless pin **(C)** (reproduced by permission of SynMed Medicinteknik AB, Sweden).

The pH-recording was initiated at the postoperative unit when the children were awake. The children and their guardians were instructed to keep the receiver attached to or within 2.5 m from the body during the 24- or 48-hour study period. All the guardians were instructed to keep a diary and to record symptoms, meal times and times for prone and supine position. The children were encouraged to engage in all normal daily activities. There were no restrictions regarding the amount of food allowed but the participants were instructed to avoid acid-containing products. In addition, the children were asked specifically about dysphagia, chest pain or other symptoms during the period the capsule was in place.

The data from the receivers were loaded into a computer and analyzed using the software Polygram^{TM}, NET (Medtronic MN, USA). No case of premature detachment of the pH-capsule was noted in our series. The DeMeester score was automatically generated by the software, with 95th percentile <14.72 as a normal value. A typical pH tracing of simultaneous pH recording for normal and pathological acid reflux activity is seen in Figure 7A and B.

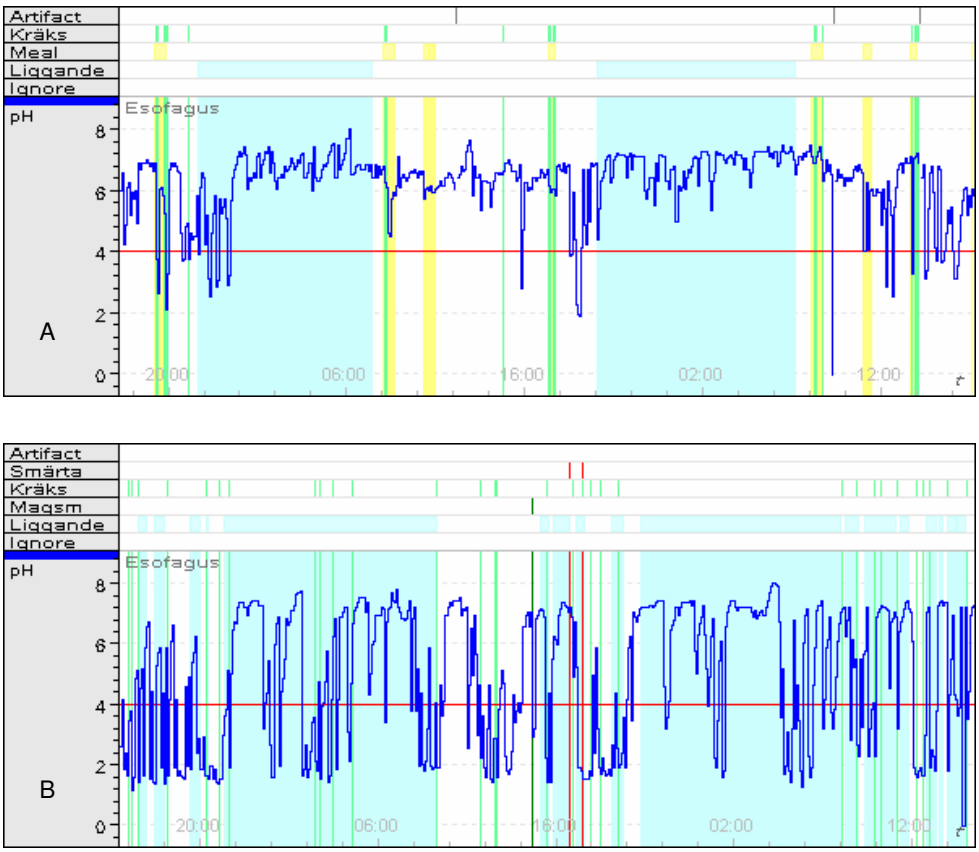


Figure 7 A and B. pH-monitoring for 48-hours showing normal acid reflux activity in (A) and pathological acid reflux activity in (B).

Statistical analysis

In Paper IV a paired-sample t-test and one way-ANOVA test were used and in Paper V a paired-sample Wilcoxon signed ranks test was used with significant *p-value* of <0.05.

RESULTS

Paper I

CART in the ganglionic vs aganglionic intestine

In normal ganglionic large and small intestine, numerous CART-IR nerve fibers were seen in the smooth muscle layers as illustrated in Figures 8A and 9A. The CART-IR nerve fibers were more abundant in the colon compared to the ileum. In the myenteric ganglia numerous CART-IR fibers and CART-IR nerve cell bodies were seen (Figure 9D). CART-IR nerve fibers were sparse in the submucosa and the mucosa. In the submucous ganglia very few CART-IR nerve terminals and cell bodies were noted (Figure 9G).

In the aganglionic colon, CART nerve cell bodies were totally absent (Figure 8B). Scattered CART nerve fibers were seen in the muscle layers. There were pathological nerve trunks, harbouring a few CART fibers, in between the circular and longitudinal muscle layers. No CART nerve fibers were detected in the submucosa, in the muscularis mucosa or in the mucosa.

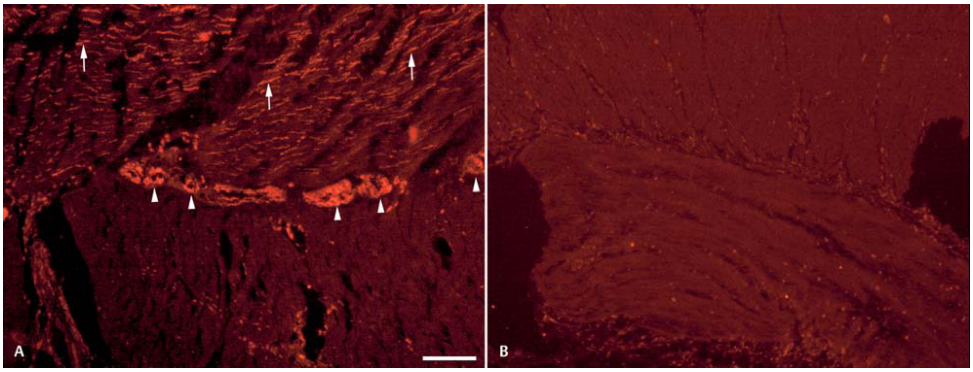


Figure 8A and B. Cryostat sections of human colon specimens from patients with Hirschsprung´s disease, showing CART-IR nerve cell bodies and nerve fibers in **(A)** ganglionic colon, where ganglia are indicated by arrowheads and fibers exemplified with arrows, and in **(B)** aganglionic colon at low magnification showing near absence of CART-IR both in and between the muscle layers.

Coexistence of CART, NOS and VIP

The main results of CART, NOS and VIP coexistence are outlined in Figure 8 and in Table 5.

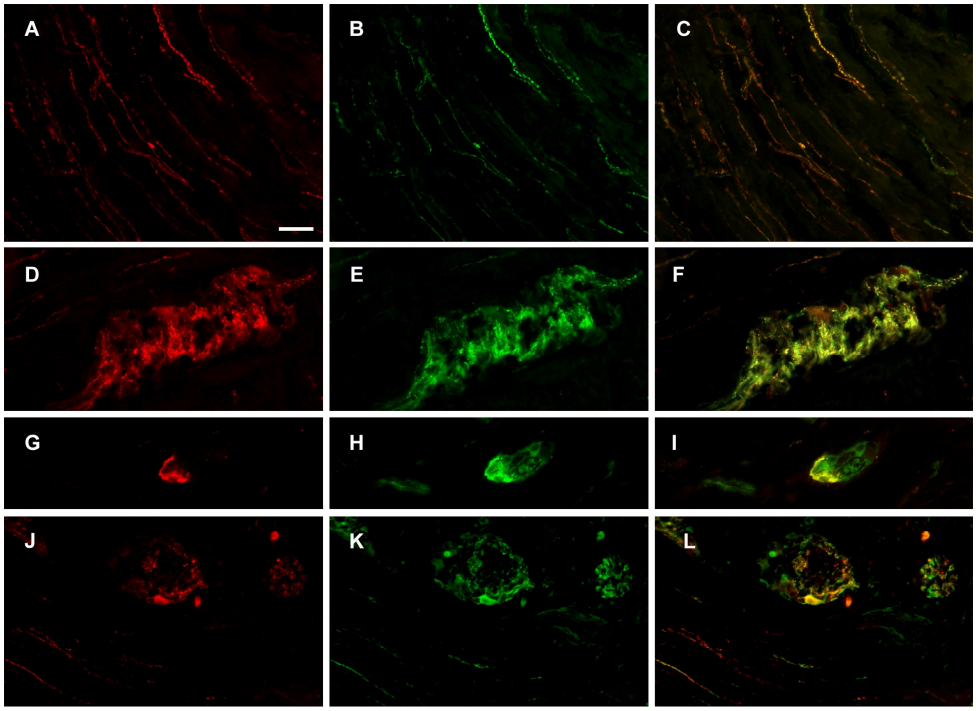


Figure 9. Cryostat sections of human colon specimens from patients with Hirschsprung's disease, showing double immunostaining for the demonstration of CART and VIP **(A–I)** and CART and NOS **(J–L)** in high magnification. Bar represents 50 µm. **(A–C)** Illustrates the muscular layer in the ganglionic part of the colon. **(A)** CART-IR nerve fibers, **(B)** VIP-IR nerve fibers and merged pictures in **(C)**. A total coexistence of CART-IR and VIP-IR nerve fibers in the muscular layer was seen. **(D–F)** Myenteric ganglia in the ganglionic part of the colon. **(D)** CART-IR in myenteric ganglia, **(E)** VIP-IR in myenteric ganglia and merged pictures in **(F)**. A high degree of coexistence of VIP-IR and CART-IR nerve cell bodies was seen. About 70% of VIP-IR nerve cell bodies also contained CART-IR and almost all CART-IR nerve cell bodies contained VIP-IR. **(G–I)** Submucous ganglia in the ganglionic part of the colon. **(G)** CART-IR in submucous ganglia, **(H)** VIP-IR in submucous ganglia and merged pictures in **(I)**. VIP-IR nerve terminals and nerve cell bodies were seen; one of them also contains CART-IR as shown in the picture **(H–I)**. All CART-IR nerve terminals and nerve cell bodies were also VIP-IR. **(J–L)** Myenteric ganglia with a neighbouring muscular layer in the ganglionic part of the colon. **(J)** CART-IR in the myenteric ganglia, **(K)** NOS-IR in the myenteric ganglia and merged pictures in **(L)**. A near total co-localization was seen between CART-IR and NOS-IR nerve fibers in the muscular layer. In the myenteric ganglia a higher number of NOS-IR nerve cell bodies than CART-IR were detected.

	Muscle layer	Myenteric ganglia		Submucous ganglia	
	Nerve fibers	**Nerve cell bodies**	**Nerve fibers**	**Nerve cell bodies**	**Nerve fibers**
CART & NOS	CART+ 90% NOS+	CART+ 100% NOS+	CART+ 90% NOS+	CART+ 100% NOS+	CART+ 100% NOS+
	NOS+ 90% CART+	NOS+ 50% CART+	NOS+ 90% CART+	NOS+ ~100%CART+	NOS+ 90% CART+
CART & VIP	CART+ 100% VIP+	CART+ ~100% VIP+	CART+ 100% VIP+	CART+ 100% VIP+	CART+ 100% VIP+
	VIP+ 100% CART+	VIP+ 70% CART+	VIP+ 90% CART+	VIP+ 10% CART+	VIP+ 10% CART+
NOS & VIP	NOS+ 90% VIP+	NOS+ 20% VIP+	NOS+ 90% VIP+	NOS+ 100% VIP+	NOS+ 100% VIP+
	VIP+ 90% NOS+	VIP+ ~100% NOS+	VIP+ 90% NOS+	VIP+ 10-20% NOS+	VIP+ 10-20% NOS+

Table 5. A high degree of coexistence between CART-, NOS- and VIP-IR nerve fibers and nerve cell bodies was confirmed in the ganglionic normal intestinal wall. This table outlines the main results of how this inter-coexistence was. Subdivided into the nerve fibers in the muscle layer, the nerve cell bodies in the myenteric and the submucous ganglia. + meaning IR-positive for the neuropeptide measured, i.e., for example “CART+ 90%NOS+” means that of all CART-IR nerve fibers, 90% of them were also NOS-IR positive and so on. The sign “~” was used when near total coexistence was encountered.

Paper II

Results from review of patient records

Of the 42 participating patients with HD (see age and sex distribution in Table 3) fifteen had concurrent disease (36%), with Mb Down (n=6), urogenital- (n=6) and neurological diseases (n=5) as the most common ones. Five patients had a family history of HD (12%). In 29 patients the aganglionic segments were located in the rectosigmoid part of the large bowel (69%) and three patients (7%) had total colonic aganglionosis. Twenty patients (48%) were operated on with a temporary enterostomy prior to the pull-through operation which was ad modum Duhamel in 38 patients (90%). The enterostomy was closed at the same time as the pull-through operation in 14 patients (2-stages) and later in five patients (3-stages). The median age at pull-through was 7 months (range 0 months-11.6 years). 63% of the pull-through operations were performed by the same surgeon and the median clinical postoperative follow-up time was 5.7 years (range 5 months-23 years).Early and late postoperative complications are listed in Table 6.

All but one patient required some kind of postoperative intervention under general anesthesia during the clinical follow-up time as shown in Table 7.

The last clinical control was performed at the median age of 7.1 years (range 8 months-24.2 years). At that time, 18 patients (43%) reported normal bowel function, 12 patients (29%) soiling, 5 patients (12%) constipation, 2 patients (5%) had recurrent enterocolitis and one patient (2%) urinary incontinence. Four patients (10%) had terminal enterostomy.

Complications	Early	%	Late	%
Anastomotic stricture	1	2	26	62
Soiling	0	0	13	31
Perianal excoriations	2	5	7	17
Enterocolitis	0	0	7	17
Constipation	0	0	5	12
Postoperative fever	4	10	0	0
Obstructive ileus	1	2	3	7
Urinary tract infection	3	7	0	0
Urine incontinence	0	0	2	5
Rectal prolapse	0	0	2	5
Wound dehiscence	2	5	0	0
Anastomotic insufficiency	2	5	0	0
Perineal abscess	1	2	1	2
Enterocutaneous fistula	0	0	1	2
Enterostomy prolapse	0	0	1	2
Respiratory distress	1	2	0	0
Wound infection	1	2	0	0

Table 6. Early (occurring before discharge) and late complications (during follow-up time) after the Duhamel pull-through operation.

Reoperations during follow-up (n=41)	n	%
Rectal examination in general anaesthesia	34	81
Division of rectal spur	26	62
-two times	10	
-three times	2	
Revision of enterostomy	4	10
Terminal enterostomy	4	10
Small bowel obstruction	3	7
Explorative laparotomy	3	7
Perianal abscess	3	7
Anal myectomy	2	5
Revision of colorectal anastomosis	1	2
Revision of rectovaginal fistula	1	2
Wound dehiscence	1	2
Revision of abdominal scar	1	2

Table 7. Reoperations during follow-up were carried out in all but one patient as shown here.

Quality of life questionnaires

SF-36

When looking at all patients as one group, they had statistically higher score for role-physical function (RF) and for the summary measure of physical health (PCS), than the age-matched norms for the general Swedish population.

The male patient group had significantly higher scores for the physical function (PF), the role-physical function (RP), bodily pain (BP) and for PCS than the age-matched males in the general Swedish population

The patient group had statistically lower scores for the general health (GH) and mental health (MH) than the age-matched females in the general Swedish population. Females had lower scores for all scales compared to males. See the result from the SF-36 questionnaire in Table 8.

	The whole group		**Males**		**Females**	
Subscales	Mean	Exp.	Mean	Exp.	Mean	Exp.
PF	93.3	94.0	96.7	94.8	87.3	92.6
RP	90.5	89.9	95.4	91.4	81.7	87.2
BP	83.5	79.7	90.9	81.3	70.3	76.6
GH	78.3	80.4	83.7	80.9	68.6	79.5
PCS	53.5	52.7	55.7	53.2	49.5	51.9
VT	72.9	70.3	78.5	72.1	62.7	66.9
SF	89.3	90.1	91.7	90.8	85.0	88.7
RE	82.5	88.7	84.0	90.1	80.0	86.1
MH	79.7	81.5	84.7	82.4	70.7	80.1
MCS	48.3	49.7	49.8	50.3	45.6	48.8

Table 8. This table shows the summarized results from the SF-36 QoL questionnaires. The mean SF-36 scores for the whole group (n=42), for the male (n=27) - and the female patients (n=15) for the different subscales compared to the expected mean value (Exp.) determined for the age– and gender-matched general Swedish population. The numbers indicated in red highlight where a statistical significance was reached, with Wilcoxon Signed Ranks Test, compared with the control group for each subscale ($p<0.05$).

GIQLI

The mean total score with SD for the whole group was 118 ± 18 (range 72-143). See results for different subscales in Table 9. The HD group scored lower than the control group for large bowel function (P= 0.013). In univariate and multivariate regression analysis, only gender was found to significantly predict the total GIQLI score. The males had a significantly higher mean total score, 123 ± 17 (range 83-143) than the females, 110 ± 18 (range 72-133) (P= 0.041).

	HD group		**Control group**		
Subcales	Mean value ± SD	Range	Mean value ± SD	Range	P value
Physical role	39.2 ± 5.9	24-44	38.5 ± 7.9	12-44	NS*
Large bowel function	18.6 ± 4.0	10-24	20.5 ± 4.1	8-24	P=0.013
Emotional role	25.3 ± 5.9	8-32	23.6 ± 6.5	2-32	NS
Upper GI-function	27.3 ± 4.1	17-32	27.3 ± 4.6	14-32	NS
Meteorism	7.7 ± 2.8	1-12	8.6 ± 2.3	3-12	NS
Total	118.2 ± 18.3	72-143	118.3 ± 21.1	52-141	NS

*NS= no statistical significance

Table 9. The mean scores from the GIQLI questionnaire for the whole patient group (n=42) with standard deviation (SD) and range, compared with the age-and gender-matched control group (n=42). The HD group had significantly lower score for large bowel function than the control group (Wilcoxon Signed Ranks Test).

Univariate and multivariate linear regression analysis found no association between the mean total GIQLI score and whether the pull-through operation was performed before or after 6 months of age, or if the operation was done as a primary pull-through or in 2-3 stages. Nor was there any association with the number of operations performed by each surgeon, or the length of the aganglionic segment when comparing patients with rectosigmoid aganglionosis with those who had a longer aganglionic segment.

When subdividing the length of the aganglionic segment into 5 groups, there was a significantly lower mean total score for patients with an aganglionic segment reaching the right ascending colon (n=4), 95 ± 21, compared with those patients with the aganglionic segment in the left descending colon (n=4), 134 ± 10 (one way ANOVA with post hoc Tukey HSD used).

Eight patients, four men and four women, had a total GIQLI score lower than the cut-off point of 105 (19%). Four of them had a complicated history after the pull-through operation requiring re-operation whereas the other four did not.

Patients with persisting bowel symptoms at the last clinical control reported significantly lower values in the physical role subscale of GIQLI (p=0.045) measured with Student´s t-test.

Paper III

TERPT vs Duhamel

With one exception there was no significant difference in the patient demographics in the two groups compared (two sided Fisher´s exact test used). A higher number of patients in the D-group had colostomy prior to the pull-through operation, 13 (72%) compared to three patients (27%) in the T-group.

The main results of pre-and perioperative data are shown in Table 10. The operative time for the two procedures was the same, but in the T-group, the operative time included waiting time for frozen section (about 45 min) with operative pause, not utilized in any patient in the D-group. The children in the T-group started oral feeding sooner after the operation, the bowel movements started sooner and they had a significantly shorter mean postoperative hospital stay, approximately 2.5 days, than the D-group (see Table 10). The need for analgesics postoperatively was higher in the D-group.

Immediate postoperative complications were few in both groups. One patient in the D-group had a major complication with leakage from the rectal stump and wound dehiscence requiring re-operation. One patient in the T-group had partial anastomotic dehiscence requiring repeated anal dilatation later on.

Variable	D-group (n=18)		T-group (n=11)		
	Mean ± SD	range	Mean ± SD	Range	P value
Birth weight (gr)	3654 ± 813	1930-4550	3445 ± 490	2760-4025	0.336
Age at diagnosis (months)	2.4 ± 4.6	0-16	2.9 ± 4.9	0-21	0.824
Age at operation (months)	5.6 ± 5.7	1-23	4.8 ± 5.2	1-24	0.351
Operative time (min)	154 ± 35	112-235	146 ± 25	101-197	0.513
Perioperative bleeding (ml)	32 ± 29	0-125**	6 ± 4	0-20***	0.053
Start of p.o. feeding (days)	2.0 ± 1.5	0-7	0.3 ± 0.5	0-2	<0.001*
First bowel movement (days)	1.1 ± 0.8	0-2	0.1 ± 0.2	0-1	0.001*
Post-op hospital stay (days)	6.9 ± 3.8	3-16	4.4 ± 1.5	2-8	0.038*

*Statistically significant. **n=13, ***n=5

Table 10. Pre- and postoperative results for the patients with colorectal Hirschsprung´s disease in the D (Duhamel) - and the T-group (TERPT). The patients in the T-group started oral feeding sooner, had their first bowel movement sooner and were discharged earlier (the Mann-Whitney test used).
p.o.: per oral, post-op: post-operative

The postoperative follow-up time was limited to 24 months. During the follow-up time, significantly more patients needed reoperations in the D-group compared with the T-group (see Table 11). Twelve patients (71%) in the D-group needed reoperation or revision of the anastomosis with transanal division of the rectal spur using a stapling device. One patient was operated on for leakage of the rectal stump and also for adhesive small bowel obstruction two months later.

Two patients (18%) of the T-group required anal dilatation of the anastomosis in general anesthesia, of which one several times during the first six months after the TERPT operation. Ten patients in the T-group (91%) had daily graduated anal dilatations at home with Hegar dilators not needed by the patients in the D-group.

Table 11 shows also the functional outcome, reported by the parents at the last clinical control, 24 months after the pull-through operation.

Main interventions during follow-up time	D-group (n=17)	%	T-group (n=11)	%	P value
Reoperations	12	71	2	18	0.018*
Routine anal dilatations	0	0	10	91	<0.001*
Perianal excoriations	4	24	5	46	0.409
Enterocolitis	2	12	2	18	1.000
Functional results reported at the last clinical control					
Constipation	10	59	3	27	0.137
Daily laxative medications	5	29	3	27	1.000
Soiling	3	18	1	8	1.000

*Statistically significant

Table 11. The incidence of reoperations as well as the main inventions during follow-up time of 2 years are shown here for the D- and the T-group. The functional results at the end of the follow-up time are also shown for the respective groups. The patients in the D-group needed more reoperations and anal dilatations were needed for the patients in the T-group, not utilized in the patients in the D-group (The two-sided Fisher´s exact test used).

In general, the children in both groups had frequent bowel movements the first months after the operation, from 10-30 bowel movements/day early on postoperatively to about 1-5 bowel movements/day after three-five months.

Paper IV & V

Wireless pH-monitoring in children

The most common indication for pH-monitoring was vomiting (n=38) and abdominal and/or chest pain (n=22). Ten children were neurologically impaired. Nine children had the measurement after anti-reflux surgery and five children were operated on later with fundoplication. The upper endoscopy revealed macroscopic esophagitis in 10 patients and a hiatus hernia in three patients.

Three children described signs of dysphagia during the measurement time; none required endoscopic removal of the capsule. In one patient we experienced a failure in the contact with the TM receiver and in two children we had a technical problem with fastening the capsule, requiring three capsules in one of them. There were no complications related to the endoscopic placement of the capsule. The characteristics of esophageal acid exposure during the 24-hour pH-monitoring are presented in Table 12.

Variable	24-hour pH-monitoring	
	Median	Range
Percent time pH<4	7	0-61
No. of reflux episodes	57	0-203
No. of reflux episodes ≥ 5 min	3	0-25
Longest reflux episode, min	20	0-200
DeMeester score*	23	0-112

*95th percentile <14.72

Table 12. The results of the pH-monitoring for 24 hours are shown here for the whole patient group.

Esophageal biopsies were taken in 49 cases (79%). The results according to the pathology examination (PAD), endoscopic findings and the Demeester score were as follows:

1. 18 patients with esophagitis according to the PAD, of which;
   8 patients (44%) had also pathologically high DeMeester score
   7 patients (39%) had also esophagitis according to the endoscopic findings
2. 10 patients with esophagitis according to the endoscopic findings, of which:
   7 patients (70%) had also pathologically high DeMeester score
   7 patients (of nine) (78%) had also esophagitis according to the PAD
3. 31 patients had no esophagitis according to the PAD, of which:
   17 patients (55%) had pathologically high DeMeester score
   2 patients (6%) had esophagitis according to the endoscopic findings

When comparing the 18 patients with esophagitis according to PAD with the 31 patients without esophagitis, we found no statistical significant difference in the 24-hour pH-measurement findings, see Table 13.

Variable	Normal biopsies (n=31)		Esophagitis (n=18)	
	Median	Range	Median	Range
% time pH<4	7	0.2-61	8	0-32
No of reflux episodes	56	6-121	67	0-203
No of reflx episodes >5 min	2	0-11	4	0-25
Longest reflux episodes (min)	18	2-109	25	0-200
DeMeester score	19	1-78	27	0-112

Table 13. The results of pH Monitoring during 24 hours in those patients with pathological verified esophagitis (n=18) and those with normal biopsies (n=31). No statistical significance was between the groups in any of the factors studied (one-way analysis of variance used).

Are two days better than one?

The median time of pH-registration was 47 hours and 18 minutes (range 43:36-47:59) and no signal interruption episodes in the registration were noted.

The characteristics of esophageal acid exposure during the 48-hour study period are presented in Table 14. Ten children had a pathological DeMeester score after both 24 and 48 hours. The median percentage time with pH <4 did not differ significantly between the first 24 hours and the 48-hour period, 5% and 6% in respective group. Neither was there any statistical difference in the DeMeester median score between the first 24 hours and the 48-hour periods 21 and 22 respectively (see Table 14).

Variable	Day 1		Day 2		Day 1+2	
	Median	Range	Median	Range	Median	Range
% time pH<4	5	0-32	6	0-41	6	0-37
No. of reflux episodes	60	0-203	59	0-258	Of no relevance	
No. of reflux episodes ≥ 5 min	3	0-25	3	0-26	Of no relevance	
Longest reflux episode, min	13	0-70	17	0-83	Of no relevance	
DeMeester score*	21	0-112	23	0-141	22	0-127

*95th percentile <14.72

Table 14. The results of the pH-monitoring during 48 hours, comparing the first with the second day. There were no statistical significant findings between the two days (Wilcoxon Signed Ranks Test used).

However, a day-to day variation was noted in the DeMeester score: we found that four children had a pathological value on day 2 and not on day 1. Three children had a pathological value on day 1 but not on day 2. For three of these seven children, their total DeMeester score (the whole study period) was pathologically high. This variation had clinical significance only in two children (8.7%) with a pathological DeMeester score when considering the entire 48-hour period, but not during the first 24 hours.

GENERAL DISCUSSION

This thesis deals with several aspects on two gastrointestinal motility disorders in childhood, i.e. Hirschsprung´s disease and gastroesophageal reflux disease. There has been continous development over the years of both operative techniques and diagnostic tools used for both these disorders, striving towards minimal invasive procedures for the benefit of the patients, without compromising the results and diagnostic accuracy on the way. The term minimal invasive surgery (MIS) is used for any procedure that is less invasive for the patient than traditional open surgery. It most often involves laparoscopy, endoscopy or robotic surgery and usually implies less surgical trauma for the patient, faster recovery and shorter hospital stay. It also includes superior cosmetic results for the patient. The latest addition to MIS in gastrointestinal surgery today is natural orifice translumenal endoscopic surgery (NOTES) which approaches intraabdominal problems through natural orifices with transvaginal or transgastric access. Laparoscopic surgery was used first by gynecologists in the 70s and adopted by the general surgeons in the 80s. Pediatric surgeons have since then increased their use of both laparoscopy and endoscopy, with or without robotic help, for their patients. Because of the small size of our pediatric patients, combined methods are often chosen, i.e., laparoscopic assisted surgery. This evolution has changed the surgical management of both HD and GERD considerably during the last decade.

Hirschsprung´s disease

When looking ahead, and in the process of taking up new methods and surgical techniques it is imperative that we, healthcare professionals, constantly evaluate the outcome of the methods and techniques previously used, in order to see where we can improve our methods to benefit our patients. In pediatric surgery this can unfortunately take a long time. From our first short-term results it is of great value to see the immediate outcome of different operations for our pediatric patients as we have shown in Paper II. However, there is also another, equally important question, i.e., how are our pediatric patients doing as grown adults? To answer such question we have to wait for up to 20 years to see the final results of our operations, and this is what we report in Paper II.

In order to assess long-term surgical results, a uniformly operated patient cohort and a sufficiently long follow-up period are crucial. Most long-term studies after pull-through operations for HD are limited to 15 years after the operation, the majority of them indicating improvement of bowel symptoms with time, especially after adolescence (Livaditis 1981; Rescorla et al. 1992; Bjornland et al. 1998; Yanchar and Soucy 1999; Athanasakos et al. 2004; Menezes and Puri 2006b; Mills et al. 2008; Niramis et al. 2008).

Mills et al found that fecal continence is an important predictor of overall QoL in children operated for HD (Mills et al. 2008). Few studies, however, report QoL results from adults operated on as a child for HD. Heikkinen et al reported good fecal continence for 100 adults operated on for HD and that all patients with a good continence score reported no limitations in their occupation, social life, or physical activities (Heikkinen et al. 1995). Swenson et al reported good bowel function in 90% of their 282 patients of which 104 were followed for 15-25 years (Swenson et al. 1975). Puri et al reported that 99% of adults operated on with Swenson´s pull-through procedure had normal bowel control and good health 18-27 years after surgery (Puri and Nixon 1977). However, these studies focus on bowel function and continence, but bowel function alone is not necessarily a good indicator of quality of life for adult individuals with HD. On the contrary, Hartman et al showed in their study of 320 patients operated on for anorectal malformations (ARM) and HD that generic QoL is particularly affected by psychosocial function but not physical symptoms, such as fecal incontinence and constipation (Hartman et al. 2004).

But what is Quality of Life? There is no consensus regarding a gold standard definition of QoL and there are many factors affecting it for each individual. Most questionnaires for QoL rely on self judgement of health, capability, disabilities and happiness and are dependent on different internal and external factors such as employment, education, leisure time, social belonging, physical and mental health which differ greatly and are valued differently between individuals. Therefore, standardized and validated QoL questionnaires are imperative for the interpretation of results and comparison with the normal population.

In paper II, two validated questionnaires were used, SF-36 and GIQLI, and we found that QoL in adulthood for patients operated on for HD in childhood was overall good, with ratings of most scales equal to or even higher than in the Swedish age- and gender-matched general population. Our results from the SF-36 questionnaire showed that the patient group had a higher mean score than expected for role-physical function (RP) and for the summary measure for physical health (PCS); this was found particularly in men, who also had higher mean scores for physical function (PF) and bodily pain (BP) than expected. This supports Hartman's conclusion that it is not the physical symptoms that mostly affect the

QoL of adult HD patients (Hartman et al. 2004). Gender subdivision, however, showed that the QoL outcome for women was poorer than for men in all subgroups and women scored significantly lower in general- (GH) and mental health (MH) compared to the values for females in the age-matched general Swedish population. A review of their medical records did not reveal any plausible explanation for this.

Gender differences are known from various health surveys, showing that women have increased morbidity, with higher rates of illness and disability days, seek medical help more often and have poorer psychosocial health than men in spite of their longer life expectancy; hence the phrase "*women are sicker, but men die quicker*" has been upheld (Verbrugge 1982; Wingard 1984; Lahelma et al. 1999). Various explanations have been offered for this phenomenon which mainly include that women may be more sensitive to discomfort, report discomfort more easily and are more willing to seek medical help (Verbrugge 1982; Wingard 1984). Others believe that psychological gender images and identities are relevant i.e. femininity-masculinity issues where masculinity is more likely to relate to health and femininity to illness, see review in (Lahelma et al. 1999). However, some researcher have not been able to confirm these gender differences and state that this does not apply today even though it might have in the 1970s (Macintyre et al. 1999).

The mean GIQLI score for the whole group was 118. Eight patients (19%) had scores lower than the cut-off score of 105, chosen in accordance with other studies (Koivusalo et al. 2005 (a and b)). Half of those patients had complicated postoperative histories which could explain their poor results. There was no significant difference between the HD group and the age- and gender-matched control group for the total GIQLI score. The only GIQLI subscore that differed between the HD group and the control group was the large bowel subscale ($p = .013$) where the HD group had a lower score. This may reflect persisting problems with soiling, constipation and enterocolitis. Even patients with persisting bowel symptoms reported quality of life equal to those without symptoms in all aspects except physical role subscore of GIQLI. The high overall GIQLI score together with the results of the SF-36 health survey indicate that with respect to overall QoL, most of the operated patients had managed to cope with these symptoms as adults.

The paradoxically higher scorings of patients in our cohort than the age- and gender-matched population may be explained by lower expectations. A person with a congenital disease usually adjusts to the disease better than patients with acquired conditions, and may thus rate their function scores relatively high in order to compensate for the stigmatisation often associated with a presumably disabling disease. One of the participants in the study puts it nicely in an

accompanying letter stating "*that in spite of my occasional symptoms from my belly, which I've learned to live with, I have lived a fulfilling life with education, working abroad, having a family with two wonderful children. I can state that no one notices that my bowel function is different from anyone else's, except for these ugly scars on my skin*" (author's translation).

The short- and long-term postoperative results described in Paper II are in accordance with other reports with the exception that 62% of the patients in our material required division of a rectal spur which is a higher proportion than reported by others (Heikkinen et al. 1995; Minford et al. 2004). At the last clinical follow-up registrated in the patient records, the median age was 7.1 years (range 8 months-24.2 years). 31% patients had problems with soiling, 12% had constipation and 17% recurrent enterocolitis which is similar to other studies (Langer et al. 2003; Minford et al. 2004).

However, as seen in Table 7, all (98%) but one patient needed some kind of operative intervention requiring general anesthesia during the follow-up time. Reoperations were most commonly due to stricture formations in the anastomosis, but also laparotomies for small bowel obstructions, fistule formation or explorative laparotomies for other causes. This we hope to be able to scale down by a more minimal invasive approach, as the TERPT method.

Since first described in 1998, the TERPT has become widely used because of many advantages. It does not require laparotomy or laparoscopy, especially for aganglionosis defined to the rectosigmoid area (De la Torre and Ortega 2000). One center has, however, described high incidence of anastomotic dehiscence not seen in other reports (Jester et al. 2009). The risks of and intra-abdominal contamination and adhesion formation are minimalized and the procedure reduces the risk of damaging the pelvic structures, is less expensive, and has the most optimal cosmetic results with no visible scarring (Langer et al. 2000; Langer et al. 2003; Elhalaby et al. 2004). The effect of scarring should not be disregarded as this seems to have a great effect on the growing individual. In our QoL study in Paper II, seven patients (17%) wrote an additional comment on that issue stating how this had affected them in a negative way. The typical scarring in children and adults after 2-staged Duhamel procedure is shown in figure 10.

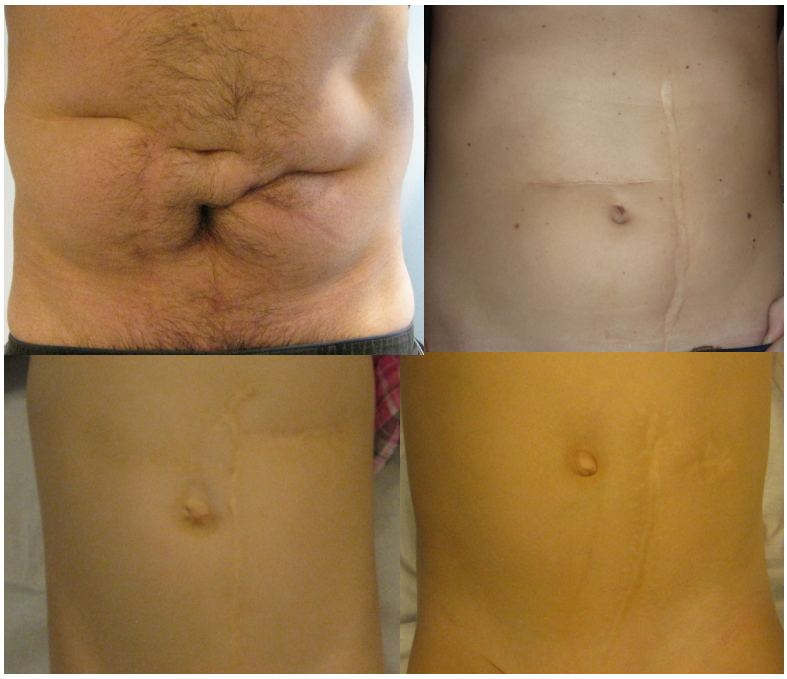


Figure 10. A typical scarring on the abdominal wall in adults (above) and in 2-3 year old children (below) after 2-staged pull-through for HD i.e., colostomy and Duhamel pull-through operation with concurrent closure of the colostomy at the second operation (pictures published with kind permission from the patients and their parents).

The patients in the T-group started oral feeding earlier and had their first bowel movement sooner than those in the D-group. The hospital stay was also significantly shorter for the patients in the T-group and the use of analgesics after surgery was also less. The incidence of enterocolitis was similar in both the groups (12% and 18% respectively). The patients in the T-group also had fewer re-operations during the two-year follow up time (18% vs. 71%).

Continence is difficult to value in an early outcome as in this study, most of the children were two-three years of age at the end of the follow-up and needed to be valued again later on. In spite of frequent defecations during the immediate postoperative period, our findings suggested that there was a trend towards decreasing numbers of bowel movements per day and improved continence in both groups in time, which is also reported by others (Martucciello et al. 2005; Zhang et al. 2005). Three patients in the T-group had constipation at the end of the follow-up time (27%) and 10 patients in the D-group (59%), this was without statistical significance.

Our incidence of anastomotic problems in the D-group was very high with 71% (n=12) of the patients requiring later division of the rectal spur. The transanal division was done with a stapling device. This incidence is higher than published elsewhere and might partly be explained by the low threshold at our center for performing a division of the rectal spur, even for minor symptoms (Minford et al. 2004).

With the advantages in surgical treatment mentioned above we constantly have to ask ourselves: have we reached our limits in treatment for HD? Can we get more minimally invasive in our treatment for HD? There are no simple answers to these questions. The search for even more minimal invasive approaches takes us down to a cellular and genetic level. The knowledge of the ENS is imperative for that purpose. Our research group, as others, has worked with the idea of nerve cell transplantation for a long time. Is that a possible alternative minimal invasive treatment for HD in the future? As part of that work we have in Paper I studied a putative neuropeptide, the CART peptide.

In Paper I we present for the first time the presence of CART-IR nerves in the ENS of human intestine. The distribution of CART peptide together with the finding of CART-IR nerve cell bodies in ganglionic intestine suggests an intrinsic origin of the peptide. This finding might add to a more complete and comprehensive understanding of the function of the ENS and its role in the pathogenesis of various diseases causing disturbances in gut motility.

In ganglionic intestine the topographic distribution of CART peptide-containing nerve fibers in the muscle layer and the CART-IR nerve cell bodies in the myenteric ganglia in man was found to be similar to that previously described in rat, guinea pig and pig (Tornoe K 1999; Ekblad et al. 2003; Ellis and Mawe 2003; Wierup et al. 2007). In the submucosa, the mucosa and the submucosal ganglia the CART-IR nerve fibers and nerve cell bodies were more seldom seen compared to results from other animals studied (Ekblad et al. 2003; Wierup et al. 2007). A certain difference in the distribution and topography of CART-IR nerve fibers was, thus, noted between man and other animals studied. It should, however, be emphasized that the human specimens used in our study all originated from children less than four years of age. Further studies of the distribution of CART-IR nerve fibers and cell bodies also in adult man are needed to determine if the noted differences are age- or species-related.

The role of CART peptides in the GI tract is still somewhat enigmatic. In humans, the peptide is found mainly in nerve fibres supplying the muscular layers. The coexistence of CART with both NOS and VIP as well as a high degree of coexistence of NOS and VIP strongly suggest an interplay between CART, VIP and/or NO in the intestinal wall.

Motility studies indicate that CART influences gut motility by both central and peripheral pathways for it is established that centrally administered CART decreases colonic transit time (Tebbe et al. 2004) and that in rat colon NO-evoked relaxations are attenuated by the simultaneous presence of CART (55-102) *in vitro*, thus suggesting that CART modulates NO activity (Ekblad et al. 2003).

Some reports have suggested a neurotrophic effect of CART peptide *in vitro* with increased survival of central motor neurons (Louis 1996) as well as for myenteric neurons in the ENS (Ekblad et al. 2003; Ekblad 2006). If CART peptide is shown to possess neurotrophic effects, use of this peptide as a neuroprotective agent could be possible. The finding of factors that promote and increase the survival of nerve cells would be beneficial for future research in enteric nerve cell transplantation in, for example, patients with Hirschsprung's disease.

GERD and wireless esophageal pH-monitoring in children

There are difficulties when evaluating the accuracy of diagnostic tests in children with GERD due to the imprecise definition and the heterogeneity of the disease as well as the lack of a gold standard to establish the diagnosis. According to the recommendations from the international Genval workshop and the updated guidelines from the NASPHGAN/ESPGHAN the diagnosis of GERD is evident in symptomatic patients with a combination of typical symptoms, such as heartburn and regurgitation, and erosive injury on esophagoscopy (Dent et al. 1999; Vandenplas et al. 2009). In our patient group of children, a symptom description is often unreliable due to the fact that the children are young and some are neurologically handicapped and therefore unable to describe their symptoms. Therefore, we have to rely on other parameters to evaluate their GER. The combined MII/pH measurement is increasingly used in clinical practice in children but the "*gold standard*" for measuring the acid reflux is still a traditional pH-monitoring for 24-48 hours.

To our knowledge, in our study in Paper IV, we have the largest published series using the wireless Bravo^{TM} pH-monitoring system in children (Bothwell et al. 2004; Hochman and Favaloro-Sabatier 2005; Croffie et al. 2007). During a two-year period we performed 62 measurements which we found feasible and well-tolerated by the children and not associated with any severe complications. In three patients we had technical difficulties, which all occurred in the learning period of applying the instrument; this might be expected with new technology and instruments. Three patients complained of discomfort during the study period. It must, however, be emphasized that ten of our children had neurological or

chromosomal abnormalities limiting their possibility to express their discomfort. There was no need for analgesics in this group. The children were unaffected by the measurement, being catheter-free, and could return to their normal activities as soon as they had recovered after the general anesthesia. In the light of our positive experience with the wireless pH-measuring system; we recommend its use in children.

It has been demonstrated that during catheter-based pH-monitoring in patients, the reflux provoking activities are significantly reduced compared with those during a normal (off-test) day (Fass et al. 1999). A recent record has indicated that pH-monitoring using the wireless system may have less effect on daily routine diet and activity level than the catheter-based technique (Pandolfino et al. 2003). As the wireless capsule is attached to the mucosa only, interference with the lower esophageal sphincter function or esophageal motility seems unlikely. This can be compared with the use of a catheter with a pH-electrode, which is not attached to the mucosa, and can move up and down the esophagus and intervene with the lower esophageal sphincter function or esophageal motility (Aksglaede et al. 2003). Thus, the presence of mucosal metal clips should not influence the esophageal acid reflux (Fletcher et al. 2001).

Our study emphasizes, as also noted by others, that no one test is totally reliable in the diagnosis of GERD in children as outlisted in the results section, this has also been noted by others (Salvatore et al. 2005; Salvatore et al. 2009). Endoscopic findings showed the highest sensitivity in our study, i.e., of those who had endoscopic esophagitis, 70% had also pathological DeMeester scores and 78% had pathological esophagitis as well. They were, however, few in number, only 10 patients, possibly indicating a more severe disease in these patients.

There is no study establishing normal values for oesophageal acid exposures using the wireless pH-system in children. This lack of normal values does not influence our results when comparing the first 24-hour with the 48-hour measurements in the same child since we used the same instruments for both occasions, in Paper V. Adult studies have shown varying results i.e., higher, lower and the same acid exposure measured with the wireless system compared with traditional catheter pH-monitoring (Portale et al. 2003; Bortolotti et al. 2004; des Varannes et al. 2005; Wenner et al. 2007b). Day-to-day variability is well-known in the monitoring of esophageal acid exposure in the same individual and thus to be expected. This was also seen in our study, but with only clinical significance in two patients (8.7%) (Wiener et al. 1988; Franzen and Grahn 2002; Ahlawat et al. 2006). We found no statistical significance in the median fraction of time with pH<4 nor in the median DeMeester score between the first 24 hours and the entire 48-hour measurement in our patient group. These results suggest that extended pH-monitoring for 48 hours does not add any significantly valuable information to

the results of the traditional 24-hour pH-measurements. Recognition of individual day-to-day variations must, however, be acknowledged and in some cases a prolonged pH-monitoring might give additional information.

CONCLUSIONS

Based on Papers I-V the following conclusions were reached:

This is the first report on the presence of CART-IR nerves in the enteric nervous system (ENS) of human intestine. We also demonstrate that the majority of the CART-containing neurons have intrinsic origin. This finding might add to a more complete and comprehensive understanding of the function of the ENS and its role in the pathogenesis of various diseases causing disturbances in gut motility.

The function and long-term quality of life after the Duhamel procedure are satisfactory. The females´ scores are somewhat lower than the males' and they should be given special consideration at follow-up. Our study also indicates that the longer the aganglionic segment, the higher is the risk of the disease affecting the patient's quality of life in adulthood.

The transanal endorectal pull-through proved to work well for patients with Hirschsprung´s disease defined to the rectosigmoid part of the colon. They had less postoperative pain, started to feed sooner, their first bowel movement occurred sooner and they had a significantly shorter hospital stay with fewer postoperative interventions than the patients operated on with the Duhamel pull-through. Our short time results support the use of the TERPT method in favor of the Duhamel pull-through for rectosigmoid HD.

The use of the wireless esophageal pH-monitoring system was feasible in use and well-tolerated by the children and is to be recommended.

It is the combination of symptoms, endoscopic findings, pH-measurement results and pathological examination that give us the most reliable diagnosis of GERD in children.

Our results find no clear advantage of esophageal pH-measurement for 48 hours compared to 24 hours in the diagnosis of GERD in children.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Det enteriska nervsystemet (ENS) utgör mag-tarmkanalens eget nervsystem, ibland refererat till som "the second brain" då det uppvisar stora likheter med det centrala nervsystemet (CNS). Rubbningar i ENS ger bland annat störningar i tarmens motorik. Denna avhandling handlar om två sjukdomar i mag-tarmkanalen hos barn som bägge är uttryck för en rubbad motorik; Hirschsprung´s sjukdom och gastroesophageal reflux sjukdom.

Hirschsprung´s sjukdom (HD) är en modellsjukdom för en defekt i ENS. Vid denna saknas nervceller i tarmväggen oftast i den sista delen av tjocktarmen. Sjukdomen är medfödd och drabbar cirka 1/5000 nyfödda. Missbildningen medför en mycket allvarlig tarmfunktionsrubbning som kräver en operation ofta direkt efter födelsen för borttagande av den sjuka delen av tarmen, och ibland krävs en tilfällig kolostomi (tarmöppning på magen).

I delarbete II har vi utvärderat livskvaliteten (QoL) hos vuxna individer som under barndomen har blivit opererade för HD. 42 patienter av 47 (89%) deltog i studien genom att besvara två frågeformulär (SF-36 och GIQLI). Det visade sig att vuxna opererade för HD i barnaåren hade lika bra QoL som jämnåriga. Kvinnor som opererats hade dock en sämre allmän och mental hälsa jämfört med jämnåriga kvinnor. Det visade sig även att ju större del av tjocktarmen som varit drabbad desto mer sannolikt var att det påverkade patientens livskvalitet som vuxen.

Olika operationsmetoder har varit beskrivna för HD. På Barnkirurgiska kliniken har vi tidigara använt oss av en metod (Duhamel) för att avlägsna den sjuka delen av tjocktarmen och återställa ändtarmsfunktionen vilket krävt att buken öppnas. Sedan år 2005 har vi använt en metod (TERPT) som innebär att den sjuka delen av tjocktarmen avlägsnas via ändtarmsöppningen utan att öppna bukhålan. I delarbete III har vi jämfört kort-tids (2 år) resultat efter de två olika operationsmetoderna för HD i nedersta delen av tjocktarmen. Denna visade att patienterna som blev TERPT opererade hämtade sig snabbare, kunde börja äta tidigare och krävde mindre smärtstillande behandling efter operationen. De hade även kortare vårdtid på sjukhuset och krävde färre re-operationer på de två åren jämfört med de patienter

som opererats med den äldre metoden (Duhamel). Kosmetiskt är det även en stor vinst för patienten med TERPT operationen då man slipper ärr på magen.

En del i vårt övergripande projekt har varit att experimentell kunna utvärdera möjligheten av att ersätta stympande tarmingrepp hos barn med autotransplantation av nervceller till den sjuka tarmen. Som del i det arbetet har vi i delarbete I studerad en möjlig tillväxtfaktor, den s.k. CART peptiden. Där har vi för första gången kunnat visa att CART peptiden förekommer i human tarm. Den samexisterar i tarmens nervceller med andra kända signalsubstanser (VIP och NO) vilket indicerar att den har en roll i ENS hos människa. CART peptiden förekommer inte i sjuk tarm vilket stödjer att den har ursprung i tarmens eget nervsystem.

Gastroesofageal refluxsjukdom (GERD) är vanlig hos barn, förekommer hos 60-70% av barn vid fyra månaders ålder men försvinner hos de flesta och drabbar ung. 5% av ettåringarna. Den innebär att maginnehåll backar upp från magsäcken till matstrupen på ett onormal sätt (refux). För diagnos av GERD används en mätning av syrahalten i matstrupen med en slang via näsborren. Sedan år 2005 har vi på Barnkirurgiska kliniken i Lund använt oss av en trådlös metod som innebär att man fäster en kapsel (Bravo^{TM}) i slemhinnan längst ner i matstrupen. Kapseln sitter fast där i 2-3 dagar medan syrahalten kontinuerligt registreras. Hos vuxna har denna metod ansetts ge mindre obehag för patienten under registreringen. I delarbete IV och V har vi utvärderat denna teknik för barn. I delarbete IV har vi undersökt 58 barn i åldern 9 månader till 15 år. Allmänt tolereras kapseln väl av barnen. I delarbete V har vi jämfört att registrera syrahalten i 24 timmar med 48 timmar. Vi fann en viss variation mellan resultaten hos barnen men endast av kliniskt vikt hos två barn. Vi fann även att det fortfarande krävs en individuell bedömning utifrån kliniska symptom, resultat av undersökningar som syrahaltsmätning, endoskopi och vävnadsprov för att komma fram till rätt diagnos.

ALMENN SAMANTEKT Á ÍSLENSKU

Starfrænar sjúkdómar í meltingarvegi barna eru algengir og geta birst í ýmsum myndum t.d. sem bæði truflanir á hreyfanleika garnarinnar og á flæði fæðunnar um görnina. Þessi doktorsritgerð fjallar um rannsóknir á tveimur þessara sjúkdóma, þ.e.a.s Hirschsprung´s sjúkdómi (HD) og vélindabakflæði (GERD). Sem aðra starfræna sjúkdóma í meltingarvegi barna má nefna hægðatregðu, þykknun á neðra magaopi (pyloric stenosis), niðurgang og starfrænar truflanir vegna annara sjúkdóma t.d. sykursýki. Taugakerfi meltingarvegarins (ENS) er hluti af ósjálfráða taugakerfinu og er stærsti hluti úttaugakerfisins. Garnaveggurinn er samansettur úr fjórum lögum og eru þar tvær taugaflækjur (plexuses) sem eru nauðsynlegar fyrir eðlilega hreyfingu garnarinna.

Hirschsprung´s sjúkdómur er meðfæddur galli og hrjáir um 1/5000 nýfæddra barna. Hann einkennist af skorti á taugahnoðafrumur (ganglion cells) í bæði vöðvahjúps- og slímhúðarbeðstaugaflækjum og veldur samdrætti og starfrænum truflunum í þeim hluta garnarinnar sem er sjúk. Sjúkdómurinn er oftast bundinn við neðsta hluta ristilsins. Meðferðin er í dag fólgin í aðgerð þar sem sjúki hluti garnarinnar er fjarlægður.

Grein II er lífsgæðarannsókn þar sem við höfum með aðstoð tveggja lífsgæðakannana (SF-36 og GIQLI) metið lífsgæði hjá fullorðnu fólki sem gekkst undir aðgerð vegna HD í barnæsku. Góð svörun var í rannsókninni þar sem 42 einstaklingar af 47 tóku þátt (89%). Kom í ljós að þeir mátu lífsgæði sín almennt góð og til jafns við og jafnvel betri en jafnaldra samanburðarhópur. Konur komu þó verr út og mátu almenna og geðræna heilsu sína verri en jafnaldra konur. Einnig kom fram að því stærri hluti ristilsins sem var sjúkur frá upphafi, því neikvæðari voru áhrifin síðar á lífsgæði fóksins.

Í grein III er borið saman skammtímaárangur, 2 ár, eftir tvær mismunandi tegundir aðgerða við HD sem er bundinn við neðsta hluta ristilsins. Sú aðgerð sem áður hefur verið notuð við Barnaskurðdeildina í Lundi heitir Duhamel aðgerð (D-hópur). Þar er gerður kviðarholsskurður og sjúki hluti ristilsins fjarlægður, oft með ristilraufun (colostomy) á undan. Árið 2005 byrjuðum við að nota aðra tegund aðgerðar sem hefur rutt sér rúms s.l. 10 ár og felst í því að sjúki ristilhlutinn er

fjarlægður í gegnum endaþarmsopið (TERPT, T-hópur). Þannig sleppur barnið við kviðarholsskurð og þar með verða engin ör á kviðveggnum. Helstu niðurstöður sýndu að börnin í T-hópnum náðu sér fyrr eftir aðgerð, gátu byrjað að borða fyrr og notuðu minna af verkjalyfjum. Sjúkralega þeirra var marktækt styttri og var sjaldnar þörf á enduraðgerðum fyrstu tvö árin. Við mælum því með að TERPT aðgerðin sé notuð við HD þar sem sjúki hluti ristilsins er bundinn við neðsta hluta ristilsins.

Hluti af rannsóknarvinnunni var gerð með dýratilraunum þar sem við höfum lengi unnið að rannsóknum á ígræðslu á taugafrumum frá görn, sem hugsanleg framtíðarmeðferð fyrir börn með HD. Sem hluti af þeirri vinnu höfum við, í grein I, skoðað tiltölulega nýuppgötvað taugafrumupeptíð (CART-peptíð). Þar gátum við, fyrst af öllum sýnt fram á að CART-peptíðið er til staðar í eðlilegri görn manna. Mest var af peptíðinu í vöðvahjúpstaugaflækjum og var það að mestu leyti á sama stað og önnur vel þekkt taugafrumupeptíð (VIP og NO) sem bendir til að það hafi áhrif á taugafrumusamskipti í taugakerfi meltingarvegarins. Hvert það hlutverk er, er þó óþekkt í dag. Sýni voru tekin frá sjúklingum með HD og kom einnig fram að CART-peptíðið var ekki til staðar í sjúkri görn sem staðfestir að um eðlislægt peptíð sé að ræða í garnavegg manna.

Annar hluti ritgerðarinnar fjallar um sýrustigsmælingar í vélinda hjá börnum með nýlegri þráðlausri mælingu. Vélindabakflæði er algengt hjá ungabörnum, en það er til staðar hjá um 60-70% barna við fjögurra mánaða aldur en minnkar hratt á fyrsta árinu, en um 5% barna hafa einkenni um vélindabakflæði við eins árs aldur. Sýrustigsmælingar í vélinda er mikilvægt greiningartæki í greiningu á súru bakflæði og kannski sérstaklega hjá börnum, þar sem þau geta ekki sagt til um hefðbundin einkenni á sama hátt og fullorðnir. Sýrustigsmælingin hefur verið framkvæmd með legg (catheter) sem er þræddur í gegnum nefið og látinn liggja í vélindanu í 24 klst til að mæla sýrustig. Þetta hefur ýmis óþægindi í för með sér, ekki síst hjá börnum. Í grein IV og V höfum við tekið saman árangur af nýlegri þráðlausri mælingartækni sem hófst á Barnaskurðdeildinni í Lundi árið 2005. Þar er notað hylki (Bravo^{TM}) sem er fest á slímhúðina í vélindanu og situr hylkið þar í 2-3 daga. Þannig er hægt er að mæla sýrustigið í vélindanu í a.m.k. 48 klst. Nauðsynlegt er að festa hylkið í svæfingu og er gerð vélinda- og magaspeglun samtímis. Í okkar rannsókn á 58 börnum á aldrinum 9 mánaða til 15 ára kom í ljós að börnin þoldu rannsóknina vel og voru óheft meðan á mælingu stóð. Einnig bárum við saman sýrustigsmælingu fyrstu 24 klst saman við 48 klst mælingu og virtist 48 klst mælingin ekki bæta neinu við hefðbundna 24 klst mælingu. Þó var viss mismunur milli daga sem skipti þó ekki sköpum nema hjá tveimur barnanna. Því getum við mælt með notkun á þráðlausri sýrustigsmælingu hjá börnum. Greining á vélindabakflæði hjá börnum getur verið vandasöm og litum við á niðurstöður sýrustigsmælinga, speglunar og meinafræðiniðurstöðu úr sýnatöku og engin ein rannsókn var afgerandi til greiningar á vélindabakflæði hjá börnunum í

okkar rannsókn. Því er ljóst að við greiningu er rétt að líta á bæði einkenni, niðurstöðu sýrustigsmælingar, niðurstöðu vélindaspeglunar og sýnatöku til að komast sem næst réttri greiningu.

Gakk yrir
&
Buff!!

Lilja Björnsdóttir age 18 months

Meaning in my tri-linguistic world

Takk fyrir, Tack så mycket, Thank you
&
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APPENDIX 1 (GIQLI)

1. How often during the past 2 weeks have you had pain in the abdomen?
all of the time, most of the time, some of the time, a little of the time, never

2. How often during the past 2 weeks have you had a feeling of fullness in the upper abdomen?
all of the time, most of the time, some of the time, a little of the time, never

3. How often during the past 2 weeks have you had bloating (sensation of too much gas in the abdomen)?
all of the time, most of the time, some of the time, a little of the time, never

4. How often during the past 2 weeks have you been troubled by excessive passage of gas through the anus?
all of the time, most of the time, some of the time, a little of the time, never

5. How often during the past 2 weeks have you been troubled by strong burping or belching?
all of the time, most of the time, some of the time, a little of the time, never

6. How often during the past 2 weeks have you been troubled by gurgling noises from the abdomen?
all of the time, most of the time, some of the time, a little of the time, never

7. How often during the past 2 weeks have you been troubled by frequent bowel movements?
all of the time, most of the time, some of the time, a little of the time, never

8. How often during the past 2 weeks have you found eating to be a pleasure?
all of the time, most of the time, some of the time, a little of the
time, never

9. Because of your illness, to what extent have you restricted the kinds of food you eat?
very much, much, somewhat, a little, not at all

10. During the past 2 weeks, how well have you been able to cope with everyday stresses?

extremely poorly, poorly, moderately, well, extremely well

11. How often during the past 2 weeks have you been sad about being ill?
all of the time, most of the time, some of the time, a little of the time, never

12. How often during the past 2 weeks have you been nervous or anxious about your illness?
all of the time, most of the time, some of the time, a little of the time, never

13. How often during the past 2 weeks have you been happy with life in general?
never, a little of the time, some of the time, most of the time, all of the time

14. How often during the past 2 weeks have you been frustrated about your illness?
all of the time, most of the time, some of the time, a little of the time, never

15. How often during the past 2 weeks have you been tired or fatigued?
all of the time, most of the time, some of the time, a little of the time, never

16. How often during the past 2 weeks have you felt unwell?
all of the time, most of the time, some of the time, a little of the time, never

17. Over the past week, have you woken up in the night?
every night, 5-6 nights, 3-4 nights, 1-2 nights, never

18. Since becoming ill, have you been troubled by changes in your appearance?
a great deal, a moderate amount, somewhat, a little bit, not at all

19. Because of your illness, how much physical strength have you lost?
a great deal, a moderate amount, some, a little bit, none

20. Because of your illness, to what extent have you lost your endurance?
a great deal, a moderate amount, somewhat, a little bit, not at all

21. Because of your illness, to what extent do you feel unfit?
extremely unfit, moderately unfit, somewhat unfit, a little unfit, fit

22. During the past 2 weeks, how often have you been able to complete your normal daily activities (school, work, household)?
all of the time, most of the time, some of the time, a little of the time, never

23. During the past 2 weeks, how often have you been able to take part in your usual patterns of leisure or recreational activities?
all of the time, most of the time, some of the time, a little of the time, never

24. During the past 2 weeks, how much have you been troubled by the medical treatment of your illness?
very much, much, somewhat, a little, not at all

25. To what extent have your personal relations with people close to you (family or friends) worsened because of your illness?
very much, much, somewhat, a little, not at all

26. To what extent has your sexual life been impaired (harmed) because of your illness?
very much, much, somewhat, a little, not at all

27. How often during the past 2 week, have you been troubled by fluid or food coming up into your mouth (regurgitation)?
all of the time, most of the time, some of the time, a little of the time, never

28. How often during the past 2 weeks have you felt uncomfortable because of your slow speed of eating?
all of the time, most of the time, some of the time, a little of the time, never

29. How often during the past 2 weeks have you had trouble swallowing your food?
all of the time, most of the time, some of the time, a little of the time, never

30. How often during the past 2 weeks have you been troubled by urgent bowel movements?
all of the time, most of the time, some of the time, a little of the time, never

31. How often during the past 2 weeks have you been troubled by diarrhoea?
all of the time, most of the time, some of the time, a little of the time, never

32. How often during the past 2 weeks have you been troubled by constipation?
all of the time, most of the time, some of the time, a little of the time, never

33. How often during the past 2 weeks have you been troubled by nausea?
all of the time, most of the time, some of the time, a little of the time, never

34. How often during the past 2 weeks have you been troubled by blood in the stool?
all of the time, most of the time, some of the time, a little of the time, never

35. How often during the past 2 weeks have you been troubled by heartburn?
all of the time, most of the time, some of the time, a little of the time, never

36. How often during the past 2 weeks have you been troubled by uncontrolled stools?
all of the time, most of the time, some of the time, a little of the time, never

Calculation of the score:
most desirable option: 4 points
least desirable option: 0 points
GIQLI score: sum of the points

ORIGINAL PAPERS (I-V)