On the development of a novel device for colorectal anastomoses

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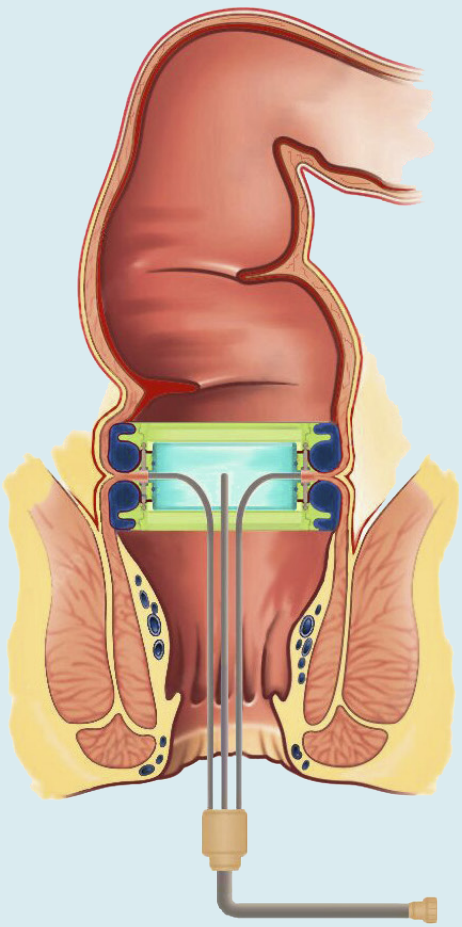

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Colorectal anastomotic leakage is a feared surgical complication and is a major cause of postoperative morbidity and mortality due to abdominal sepsis. The rate of colorectal anastomotic leakage has remained unchanged for the last decades, with leak rates as high as 20% in anastomoses performed in the lower third of the rectum. This thesis evaluates the safety and efficacy of the novel compression anastomotic devices CARP and C-REX in performing colorectal anastomoses.

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On the development of a novel device for colorectal anastomoses

Dadi Thor Vilhjalmsson

DOCTORAL DISSERTATION

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Abstract

Background: Intestinal anastomotic leakage is a feared surgical complication, constituting a major cause of postoperative morbidity and mortality due to abdominal sepsis. Furthermore, the occurrence of anastomotic leakage following colorectal cancer surgery endangers the potential postoperative oncological treatment of the affected patients. The rate of colorectal anastomotic leakage has remained unchanged for the last decades, with leak rates as high as 20% in anastomoses performed in the lower third of the rectum. Alternatives to the traditional handsewn and stapled anastomoses are needed.

Aims and methods: To evolve and assess the novel compression anastomotic devices CARP and C-REX, and evaluate their safety and efficacy in performing end-to-end colorectal anastomoses in the lower sigmoid colon and in the upper third of the rectum.

I. To assess the safety and efficacy of the CARP device in performing end-to-end colonic anastomoses following open resection of the sigmoid colon in an experimental model in pigs. To investigate the efficacy of the catheter-based system of the CARP instrument in measuring the intraoperative anastomotic contact pressure (ACP) and in evaluating the postoperative anastomotic integrity.

II. To assess the safety and efficacy of the CARP device in performing end-to-end colonic anastomoses following open left-sided colonic resection in patients with cancer in the descending- or sigmoid colon. To evaluate CARP´s ability in measuring intraoperative ACP and evaluating the postoperative anastomotic integrity in human settings.

III. To assess the safety and efficacy of the C-REX device in performing end-to-end colorectal anastomoses in the upper third of the rectum following open and minimally invasive high anterior resections in patients with cancer in the lower sigmoid colon or upper third of the rectum.

IV. To evaluate the early postoperative mechanical strength of end-to-end colorectal anastomoses in an experimental model in pigs, comparing bursting pressure of C-REX anastomoses and traditional circular stapled anastomoses following resection of the sigmoid colon.

Results and conclusions: The CARP method was safe and efficient in performing end-to-end colonic anastomoses following open resection of the sigmoid colon in a pig model (I), and after left-sided colonic resection in human settings (II). The catheter system of CARP could be used to measure intraoperative ACP and assess postoperative anastomotic integrity. The transanal C-REX method was safe and efficient in performing end-to-end colorectal anastomoses following open and minimally invasive high anterior resections in human settings (III). The C-REX anastomoses had more than 2-5 fold higher median bursting pressure in the early phase of the anastomotic healing compared to traditional circular stapled anastomoses, following open resection of the sigmoid colon in a pig model.

Key words: Colorectal anastomoses, anastomotic healing, anastomotic leakage, CARP, C-REX. Compression anastomoses, RectoAid, LapAid.

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In memory of my father, mother and sisters Ásta and Nína

To Elva, Sara, Valur and Brynjar

"The history of the intestinal suture is full of interest to the student of surgical literature. It is replete with stupendous ignorance, clever mechanical ingenuity, patient experimental research, and the careful application of pathological knowledge to the treatment of injuries and diseases of the intestinal canal".

Nicholas Senn (1844-1908)

Table of Contents

Original papers

This thesis is based on the following papers, which will be referred to by their Roman numerals I-IV:

I. Vilhjalmsson D, Olofsson P, Syk I, Thorlacius H, Grönberg A. Compression anastomotic ring-locking procedure (CARP) is a novel technique for creating a suture-less colonic anastomosis. Eur Surg Res. 2015;54:139-147. doi: 10.1159/000368354.

II. Vilhjalmsson D, Appelros A, Toth E, Syk I, Grönberg A, Mynster T, Thorlacius H. Compression anastomotic ring-locking procedure (CARP) is a safe and effective method for intestinal anastomoses following left-sided colonic resection. Int J Colorectal Dis. 2015;30:969-975. doi: 10.1007/s00384-015-2257-z.

III. Vilhjalmsson D, Lepsenyi M, Syk I, Grönberg A, Thorlacius H. Transanal formation of anastomosis using C-REX device is feasible and effective in high anterior resection. Int J Colorectal Dis. 2023;38:127. doi: 10.1007/s00384-023-04420-x.

IV. Vilhjalmsson D, Grönberg A, Syk I, Thorlacius H. Comparison of the C-REX LapAid and circular stapled colorectal anastomoses in an experimental model. Manuscript (submitted).

Abbreviations

ACP	Anastomotic contact pressure
AL	Anastomotic leakage
ASA	American Society of Anesthesiologists
bFGF	Basic fibroblast growth factor
BMI	Body Mass Index
CARP	Compression anastomotic ring-locking procedure
C-REX	Colorectal anastomosis; rejoin the intestine and validate the anastomosis; extract samples for analysis; X-ray through connected catheters
CRP	C - reactive protein
CT	Computerized tomography
DM	Diabetes mellitus
IBD	Inflammatory bowel disease
ISREC	International Study Group of Rectal Cancer
LL	LapAid-LapAid
LR	LapAid-RectoAid
MDR	Medical device regulation
MMP	Matrix metalloproteinases
PDGF	Platelet-derived growth factor
PME	Partial mesorectal excision
POD	Postoperative day
TGF-β	Transforming growth factor beta

Introduction

The intestinal anastomosis

An intestinal anastomosis is a surgical procedure that involves connecting two segments of the gastrointestinal tract, usually formed after a bowel resection for a benign or malignant disease. The formation of an anastomosis is not without risks and the process of intestinal anastomotic healing is a fine balance between insufficient healing with subsequent leakage, and excessive healing with the formation of a stricture. Anastomotic leakage is one of the most feared surgical complications in colorectal surgery and occurs when the joined bowel ends fail to heal, resulting in a spill of luminal contents into the surrounding extraluminal space.

Anastomotic leakage is a major cause of postoperative morbidity and mortality due to abdominal sepsis, leading to significant patient suffering and extended hospitalization with increased healthcare costs[1-3]. Furthermore, the occurrence of anastomotic leakage following colorectal cancer surgery endangers the postoperative oncological treatment strategy for the patient. This serious anastomotic adverse event may increase the likelihood of local recurrence and lower long-term survival rates[3-5]. However, it is worth noting that this risk can be reduced using neoadjuvant chemoradiotherapy in curative rectal cancer surgery[6].

The anatomy of the intestinal wall

The gastrointestinal tract is composed of a hollow visceral tube stretching from the mouth to the anal canal, with the task of digesting and absorbing nutrients and fluids with the help of its accessory organs. This elongated tube includes the oral cavity, pharynx, esophagus, stomach, small intestine, large intestine and anus. The largest portion of the gastrointestinal tract consists of four distinct intestinal wall layers: mucosa, submucosa, muscularis propria, and serosa (Figure 1).

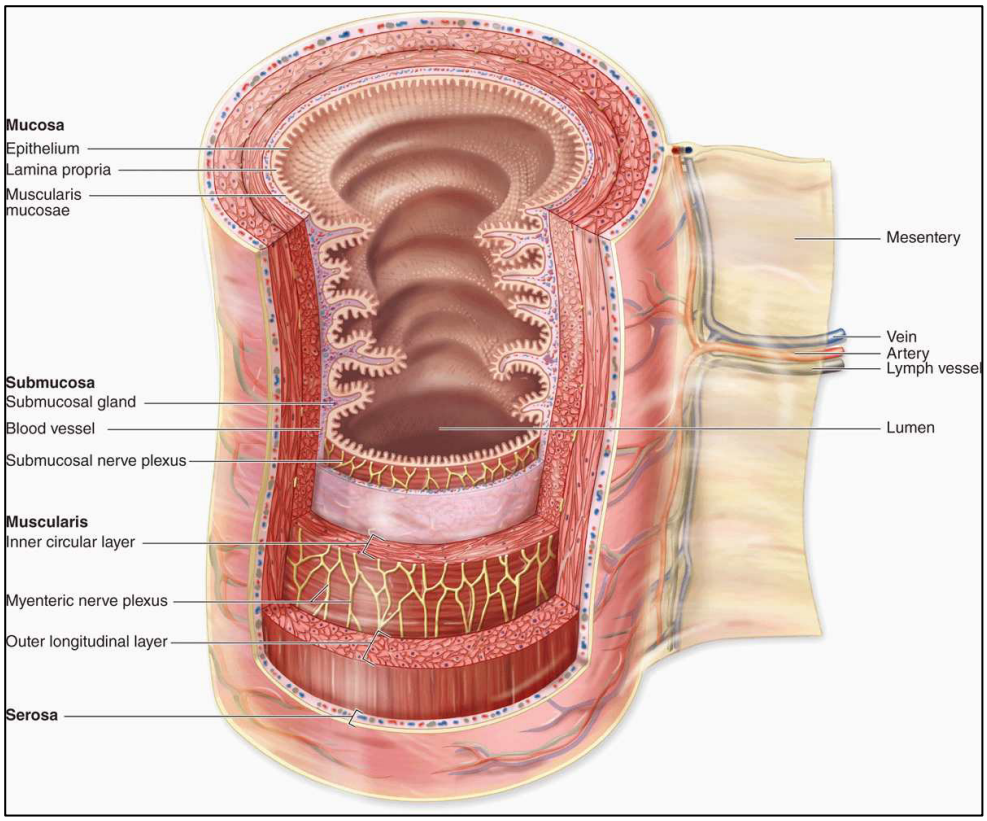

Figure 1: The layers of the intestinal wall. Copyright by McGraw-Hill Education, reproduced with permission.

The **mucosa** is the innermost layer and lines the lumen of the gastrointestinal tract. It is composed of three sublayers: the epithelium, lamina propria, and muscularis mucosa. The role of the epithelium is secretion, digestion, and absorption. The lamina propria is a thin connecting tissue layer that provides structural support, vascularization, and lymphoid drainage to the epithelium. The muscularis mucosa is a thin smooth muscle layer enabling local peristalsis in the mucosa.

The fibrous **submucosa** layer lies beneath the mucosa and connects it to the muscularis layer of the bowel wall. It is rich in collagen fibers, giving the bowel wall its structural support and acting as the skeleton of the gastrointestinal tract. The submucosa contains blood vessels, autonomic nerve plexus (Meissner´s plexus), and mucous-secreting glands. The submucosa has an important biological role in intestinal healing.

The **muscularis propria** is the main muscle layer of the gastrointestinal wall and is responsible for the peristaltic movements of the visceral tube. It is composed of two smooth muscle layers, the inner circular layer, and the outer longitudinal layer. The

stomach has an additional innermost oblique muscular layer. The myenteric nerve plexus (Auerbach´s plexus) lies between these muscle layers, enabling the peristaltic movements and transport of bolus through the gastrointestinal tube. The inner circular layer is thicker in the colon compared to other parts of the gastrointestinal tract and its longitudinal smooth muscle layer is organized into three longitudinal bands called tenia coli.

The **serosa** is the outermost layer of the gastrointestinal tract and provides a protective barrier for the bowel wall. It covers the intraperitoneal portion of the gastrointestinal tract, whereas the adventitia is the outermost layer of gastrointestinal segments lying extraperitoneal, such as the esophagus, duodenum, and the lower third of the rectum beneath the peritoneal reflection.

A brief history of the intestinal anastomosis

The art and surgical techniques of the intestinal suture (enterorrhaphy) dates back before the Roman Empire. The Roman physician Aulus Cornelius Celsus (c. 25 BC-c. 50 AD), known for his description of the cardinal signs of inflammation (*i.e.*, calor, dolor, rubor, and tumor), mentioned the intestinal suture in his medical treatise *De Medicina*, although himself being unsure of its use[7]. During ancient times and up to the late Renaissance period, physicians had probably little experience and technical knowledge in using this suture method. The first surgical techniques were developed to treat traumatic tissue injuries and these intestinal sutures were used when repairing bowel lacerations after penetrating abdominal trauma and were presumably the only performed gastrointestinal surgical procedures until the late 17th century. At the beginning of the 18th century, most surgeons considered it more successful to treat intestinal wounds with a more conservative approach instead of closing them with sutures. This was because the only reliable life-saving treatment for intestinal injuries and diseases at that time was the evolvement of ostomies, such as the enterocutaneous fistulas resulting from incarcerated hernias or penetrating intestinal trauma[8]. However, as a result of the advancements in experimental surgery and knowledge in tissue pathology, additional improvements were made in suture materials and surgical techniques. Numerous surgeons continued evolving the art and finesse of the intestinal suture, and eventually performed an intestinal anastomosis with circular end-to-end suture techniques, using a vast number of different suture methods and diverse aiding prostheses and buttons. Between 1727 and 1881, there were about 100 different suture techniques used and 90 reported surgical anastomoses[8].

In 1812, the British surgeon Benjamin Travers (1783-1858) published his work on intestinal injuries, trying to understand the healing process of penetrating intestinal wounds and strangulated hernias[9]. Travers combined historical observations of intestinal trauma in humans with his pioneering experimental animal research. He

argued that “although the acknowledged varieties in the constitution of man and animals do not permit every inference from the powers and actions of one to be applied to the other, yet are there striking features of resemblance in the phenomena ensuing upon mechanical injury, some of which have already been applied, and more admit of application to important practical purposes”[9]. Travers demonstrated that it was possible to recover spontaneously from certain types of intestinal wounds, contrary to prevailing beliefs. He addressed the surgical dilemma of when to assist intestinal wounds with sutures and when to avoid harmful interference with the natural healing process of the body. Travers recommended extensive intestinal wounds to be sutured with a silk thread near the line of the wound, with short regular intervals through the whole extent of the wound, and with an equal position of the edges included in each stitch[9].

In 1826, the French surgeon Antoine Lembert (1802-1851) introduced the significance of apposition of the intestinal serosa layer when suturing intestinal wounds. He achieved this by inverting the bowel edges, whereas previous suture methods involved everting the bowel wound by approximating the mucous membrane of the injured bowel to the peritoneal surface of the abdominal wall[7]. This novel inverting suture technique became known as the Lembert suture and was a crucial step in the technical evolution of intestinal anastomosis (Figure 2). This serosa inverting suture technique is more prone to heal compared to the everting techniques, where the everting suture line is dependent on the formation of postoperative adhesions to seal it from leakage in experimental models[10]. Furthermore, the everting suture technique has been shown to have over five times higher rate of fecal fistula formation in colorectal anastomoses compared to the inverting technique (43% versus 8%)[11].

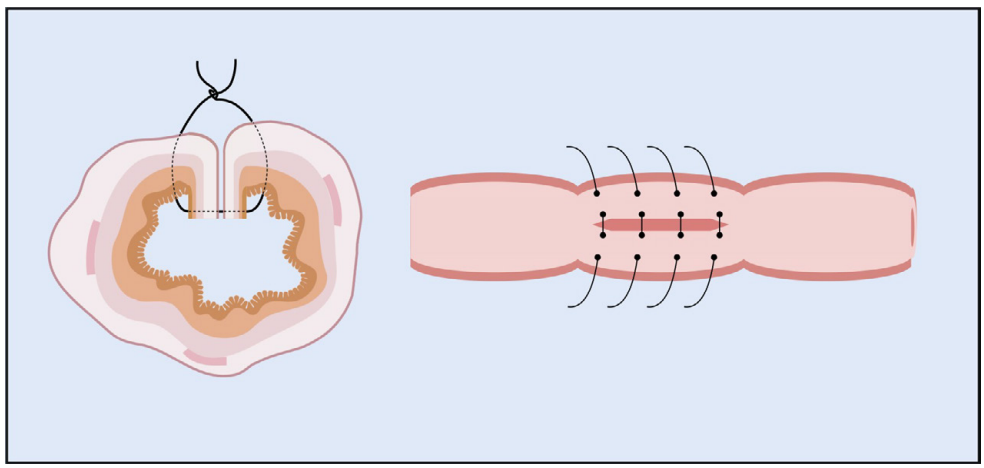

Figure 2: The Lembert suture. Copyright by author. Created with BioRender.com.

In 1867, the British surgeon Joseph Lister (1827-1912) introduced the principles of antiseptic surgery when he presented carbolic acid (phenol) for sterilizing wounds, sutures, and surgical instruments when managing tissue injuries[12]. His antiseptic method was essential for reducing infectious complications during wound management. This was a cornerstone for further development of the anastomotic techniques that followed. Before the use of Lister´s carbolic acid, wound infections were serious and life-threatening complications and the previously used non-sterile intestinal sutures tended to migrate through the bowel wall after only a few days, leading to impaired healing of the intestinal wound[8].

In 1887, the American surgeon William Halsted (1852-1922) emphasized the importance of including the fibrous submucous layer of the bowel wall when suturing intestinal wounds, as he believed the submucosa to be the only layer in the intestinal wall having sufficient structural strength capable of anchoring the sutures[7, 8]. Halsted's observations laid down one of the most important advancements in the technical evolution of the intestinal anastomosis, as the submucosa is rich in collagen fibers, giving the intestinal wall its anatomical support for its load-bearing functions and is crucial in intestinal anastomotic healing and anastomotic integrity.

In 1899, the Czech surgeon Vitezslav Chlumsky (1867-1943) was experimenting with the mechanical characteristics of intestinal anastomoses at various time intervals after surgery, using a canine model[8]. He applied and measured for the first time the breaking strength and bursting pressure of an intestinal anastomosis, determining when it had healed sufficiently with enough mechanical strength to withstand physiologic strains. Since then, the bursting pressure and breaking strength have been used as mechanical parameters for assessing the strength of the resulting anastomotic healing process. Chlumsky´s results demonstrated that the anastomoses were weakest during the first three postoperative days when they had the lowest resisting strength, followed by steadily increasing strength and reaching their initial tensile resistance at the end of the second week[8].

In 1908, the Hungarian surgeon Humer Hültl (1868-1940) developed the first surgical stapler instrument[13, 14]. His device weighed 3.6 kg and consisted of more than 100 parts that had to be assembled before the surgical procedure. The instrument required considerable physical strength to handle and reloading took two hours, which made it cumbersome to use. In 1924, the Hungarian surgeon Aladár Von Petz (1888-1956), a student of Hültl, developed a more lightweight stapler model named the Petz clamp, which was easier to use and reload[14, 15]. His novel device weighed 1.5 kg and consisted of ten parts.

Refinements in stapling instruments emerged from Russia in the 1950s, followed by further advancements from the American surgeon Mark Ravitch (1910-1989) in the 1970s, which enabled rectal anastomosis[13, 14]. The surgical staplers were further modified to incorporate interchangeable cartridges with various designs. Since then, several modifications have been undertaken and newer and simpler linear and

circular intestinal stapling devices have been constructed and are still in use today, with circular transanal stapling instruments constituting a cornerstone in rectal surgery. The configuration and technique of the intestinal staplers continue to evolve, while still maintaining the basic principles from the original design of Humer Hültl.

The art and knowledge behind intestinal anastomosis have advanced progressively since the 19th century. In the beginning, the attention was on developing more refined suture materials and improving surgical methods, followed by a subsequent contribution of knowledge of the histological healing process itself. Due to clever innovations and experimental research, the creation of an intestinal anastomosis transformed from being a life-threatening procedure to being a routinely performed step in gastrointestinal surgery in the present time. The two most crucial technical milestones for the development of intestinal anastomosis were the serosa inverting method described by Antoine Lembert and the submucosal inclusion described by William Halsted. Furthermore, surgeons gradually recognized the importance of good surgical technique, using sufficient exposure and re-joining healthy intestinal ends in an anastomosis with good tissue perfusion, free of tension and without distal obstruction.

The intestinal anastomotic healing process

A wound is a disruption of a normal tissue structure caused by an injury, and the process of wound healing is a series of well-organized and coordinated steps designed to repair the damaged tissue and re-establish the immune barrier. These steps are regulated by platelets and various inflammatory cells, orchestrated through cell-signaling by cytokines and growth factors[15]. The healing process is initiated by the inflammatory response inflicted by an injury and is followed by the recruitment of leukocytes and fibroblasts, tissue renewal with deposition of new extracellular matrix and collagen, and subsequent maturation of the scar tissue. Wound healing is further assisted by proteolysis and tissue matrix degradation mediated by tissue metalloproteinases, and a disturbance in the balance between this important family of collagenase enzymes and their inhibitors can result in impaired wound healing. The nature of wound healing has been studied extensively in the skin, since these wounds are easy to observe. The fundamental steps of skin healing apply to most tissues in the body and involve the sequence of hemostasis at the site of injury, decontamination of the wound, followed by cellular differentiation to reform and regain the morphology and function of the tissue structure[15-17]. The physiological responses of wound healing in the skin are divided into three time-dependent and overlapping phases: the inflammation phase, the proliferation phase, and the remodeling phase[15-17].

The healing process of an intestinal anastomosis remains a topic of ongoing research interest. The physiological responses involved in wound healing of the gastrointestinal tract follow similar features as wound healing in the skin, although there are some significant differences[16, 18]. The physical environment in the healing of intestinal anastomoses differs from a skin wound owing to the shear stress in the anastomotic wound secondary to the intraluminal bulk transit and the periodical wave movements of the intestinal peristalsis. In addition, the bowel lumen contains both aerobic and anaerobic bacteria compared to only aerobic bacteria in the skin. Furthermore, the skin has only two subtypes of collagen (I and III) which are produced by fibroblasts in the skin during the healing process. In comparison, the gastrointestinal tract contains three subtypes of collagen (I, III and V) produced by both fibroblasts in the bowel wall and by smooth muscle cells located in the muscularis propria of the intestinal wall and in the layer of the muscularis mucosa (Table 1)[16, 18].

Table 1: Subtypes of collagen in the skin and the gastrointestinal (GI) tract.

Skin:	GI tract:
80% subtype I	68% subtype I
20% subtype III	20% subtype III
	12% subtype V

The rate of the intestinal anastomotic healing process is also more rapid compared to wound healing in the skin, as an intestinal wound takes a few weeks to heal compared to a few months in the skin. Collagen, which is located primarily in the intestinal submucosa of the bowel wall, is the single most important molecule for determining the tensile strength of the intestinal wall, making collagen metabolism particularly interesting in understanding anastomotic healing in the gastrointestinal tract. Consequently, a newly formed intestinal anastomosis regains its strength by novel collagen synthesis and maturation, where a disturbance in this collagen metabolism can endanger the recovery of the anastomotic strength with resulting anastomotic leakage[19].

The healing process of an intestinal anastomosis can be divided into three overlapping phases (Figure 3)[16-18, 20]:

- The acute inflammatory phase (lag phase)
- The proliferative phase
- The maturation phase (remodulation phase)

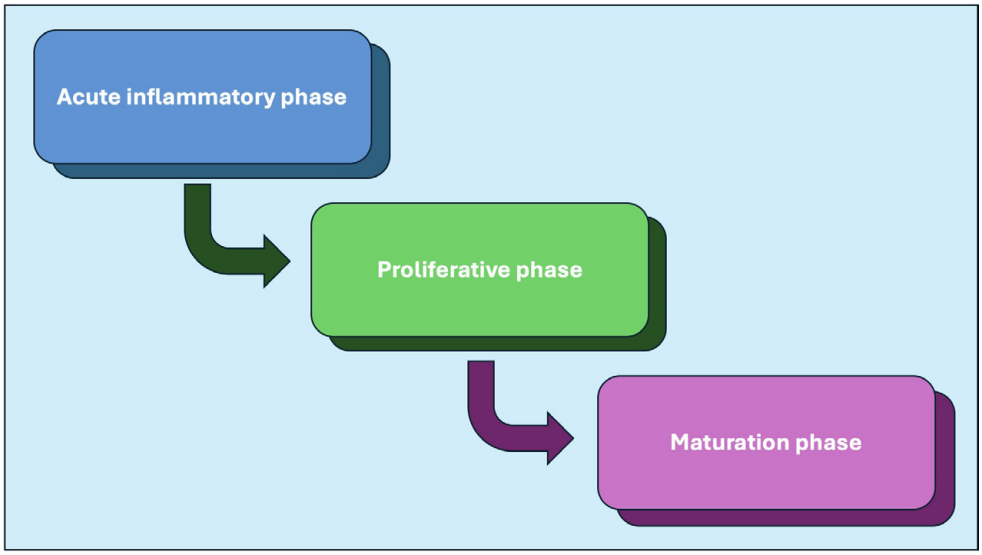

Figure 3: The overlapping healing phases of an intestinal anastomosis. Copyright by author.

The **acute inflammatory phase** (0-3 days) is essential for intestinal wound healing and its purpose is to gain hemostasis, establish an immune barrier and recruit additional cell types with the help of chemotactic signal molecules released locally in the wound by platelets, neutrophils and monocytes/macrophages. In combination with the initial hemostatic response of vasoconstriction and the intrinsic part of the coagulation cascade following the injury, platelets obtain hemostasis with the formation of a fibrin plug and provisional matrix and release chemotactic substances like platelet-derived growth factor (PDGF) and transforming growth factor beta (TGF-β)[16, 21]. Increased vascular permeability in the vicinity of the intestinal wound facilitates the chemotactic signal orchestrated efflux of inflammatory cells into the wound area. Neutrophil recruitment predominates initially and their role is to clear the wound of debris and invading microorganisms using phagocytosis. Within 2-3 days, the monocytes take over and mature into tissue macrophages. Macrophages continue with the phagocytic work and secrete growth factors that are important for chemotaxis, activation and proliferation of cell types taking part in the next step of the tissue repair. During the acute inflammatory phase, the balance of the collagen metabolism changes and collagen degradation exceeds collagen synthesis in the area of the intestinal wound, and the strength of a newly formed anastomosis is low. This leads to lower bursting pressure values during the first three postoperative days. The bursting pressure of a small bowel anastomosis is only 50% of its initial strength during the first 2-3 days after its formation, and the bursting pressure of a colonic anastomosis is only 30% of its initial strength (Figure 4)[16, 21]. Consequently, the bursting pressure values equal that of an intact bowel wall by the seventh day after

surgery, after which the intestine will generally burst some distance from the anastomotic line[16, 21].

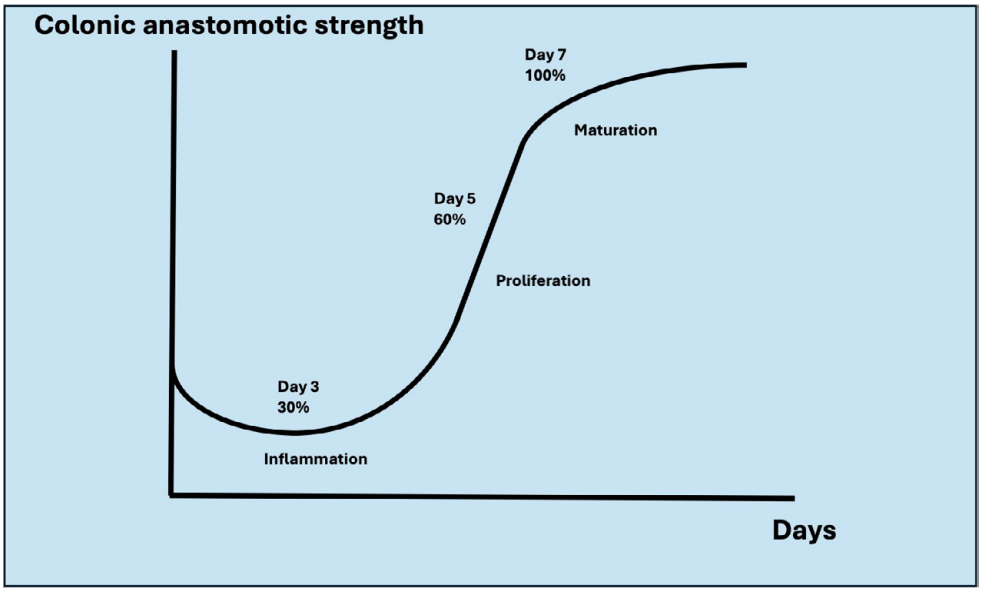

Figure 4: Bursting pressure of a newly formed colonic anastomosis. Copyright by author.

This 50-70% loss in anastomotic strength during the first postoperative days is thought to be mediated by upregulation of matrix metalloproteinases (MMPs) proteolytic activity, causing local collagen and tissue matrix degradation in the submucosa of the bowel wall. The anastomotic integrity during that period is therefore dependent on the suture- or staple-holding capacity of the remaining collagen in the submucosa, until the collagen metabolism shifts to more collagen being synthesized than degraded in the bowel wound, marking the beginning of the proliferative phase[16, 18, 21]. This regulated activation of the MMPs is important for the anastomotic healing process, as the degradation of the provisional wound matrix creates space for cell migration, novel angiogenesis, and remodeling of the extracellular matrix in the anastomotic wound[22, 23].

The **proliferative phase** (3-14 days) begins with the arrival of fibroblasts migrating to the anastomotic wound and is regulated by various growth factors like PDGF, TGF-β and basic fibroblast growth factor (bFGF). Fibroblasts initiate the production of collagen-rich granulation tissue together with the smooth muscle cells in the intestinal wall, replacing the provisional matrix laid during the acute inflammatory phase (Figure 5).

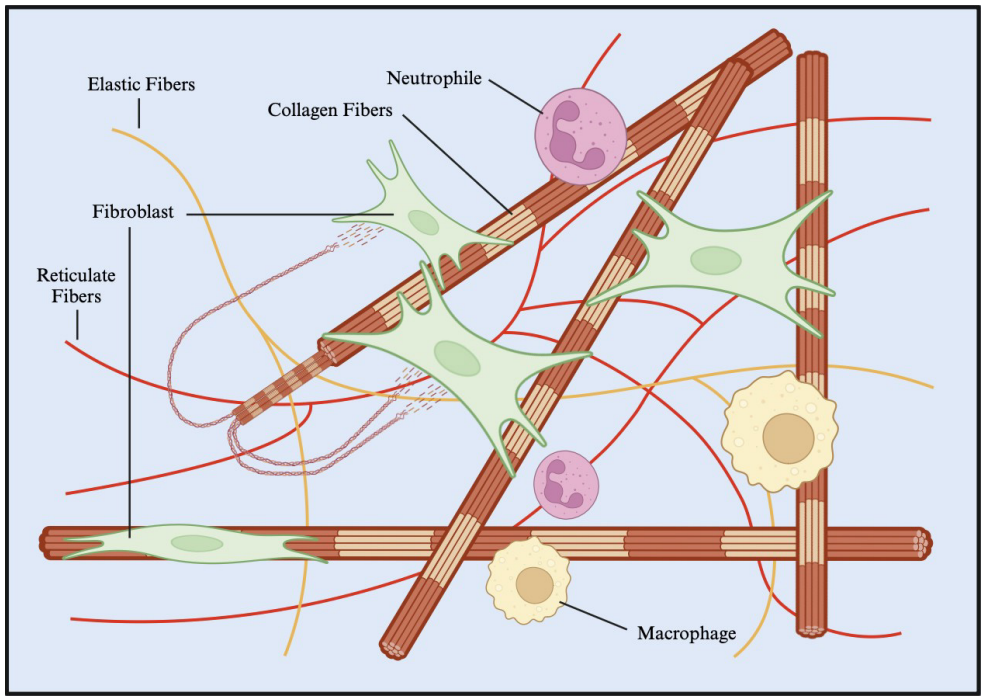

Figure 5: Fibroblasts initiate collagen production in the anastomotic wound. Copyright by author. Created with BioRender.com.

At this stage, fibroblasts and endothelial cells are the primary proliferating cells[13]. Fibroblasts become the most important cell type at the fourth day of the healing process and join the smooth muscle cells in collagen synthesis, where the post-translational hydroxylation of the amino acids lysine and proline in the collagen molecule is a crucial step in collagen maturation[24], enabling cross-linking entailing folding of the collagen molecule into a stable triple helix conformation which gives collagen its structural strength (Figure 6). Vascular regeneration occurs in this phase with endothelial cell proliferation and migration from intact venules close to the wound edges and form new capillaries to allow oxygenation, and delivers important nutrients and building blocks to the healing wound. During the proliferative phase, collagen synthesis exceeds collagen lysis and the anastomotic wound increases gradually in strength. It has been demonstrated that the novel collagen synthesis in a small bowel anastomosis is more abundant and is initiated at an earlier stage compared to a colonic anastomosis, possibly explaining the lower rate of anastomotic leakage in the small intestine compared to the large intestine[19].

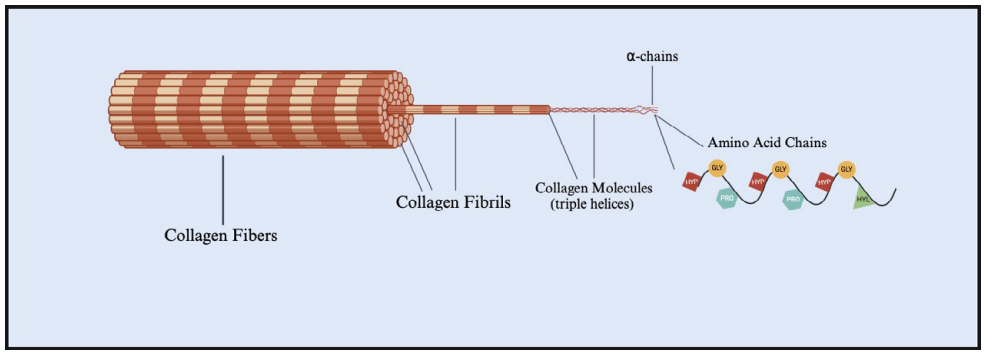

Figure 6: The structure of collagen. HYP=Hydroxyproline, GLY=Glycine, PRO=Proline, HYL=Hydroxylysine. Copyright by author. Created with BioRender.com.

The final phase of the anastomotic healing involves the maturation of the newly formed collagen-rich granulation matrix. In the **maturation phase** (2-6 weeks), the newly formed granulation tissue undergoes remodeling and the density of macrophages and fibroblasts decreases. During this phase, collagen synthesis continues and the thin collagen fibers are converted into thicker collagen bundles with the help of additional molecular cross-linking. The wound starts to contract owing to the linkage between these collagen bundles and contractile units in the tissue, and together with the orientation of the collagen fibers, creates the strength of the healing anastomosis[16, 18, 21].

Assessment of intestinal anastomotic healing

Experimental studies in animal models are necessary when studying the process of anastomotic healing, as it is essential to be able to assess the strength of the anastomotic wound and the outcome of the anastomotic repair. The strength of a newly formed intestinal anastomosis depends mainly on:

- The quality of its sutures or staples
- The suture- or staple-holding capacity of the anastomotic tissue
- The healing tissue bridging the anastomotic gap

Provided that the anastomotic sutures and staples are strong enough and correctly placed, the strength of a newly formed intestinal anastomosis depends mainly on the suture- or staple-holding capacity of the intestinal submucosa, since the newly formed provisional anastomotic tissue bridging the anastomotic gap is too weak during the first postoperative days.

There are three methods used for assessing anastomotic repair in experimental models, which are based on the analysis of histological-, biochemical- or

mechanical parameters[25, 26]. **Histological** observations are important to evaluate the outcome of the healing process, but are not useful for assessing anastomotic strength. Instead, quantitative methods based on biochemical- or mechanical analyses are used for this purpose.

The **biochemical** parameters used to assess anastomotic strength are the amount of anastomotic collagen content and concentration. This is often performed by measuring hydroxyproline content in anastomotic tissue, since this post-translational modified proline amino acid is mainly found in collagen molecules[25]. Measurements of collagen content and concentration have been reported in numerous experimental anastomotic studies[25-29]. However, most of these studies have focused on collagen mass rather than collagen structure and overlooked the aspect of collagen organization in the tissue[25, 26]. Although hydroxyproline content is informative about the amount of collagen in anastomotic tissue, it does not provide information on the quality and maturation state of collagen[25, 30-32]. Collagen quality, such as collagen subtype and cross-linking, is an important factor when assessing anastomotic repair, as the amount of collagen mass does not necessarily relate to anastomotic strength[25, 30-32].

The **mechanical** analyses used to assess anastomotic strength are breaking strength and bursting strength. Breaking strength is defined as the maximum force required to tear an anastomosis apart in its longitudinal (axial) direction, and is usually measured with the aid of a tensiometer. Breaking strength has been used to assess the anastomotic strength in numerous experimental anastomotic studies[33-36]. However, the study results of breaking strength are inconsistent and have been criticized for being too sensitive to both the surgical- and the measuring techniques used, and because it lacks the exerted force in the circular direction of the anastomosis[25, 26]. Instead, the measurement of bursting strength is considered to reflect more accurately the physiological strain of an intestinal anastomosis and is now the mechanical parameter mostly used in experimental studies of intestinal anastomotic repair[25, 26, 37].

Bursting strength is defined as the maximum intraluminal pressure needed to rupture a bowel segment when inflating it with gas or liquid and reflects the weakest site of the analyzed intestinal segment, which is usually the anastomotic line during the first few postoperative days[16]. The parameter of bursting strength can be expressed as either bursting pressure or bursting wall tension, where bursting pressure is more frequently used[25]. This is because the calculation of bursting wall tension requires measurements of the internal radius of the anastomosis according to Laplace´s law, which is not easy to achieve (Figure 7)[25].

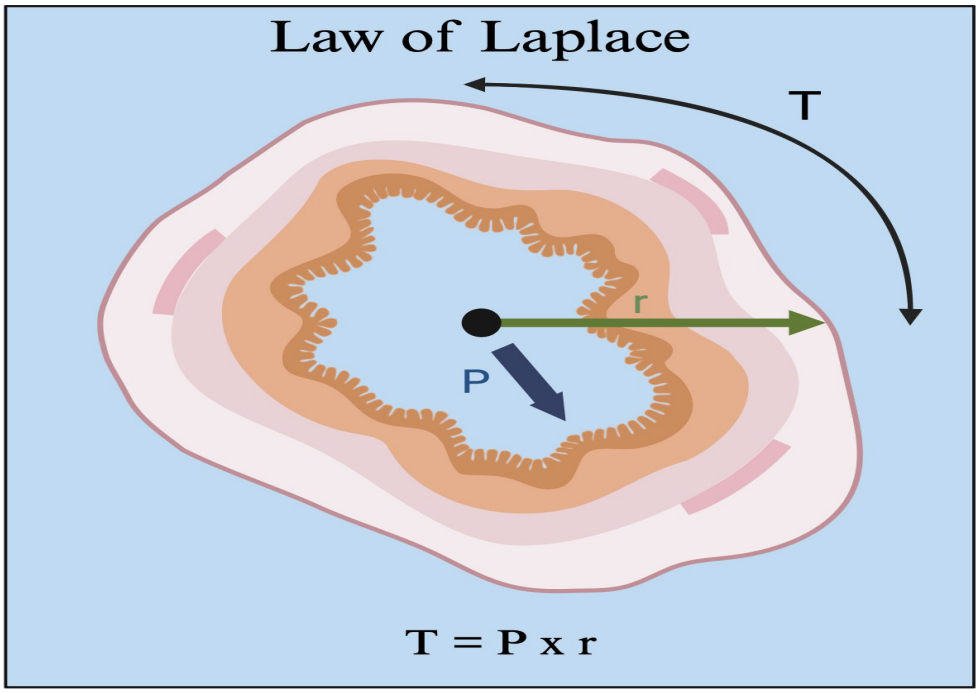

Figure 7: The law of Laplace states that the wall tension of the intestine (T) is directly proportional to the intraluminal pressure (P) and the radius of the intestinal segment. Copyright by author. Created with BioRender.com.

However, it is important to recognize the limitations of using bursting pressure as a mechanical parameter in anastomotic studies, as it has been demonstrated that bursting pressure may depend on[25, 26, 36, 37]:

- Rate of bowel inflation
- In situ or in vitro measurements
- Animal species utilized
- Intestinal segment used
- Which suturing- or staple technique used
- Preparation of the bowel segment

With that in mind, a direct comparison of bursting pressure values between studies is probably not relevant, and it has been recommended to compare the pattern of bursting pressure values instead[25]. In addition, experimental studies have demonstrated that bursting pressure is mostly valuable as a mechanical parameter

for assessing anastomotic repair during the first postoperative week, when ruptures occasionally occur within the anastomotic line during the test[25].

Intestinal anastomotic leakage

Despite this dreaded surgical adverse event, the concept of anastomotic leakage is poorly characterized and lacks a uniform definition, making it difficult to estimate the incidence of anastomotic leakage. A systematic review of 97 studies, analyzing the applied definition of anastomotic leakage after gastrointestinal resections, demonstrated that leaks were not comparably reported and the definition of a colorectal anastomotic leakage was described in 29 different ways[38]. The absence of a uniform definition prevents a reasonable comparison of the reported incidence of anastomotic leakage between studies. In 2010, the International Study Group of Rectal Cancer (ISREC) proposed a definition and a grading system for colorectal anastomotic leakage[39]. ISREC proposed that the definition of an anastomotic leakage should not only be confined to a defect of the intestinal wall at the anastomotic site, but also include defects in the suture- and staple lines of neorectal reservoirs (Box 1). Furthermore, ISREC recommended that anastomotic leakage should also be graded from A-C according to the impact of the anastomotic leakage on the clinical management of the patient (Table 2)[39].

Box 1: The International Study Group of Rectal Cancer (ISREC) definition of anastomotic leakage

An **anastomotic leakage** is a defect of the intestinal wall at the anastomotic site (including suture and staple lines of neorectal reservoirs) leading to a communication between the intra- and extraluminal compartments.

Table 2: The International Study Group of Rectal Cancer (ISREC) grading of anastomotic leakage

Grade A	No change in patient management
Grade B	Requiers active therapeutic intervention without re-laparotomy
Grade C	Requiers re-laparotomy

The incidence of intestinal anastomotic leakage varies according to their anatomical location. The reported leakage rate of small bowel anastomoses is between 1-3% and that of colorectal anastomoses between 2-20%, with the highest leakage rate in rectal anastomoses below the peritoneal reflection[40-50]. The clinical presentation of an anastomotic leakage can vary considerably, from fulminant peritonitis with sepsis and subsequent multiorgan failure and death, to an asymptomatic finding on a routine investigation with endoscopy, CT scan, and/or contrast enema before defunctioning stoma reversal. In addition, anastomotic leakage can have a more

subtle clinical presentation, as in patients with failure to thrive, who are not critically ill but are not recovering as expected postoperatively.

Clinical anastomotic leakage usually develops within the first week after the anastomosis formation and commonly presents with abdominal pain, high fever, and signs of sepsis[51-53]. However, anastomotic leakage can be detected at other time intervals and is frequently diagnosed after the initial hospital discharge. This is most often seen in patients with a rectal anastomosis following low anterior resection of the rectum with a defunctioning stoma, where 24-40% of these late presenting anastomotic leaks are diagnosed more than 30 days after the surgical procedure[42, 51, 54, 55].

Anastomotic leakage can be categorized as follows[40]:

- Simple fistulas versus large openings
- Intraperitoneal versus extraperitoneal
- Asymptomatic versus septicemia
- Early versus late

Early detection of anastomotic leakage is crucial, although sometimes difficult to achieve due to the varying anatomical location of the anastomoses and the variation of presenting symptoms. Anastomotic leakage within the peritoneal cavity usually presents with peritonitis and sepsis following the peritoneal contamination. However, anastomotic leakage which originates extraperitoneally can sometimes be more subtle and present as pain or discomfort in the pelvic area with possible urinary symptoms, enterovaginal/enterovesical fistulas, or as fecal contaminated fluids in pelvic drainage[40]. In a randomized, multicenter trial of patients undergoing low anterior resection of the rectum with a colorectal anastomosis, 116 patients were randomized to receive a diverting loop ileostomy compared with 118 patients who received no diversion. Patients with stoma diversion were less likely to present with symptoms of peritonitis and sepsis (10.3 % vs 28.0%) or require an urgent re-laparotomy (8.6% vs 25.4%)[42].

Delayed intervention in patients with symptomatic anastomotic leakage is related to poorer outcome with increased postoperative mortality[56, 57]. Patients with symptoms or suspicion of an anastomotic leakage should have their blood test taken for assessment of complete blood cell count, C-reactive protein (CRP), electrolyte panel, and coagulation screening tests, together with the assessment of pH and lactate in arterial blood. CRP, named for its interaction with pneumococcal somatic C-polysaccharide, is an acute-phase plasma protein produced by the liver in response to inflammation, infectious diseases, and tissue injuries like surgical trauma[58, 59]. CRP activates the classical complement pathway of the immune system during bacterial infections and participates as an opsonization factor during macrophage activation and phagocytosis[58, 59]. The CRP value usually peaks on the

second or third postoperative day following the tissue injury inflicted by the surgical procedure and should decline gradually in the next postoperative days in case of uncomplicated recovery. CRP has an important role in the assessment of possible postoperative adverse events, as persistent or biphasic elevated plasma or serum levels of CRP after the third postoperative day are predictive of postoperative complications, such as anastomotic leakage[60, 61].

Patients with suspicion of anastomotic leakage should be managed with a urinary catheter to enable monitoring of fluid balance and be resuscitated with intravenous fluids and broad-spectrum antibiotics. The preferred diagnostic radiological modality when suspecting an intestinal anastomotic leakage is a computerized tomography (CT) with intravenous contrast to assess the presence of excessive extraluminal gas or free fluid in the abdominal- or pelvic cavity[62, 63]. The CT scan can be supplemented with water-soluble contrast given orally or as a rectal enema depending on the anatomical location of the anastomosis, to indicate possible extraluminal leakage of contrast medium from the anastomosis. A systematic review of eight studies assessing the value of 221 abdominal CT scans in the diagnosis of an anastomotic leakage after colorectal surgery, demonstrated a relative low sensitivity (68%) of the CT scans, where the author argued that the overall methodological quality of the studies was insufficient[64]. Another retrospective study of 600 patients undergoing colorectal resections with an anastomotic leakage rate of 10% (60/600), demonstrated 75% sensitivity of the CT scans in detecting an anastomotic leakage (33/44), with 25% false-negative CT findings[48]. Poor CT scan sensitivity with resulting false-negative CT findings is a major concern when dealing with the consequences of anastomotic leakage, as delayed intervention is associated with higher mortality rates and prolonged hospital stay[1-3, 40, 48, 65]. In a prospective study, true-positive CT scans (24/35) showing anastomotic leakage led to faster intervention with a mortality rate of 4.2% (1/24) and a mean hospital stay of 28 days compared to false-negative CT scans (11/35) with delayed intervention, mortality rate of 45.5% (5/11) and median hospital stay of 54 days[65]. Other studies assessing standardized diagnostic methods using CT scans with rectal contrast in the diagnosis of anastomotic leakage in rectal anastomosis after low anterior resections of the rectum have reported up to 97% sensitivity[66]. Digital rectal palpation and a careful rectoscopy using a flexible sigmoidoscope are also useful diagnostic tools in accessing the integrity of an extraperitoneal colorectal anastomosis in patients with low rectal anastomosis and defunctioning stoma[67]. In addition, a diagnostic laparoscopy can be performed to assess the abdominal cavity and the anastomotic area in patients who have undergone minimally invasive colorectal resection with an anastomosis, if there is a clinical suspicion of anastomotic leakage.

Once an anastomotic leakage has been diagnosed, the management depends on the extent of the leakage and the physiological status of the patient, where controlling septic condition is the highest priority. Localized minor anastomotic leaks without

symptoms of sepsis (grade B) can often be managed conservatively with fasting, fluid resuscitation, total parenteral nutrition, antibiotics, and ultrasound- or CT-guided percutaneous drainage of the resulting fluid and air collection[48, 50, 66]. Patients with extraperitoneal anastomotic leakage from a minor defect in a low rectal anastomosis and a defunctioning stoma can be treated with endoluminal vacuum therapy as a therapy option, where some studies have demonstrated an anastomotic salvage rate of up to 79-90% in selected cases[68-70]. On the other hand, patients with anastomotic leakage with peritonitis and signs of sepsis (grade C) should be managed with surgical intervention with laparotomy, or in selected cases with laparoscopy[71], to ensure source control of the contamination which often requires taking down the anastomosis with construction of a proximal intestinal stoma[48, 69]. A retrospective study of patients undergoing colorectal resections with an anastomotic leakage rate of 10% (60/600) showed that 76.3% of patients with anastomotic leakage were reoperated with anastomosis take down[48]. A nationwide prospective study on colon cancer surgery in Denmark with overall leakage rate of 6.4% (593/9333), demonstrated that 85.4% of the patients with Grade C anastomotic leakage (433/507) were treated with anastomosis take down[72]. The anastomotic salvage rate was 14.6% (74/507), and was in patient with minor anastomotic defects.

Intestinal anastomotic leakage following colorectal cancer surgery endangers the potential postoperative oncological treatment strategy for the patient. Adjuvant oncological treatment is contraindicated in case of suboptimal source control of septic conditions or localized abscesses that are difficult to reach and drain. In addition, the patient has to be receptive to adjuvant treatment and a malnourished patient in poor physiological condition is often not fit to receive this treatment. Consequently, anastomotic leakage after colorectal cancer surgery may be associated with a higher frequency of local recurrence and lower overall and cancer-specific survival[1-5, 73-77]. However, neoadjuvant chemoradiotherapy seems to reduce this risk significantly in rectal cancer surgery[6].

A defunctioning loop ileostomy is often performed in patients with high-risk colorectal anastomoses to reduce morbidity and mortality rates following the event of an anastomotic leakage. While defunctioning stoma may decrease the impact of anastomotic leakage in these patients, they are not without risks and are associated with a specific complication profile[78-82]. The most common complication of a defunctioning loop ileostomy is dehydration with electrolyte disturbances following high stoma output, which in some cases can develop into acute renal failure or even progress to chronic kidney disease[78-82]. Other complications associated with loop ileostomy are skin irritation, stoma necrosis, prolapse, and retraction which may require reoperation. Moreover, patients with temporary loop ileostomy have to undergo a second operation for the ileostomy reversal, which is not without risk. A systematic review of 48 studies including 6107 patients reported an overall morbidity rate associated with ileostomy closure of 17.3% and a mortality rate of 0.4%[78]. Although the defunctioning loop ileostomy is meant to be temporary until

the anastomotic integrity is achieved, not all patients receive stoma reversal, and the incidence of permanent ileostomy has been reported to be as high as 21.6% following low anterior resection of the rectum[78, 83].

Risk factors for intestinal anastomotic leakage

The rate of colorectal anastomotic leakage has remained unchanged for the last few decades despite improvements in surgical techniques and advances in anastomotic devices. The etiology of anastomotic leakage is most likely multifactorial and the pathological process behind the leakage is poorly understood. The potential risk factors for anastomotic leakage are believed to interfere with the anastomotic healing process by prolonging the duration of the acute inflammatory phase and thereby extending the period of collagen degradation in the intestinal submucosa. This affects the suture- and staple anchoring capabilities of the anastomotic tissue with the risk of subsequent anastomotic leakage. Numerous studies have identified various risk factors for developing anastomotic leakage. The integrity of any intestinal anastomosis results from a complex interaction between the surgical procedure, the patient characteristics, and the nature of the underlying disease.

In a simplified way, these risk factors can be divided into three groups[84]:

- Risk factors related to the surgeon
- Risk factors related to the patient
- Risk factors related to the disease

The technical aspect of an intestinal anastomosis should not be neglected. Surgical residents are taught early during their training the importance of using good surgical techniques and practice. The basic surgical principle behind a successful intestinal anastomosis is connecting two healthy bowel ends together. The anastomosis should have adequate blood circulation and without distal obstruction or tension in the bowel wall or its mesentery, as technical failure due to disruption of the anastomotic blood supply or tension at the anastomotic site are well known causes of intestinal anastomotic leakage[84, 85]. In addition, the **surgeon** must aim for a minimum blood loss and short operating time, as these are known risk factors for anastomotic leakage[84-90], although they may be markers for demanding surgical procedures or poor surgical techniques. Furthermore, the surgeon has to minimize the number of staple cartridges used during minimally invasive rectal surgery, as the use of three or more staple cartridges during rectal transection increases the risk for anastomotic leakage[85-87, 90]**.** The surgeon has to place sutures and staples correctly in the anastomotic tissue, as anastomotic sutures or staples without submucosal inclusion (seromuscular) are unreliable. A study on intestinal tensile strength properties of 471 human cadaveric and 98 surgically removed bowel specimens demonstrated

that the transverse mechanical strength of an intact intestinal wall was in 70-75% provided by the submucosa and 15-20% by the muscularis propria layer, whereas the serosa and mucosa did not contribute to significant strength[91]. When comparing the axial tensile strength, both the intact intestinal wall and the sutured anastomosis demonstrated that only the submucosa layer supplied mechanical strength. A prospective multicenter study of 1466 patients undergoing resection of colonic adenocarcinoma with intestinal anastomosis, performed by 84 surgeons in 23 hospitals, demonstrated an overall clinical anastomotic leakage rate of 13%, where the incidence varied vastly between the surgeons from 0.5-30%[92]. In this study, surgeons with fewer than 20 anastomoses were excluded from the analysis.

The known risk factors for anastomotic leakage related to the **patient** are male gender (particularly in low anterior resection), increasing age, smoking and excessive alcohol consumption[84, 90, 93-100]. Furthermore, high body mass index (BMI), malnutrition, and certain patient comorbidities such as diabetes, chronic obstructive lung disease, and renal failure, are all known risk factors for anastomotic leakage[84, 93-96, 101, 102]. In addition, an American Society of Anesthesiologists (ASA) score ≥ 3, which in turn reflects the patient's comorbidity and current physical condition, is a known risk factor for anastomotic leakage[84, 90, 93, 94, 103].

The first evidence linking microbiota involvement as a potential risk factor for intestinal anastomotic leakage was published in 1955[104]. The classical bacterial pathogens implicated in an anastomotic leakage after colorectal surgery belong to the collagenase-producing species of Pseudomonas aeruginosa, Enterococcus faecalis, Escherichia coli, Proteus mirabilis, and Klebsiella pneumonia[105-107]. These opportunistic bacterial pathogens can sense changes in their environment, such as chemotactic signal molecules released locally in a healing anastomotic wound, as a mechanism for nutrient acquisition and survival. These bacterial pathogens are then capable of colonizing the anastomotic tissue in response to these favorable local environmental changes by enhancing their virulence factor by expressing collagenolytic phenotypes[106, 107]. Enterococcus faecalis can colonize anastomotic tissue by producing adhesion proteins that bind to fibrinogen and collagen which are abundantly exposed in the anastomotic wound, and then express its collagenase-producing phenotype and degrade anastomotic subtype I collagen[106]. Enterococcus faecalis virulence factor can also activate the tissue matrix metalloprotease from its inactive proform, causing over-expression of collagenase function, which increases the proteolytic activity locally in the anastomotic tissue even further[106]. This bacterial virulence expression of collagenase is not always detected in the luminal contents of the bowel during bacterial fecal cultures and is often found only in anastomotic tissue samples, which strongly suggests that the microbiome found in the anastomotic wound is more likely to interfere with the anastomotic healing, rather than other types of bacteria that are detected in the luminal content alone[106, 107].

The intestinal mucus system is important for the luminal protection of the epithelium (enterocytes) and assists in the regulation of microbiome homeostasis. The mucus

is mainly built up of highly glycosylated proteins called mucins, where mucin 2 (Muc2) is particularly prominent in the gastrointestinal tract[30, 108, 109]. The colon has a two-layered mucus system, where the inner layer normally remains impenetrable to bacteria and the outer layer is the habitat for commensal bacteria[108]. It has been demonstrated in an experimental model that Muc2 knockout mice are more prone to develop colorectal anastomotic leakage than control mice, indicating that the mucus layer plays a role in the anastomotic healing process in mice[30, 109]. Hence, the pathogenesis of an anastomotic leakage may include both microbial and host elements in the gastrointestinal tract interacting during the healing process.

The current **disease** itself may act as a risk factor for intestinal anastomotic leakage. Advanced gastrointestinal tumor stage and neoadjuvant chemoradiotherapy have been reported as risk factors for leakage, as is the tumor size and its location, with a higher risk for anastomotic leakage in the lower gastrointestinal tract[84-88, 93, 94, 96-98, 110, 111]. In addition, inflammatory bowel diseases, such as ulcerative colitis and Crohn's disease, are known risk factors for anastomotic leakage, as is the use of cortisone or other immunosuppressive drugs[50, 84, 93, 94, 101, 112-114]. Furthermore, emergency conditions requiring surgery and bowel resection can affect the anastomotic healing process, such as pre-existing mechanical bowel obstruction or intestinal perforation with peritonitis, which often lead to deteriorated physical status of the patient with ongoing catabolism. These emergency conditions can lead to systemic inflammatory response syndrome (SIRS), which is an exaggerated defense response mechanism in the body, possibly igniting and escalating the acute inflammatory phase of the anastomotic healing process and risking anastomotic leakage. It has been demonstrated in experimental models that acute colonic obstruction decreases collagen levels rapidly in the submucosa[115], which correlates with the local MMPs activity in the bowel wall[116] and increases with the degree of colonic dilation[117]. During a histological examination, this colonic distension was shown to cause pronounced edema and inflammation in the colonic wall proximal to the obstruction[115], where neutrophils and macrophages were probably the major cellular sources of MMP production[118]. This marked reduction in collagen concentration during this experimental acute colonic distention, was restored to normal collagen concentration 10 days after decompression of the colonic obstruction, which may have a clinical implication for the timing of a surgical procedure[117]. It has also been demonstrated that MMPs activity is higher in coexisting bacterial peritonitis, generating additional deterioration of anastomotic strength under such conditions[119].

Another potential risk factor for anastomotic leakage is related to a foreign body reaction in the anastomotic line. Sutures and staplers can cause pathological inflammation in the anastomosis, endangering the anastomotic healing process[120, 121]. This foreign body granulomatous reaction in the anastomotic tissue can prolong the acute inflammatory phase of the anastomotic healing process and cause excessive breakdown of collagen in the intestinal submucosa. Furthermore, the

tensile forces created in a moving sutured- or stapled anastomosis are not evenly distributed, as sutures and staplers cause a local mechanical strain in their vicinity and it has been reported that excessive collagenase activity is mainly seen around these sutures[118]. A microscopic study has demonstrated the presence of foreign body reaction in stapled human gastrointestinal anastomoses, where the source of the foreign materials found in the anastomotic tissue and eliciting this pathological reaction, came from the stapler cartridges. These foreign bodies were rich in fluorine, carbon, oxygen, calcium, sodium, potassium, magnesium, aluminum, and silicon, where even fragments from the cartridges could be found on the surfaces of the staples[121]. This pathological foreign body reaction is also associated with the risk of a prolonged release of profibrotic chemokines at the anastomotic site that may cause excessive deposition of collagen in the anastomotic scar tissue during the maturation phase of the anastomotic healing process, resulting in an anastomotic stricture formation[17, 122-124], similar to the process of a hypertrophic keloid scar tissue formation in the skin[15].

Risk factors for intestinal anastomotic leakage can also be divided into modifiable or nonmodifiable risk factors. An awareness of nonmodifiable risk factors (*e.g.* male gender, tumor location, and neoadjuvant treatment) may influence intraoperative decision-making, such as the formation of an end colostomy instead of colorectal anastomosis. An example of this dilemma is found during low anterior resection in an obese male patient with a narrow pelvis, with significant comorbidities who has undergone neoadjuvant radiation therapy. On the other hand, modifiable risk factors offer an opportunity to intervene before the surgery to improve clinical outcomes, such as encouraging patients to stop smoking and using alcohol and treating preoperative anemia and malnutrition.

Current intestinal anastomotic methods

There are two commonly used techniques for creating an intestinal anastomosis, the handsewn sutured method and the mechanical stapled method, where the circular staple technique dominates in rectal surgery. A Cochrane review published in 2012 comparing the safety and effectiveness of stapled versus handsewn colorectal anastomosis, analyzed the outcome of 1233 patients from nine randomized controlled trials who underwent colorectal resection, of which 611 received a handsewn anastomosis and 622 received a stapled anastomosis[125]. This meta-analysis did not demonstrate the superiority of either technique, regardless of the level of the anastomosis. However, the meta-analysis showed the stapled technique to have a higher frequency of anastomotic stricture formation compared to the hand-sewn method (8% vs 2%). The overall anastomotic leakage rate in both techniques was 13% (risk difference 0.2%, 95% CI -5.0% to 5.3%). Another meta-analysis of thirteen randomized controlled trials assessing handsewn and stapled colonic and

rectal anastomoses in 4917 patients showed no difference in effectiveness or complication rates between the techniques, and it was argued that the surgeon's choice of method might be based on personal preference[126].

The choice of the anastomotic suture material has also been reviewed. The ideal suture material should cause minimal inflammation and tissue reaction while providing sufficient strength during the acute inflammatory phase of the anastomotic healing process. Although monofilament sutures are believed to elicit less inflammatory response than braided sutures and are used more frequently in practice, there is currently no evidence that one is better than the other[20, 84]. No randomized trials have addressed the issue of comparing interrupted sutures to continuous sutures. However, retrospective reviews have not demonstrated the superiority of either technique[20, 123].

A meta-analysis of six randomized controlled trials published in 2006, analyzing 670 patients with colorectal anastomosis, demonstrated no evidence that double-layer anastomoses resulted in lower anastomotic leakage rates compared to single-layer anastomoses[127]. A single-center prospective randomized controlled study of 97 patients undergoing intestinal resection and anastomosis compared 50 patients randomized for single-layered extra-mucosal continuous anastomosis to 47 patients randomized for double-layered anastomosis[128]. The study demonstrated that the methods were equally safe, but the single-layered continuous anastomosis was less time-consuming compared to the double-layer method. A Cochrane review published in 2012 that included seven randomized controlled trials with 840 patients undergoing gastrointestinal resection with anastomosis compared 408 patients with single-layer intestinal anastomosis to 432 patients with double-layer intestinal anastomosis[129]. The study assessed both methods to be equally safe and did not demonstrate the superiority of either technique. However, the single-layer technique resulted in shorter operative time.

In a cohort study designed to determine the value of intraoperative anastomotic air leak testing of left-sided colorectal anastomoses and the development of anastomotic leakage, a total of 998 left-sided colorectal anastomoses were analyzed, 90% stapled and 10% handsewn, all without diverting stoma[130]. Intraoperative air leaks were noted in 7.9% of the tested anastomoses (65/825), in 7.8% of the stapled anastomoses, and in 9.5% of the handsewn anastomoses. A clinical leak developed in 4.8% of the patients and was noted in 7.7% of anastomoses with positive air leak tests compared to 3.8% of anastomoses with negative air leak tests and 8.1% of all untested anastomoses (14/173). In addition, the study demonstrated that if the anastomoses with positive air leak tests were managed with suture repair alone, they were associated with 12.2% clinical anastomotic leakage compared to no event of clinical leakage after re-anastomosis or stoma diversion.

Although handsewn and stapled anastomoses appear to be similar methods in terms of clinical safety and efficiency, numerous patients are affected by disturbed

anastomotic healing leading to anastomotic leakage or stenosis formation. Apart from inert substances, most foreign materials will induce an inflammatory reaction in tissues. Surgical sutures and staples are no exception. Both techniques leave foreign bodies in the anastomotic line that can interfere with the healing process of the newly formed anastomosis. In addition, the concentrated forces created locally around the sutures, and presumably around the anastomotic staples as well, trigger additional collagenase activation with further collagen degradation (Figure 8).

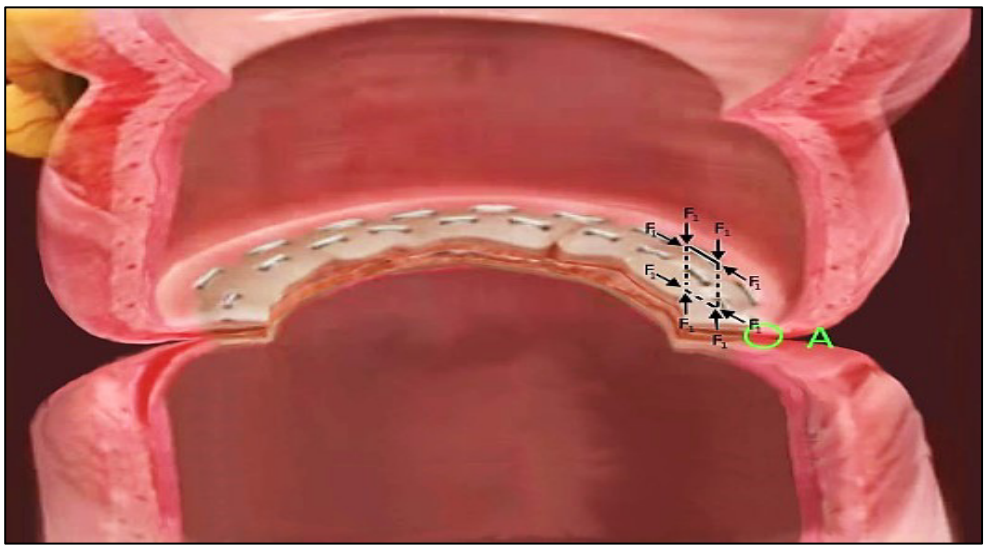

Figure 8: Stapled anastomosis. The staplers create a parallelogram of forces (F1) during bowel wall movements. The green circle marks the healing area. Copyright by CarpoNovum, reproduced with permission.

These limitations concerning the current anastomotic methods have generated an interest in alternative suture- and staple-free anastomotic techniques, such as compression anastomosis, which leaves no foreign material in the anastomosis. The technology behind intestinal compression anastomosis is not new and dates back to the 19th century.

Intestinal compression anastomosis

In 1826, the French surgeon Felix-Nicholas Denans (1768-1832) introduced the concept of intestinal compression anastomosis at the meeting of the Société Royale de Medicine de Marseilles. He had previously performed a sutureless end-to-end, ileo-ileal anastomosis in a dog using a metallic ring[21, 131-133]. His instrument relied on two opposing silver rings connected by a snap function that captured and compressed the intestinal ends of the transected intestine. The resulting compression

induced a healing area between the intestinal ends and an adjacent ischemic collar away from the healing process, which together with the silver rings, detached from the anastomosis and was expulsed by the natural route. After Denans device introduction, other surgeons started developing novel compression anastomotic instruments that were essentially minor refinements of Denans original rings.

In 1892, the American surgeon John Benjamin Murphy (1857-1916), known for his early surgical intervention for appendicitis and description of the clinical signs of acute cholecystitis, introduced a compression anastomotic ring called Murphy's button (Figure 9), which was simpler than Denans rings despite being based on similar principles[21, 131-133].

Figure 9: Murphy´s button. Copyright by Baishideng Publishing Group Inc, reproduced with permission.

Murphy's button was widely used for a few decades, but its clinical success was limited and bowel obstruction and anastomotic stenosis were relatively common complications, presumably due to the instrument's narrow inner diameter.

The technique of intestinal compression anastomosis was then forgotten for almost a century until Kanshin and colleagues in Russia designed a new compression anastomotic device in 1984 called AKA-2 (Figure 10), which was made for transanal colorectal anastomosis during low anterior resection of the rectum[21, 131-133]. The AKA-2 was constructed of one plastic and one metal ring with metallic springs and contained a circular blade that cut an opening between the intestinal

ends in the anastomosis, similar to the conventional transanal stapler instrument used today. The device was made in 3 different sizes, 25, 28, and 31 mm.

Figure 10: The AKA-2 rings. Copyright by Elsevier, reproduced with permission.

In 1985, Hardy and colleagues introduced a new compression anastomotic device called Valtrac BAR (Biofragmentable Anastomotic Ring)[21, 131-133]. The BAR was constructed from two identical rings made of 87.5% absorbable polyglycolic acid and 12.5% barium sulfate, making the rings radiopaque (Figure 11).

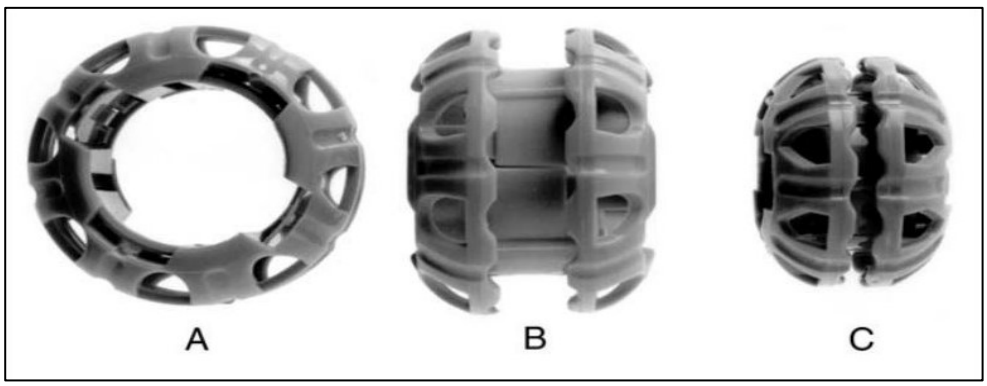

Figure 11: The BAR rings. (A) Front view. (B) Rings open. (C) Rings closed. Copyright by Elsevier, reproduced with permission.

Contrary to other compression devices, the BAR was designed to have an adjustable space between the rings after they were locked in position with the captured and compressed bowel ends. This space could be set to 1.5-2.5 mm in width, avoiding too much tissue compression with the risk of premature tissue necrosis and early detachment of the rings as a result. BAR was produced in four different sizes, 25, 28, 31, and 34 mm, and the inner diameter variated in size from 11-20 mm, depending on the ring sizes used. Approximately 2-3 weeks after the formation of

the BAR anastomosis, the fragmented pieces of the anastomotic rings passed out with the fecal stream.

Nitinol is a metal mixture of nickel and titanium which has a temperature-dependent shape memory. This metal mixture is formed into the desired shape during high temperatures, and when the mixture is cooled down to 0°C, it loses its rigidity and becomes more flexible. At room temperature, the metal mixture resumes its original form. Nitinol has been used in two compression anastomotic devices, the NiTi CAC (Compression Anastomotic Clip) and the NiTi CAR (Compression Anastomotic Ring) (Figure 12)[21, 131-133].

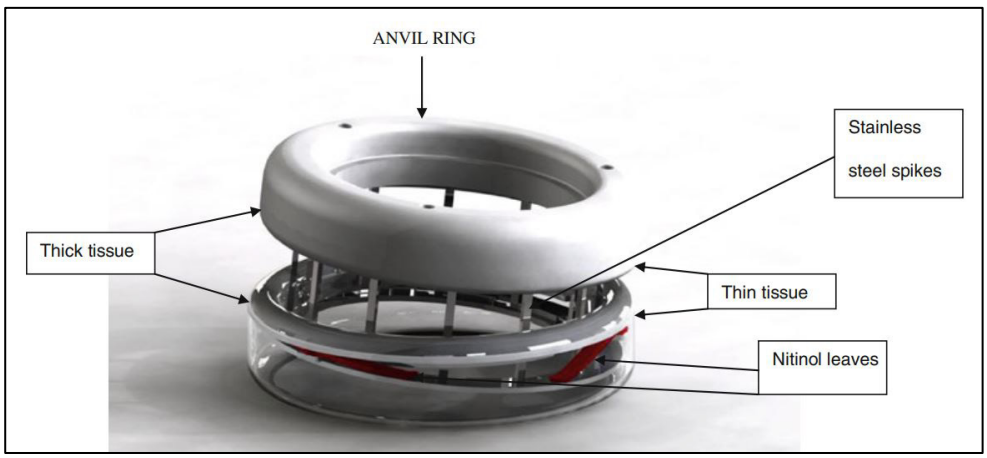

Figure 12: The NiTi CAR. Copyright by Springer, reproduced with permission.

There are some published randomized prospective studies and several prospective follow-up studies that have demonstrated the safety and efficiency of compression anastomotic devices in performing colorectal anastomoses, and they seem to be safe during both elective and emergency surgery procedures[21, 131-150]. The reported anastomotic leakage rates in these studies range between 0.5-15% when using the BAR, AKA-2, and NiTi devices, and the reported anastomotic stricture formation ranges between 0.2-10 %[21, 131]. In the larger studies containing 101-1180 patients, the reported anastomotic leakage rates are much lower, ranging from 0.5-6.7 percent, with an anastomotic stricture formation between 0.2-3.3 percent.

A systematic review and meta-analysis of ten randomized controlled trials including 1969 patients comparing 992 BAR compression anastomoses to 977 conventional anastomoses (752 sutured and 225 stapled), demonstrated no significant difference in anastomotic leakage rate (OR 0.80; 95% CI 0.47-1.37, p=0.42) or stricture formation (OR 0.54; 95% CI 0.18-1.64, p=0.28)[151]. However, this meta-analysis showed an increased risk of postoperative bowel obstruction in the BAR group (OR 1.87; 95% CI 1.07-3.26, p=0.03).

An experimental study in a porcine model of 31 pigs compared the bursting pressure values in BAR anastomoses to the traditional sutured and stapled anastomoses. The bursting pressure was measured directly after the formation of the anastomoses (time zero) and again 7 days after the procedure. The study showed significantly higher bursting pressure in the BAR anastomoses at time zero compared to the sutured and stapled anastomoses, whereas there was no significant difference at day 7 (unpublished data)[21, 133].

An experimental study using the NiTi CAR device in a porcine model of 9 pigs measured a mean bursting pressure value of 330 mBar at time zero, with a range of 133-400 mBar[21]. In another experimental animal study comparing bursting pressure in NiTi CAR and stapled anastomoses in 18 pigs, there was a significantly higher mean bursting pressure in the NiTi CAR anastomoses in comparison to the double stapled anastomoses at time zero, 137 mBar versus 31 mBar respectively[21, 150]. During the bursting pressure test, four out of nine NiTi CAR failed at the anastomotic line, whereas all nine stapled anastomoses failed at the staple line.

An experimental study in a canine model using a circular compression anastomotic device designed at the University of Milan, consisting of three polypropylene rings, compared bursting pressure in 24 compression anastomoses to 19 stapled anastomoses in the colon[149]. At time zero, the bursting pressure in the compression anastomoses ranged between 320-660 mBar and in the stapled anastomoses between 133-200 mBar. The bursting pressure decreased gradually the following days and by the third postoperative day, the bursting pressure in the compression anastomoses ranged between 88-167 mBar, whereas the bursting pressure of non-operated colon parts used as controls ranged between 368-424 mBar during the same period. By postoperative day 7, the bursting pressure of the compression anastomoses had increased to 373-535 mBar and in the stapled anastomoses up to 464 mBar. The compression anastomoses in the study ruptured at the anastomotic line during postoperative days 2-5 and outside the anastomotic line at time zero and postoperative day 7, whereas the stapled anastomoses failed at the staple line at postoperative days 0-5 and outside the staple line at day 7.

A blinded comparative experimental study in a porcine model compared the histopathological appearances of NiTi CAR compression anastomoses to circular stapled anastomose[122]. The anastomoses were constructed 20 cm from the anal verge in 50 pigs and the microscopic histological architecture of the healing process was assessed at postoperative days 3, 7, 30, and 90. The study demonstrated that the healing process of the compression anastomoses progressed faster and with less granulation formation compared to the circular stapled anastomoses. The compression anastomoses showed lesser scarring with narrower anastomotic lines, significantly lesser foreign body response, and lower inflammatory cell counts. In addition, the compression anastomoses revealed a more accurate alignment of the layers in the intestinal wall with adequate mucosal re-epithelialization of the anastomotic line with minimal inflammatory reaction.

In summary, there are no existing compression anastomosis devices that have been proven to yield superior results compared to the standard anastomotic methods and experimental studies show somewhat heterogeneous results. Hence, no such device has become clinically established although the anastomotic compression method is theoretically appealing. There is thus a need for further development.

An ideal intestinal anastomosis

An ideal intestinal anastomosis should be formed with good surgical technique, using healthy intestinal ends with good tissue perfusion of both bowel limbs, free of tension and without distal obstruction. This ideal anastomotic technique should provide the intestinal anastomosis with enough mechanical support during the acute inflammatory phase of the healing process to avoid anastomotic leakage and at the same time reduce the risk of excessive tissue fibrosis in the maturation phase resulting in the formation of anastomotic stricture. It should also allow quantitative measurement of the anastomotic strength, thereby giving the surgeon direct intraoperative feedback on the quality and strenght of the anastomosis and its integrity.

Staples and sutures remain in the anastomotic site long after their requirements for tissue support are over, creating locally concentrated forces in the anastomotic line and foreign body reaction, possibly eliciting an additional collagenase activity or prolonged profibrotic chemokines production.

A sutureless compression anastomosis generates an evenly distributed pressure over the anastomotic line and induces a healing area between the intestinal ends and a collar of necrotic tissue not far from the healing process. This close vicinity between these two separate tissue processes might be a weak point in the compression anastomotic technique, as the resulting necrotic tissue could theoretically reach the ongoing healing area of the anastomosis and disturb the healing process.

The previously described compression anastomotic devices might not separate the ischemic collar enough from the healing area of the anastomotic line, which could explain the results of the compression anastomotic studies. Moreover, Murphy's button and NiTi devices were not designed to allow an adjustable width between the anastomotic rings when locked in position. As a result, these devices compress the bowel wall with high pressure, risking premature tissue necrosis and early detachment of the rings before the healing process is completed (Figure 13).

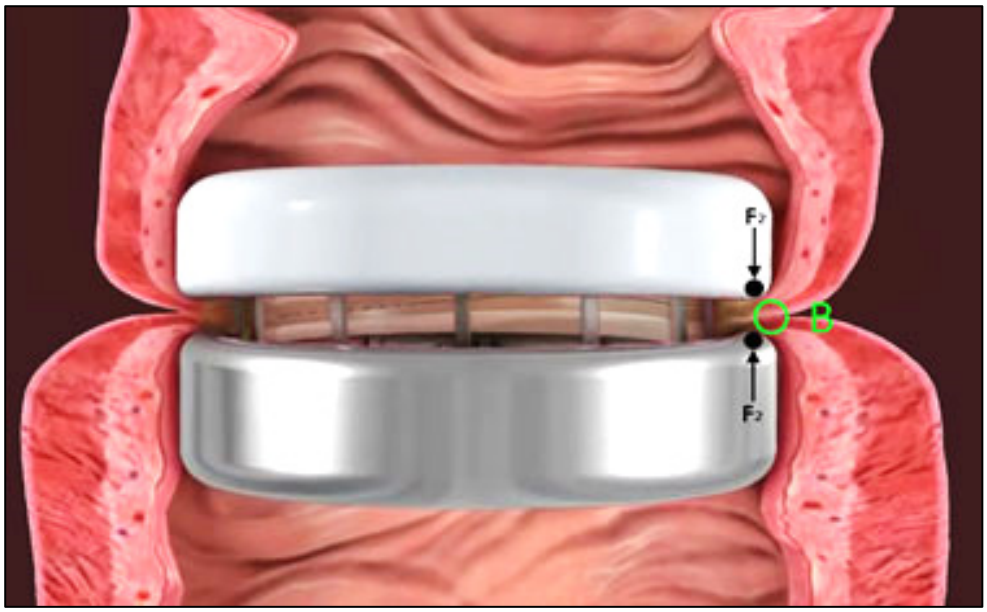

Figure 13: The NiTi CAR anastomosis. The close proximity of the maximum bowel wall pressure (black circles) and the healing area (green circle). Copyright by CarpoNovum, reproduced with permission.

The intestinal compression devices have been reported in numerous studies as feasible and safe and at least comparable to the standard suturing- and stapling techniques in terms of adverse events, although none of them have been established as a routine procedure in colorectal surgery today. These compression instruments have been cumbersome to use, with increased risk for postoperative bowel obstruction and have mostly been abandoned.

There are presently no anastomotic techniques with the capacity to assess the quality of an anastomosis immediately after its formation. Surgeons are left to evaluate colorectal anastomoses by morphological inspection and with the help of air leak test[130]. In addition, there are no anastomotic techniques that allow postoperative monitoring of the anastomotic integrity.

CARP compression anastomosis

A new sutureless anastomotic compression instrument has been developed during the last decades and the surgical procedure associated with the use of this device is called compression anastomotic ring-locking procedure (CARP).

CARP´s unique catheter system allows an intraoperative measurement of the anastomotic contact pressure (ACP) during surgery, thereby giving the surgeon feedback on the strength of the anastomosis immediately after its formation. This

catheter-based system has a direct access to the healing area within the anastomosis, allowing sampling of fluids from the anastomosis to investigate the dynamic aspects of the anastomotic healing process. It could also be used for modulating the healing process by local administration of specific compounds through the catheters at the anastomotic site. This direct access can theoretically allow gentle intermittent suction through the catheters that would help to maintain a fixed compression pressure value of the anastomotic tissue in the first postoperative days during the acute inflammatory phase.

CARP´s anastomotic ring construction has a more ovular shape compared to the previous compression devices, intending to separate the compression ischemic collar more efficiently from the anastomotic healing area. This design creates a gradual pressure gradient reduction from the highest compression to lower when approaching the location of the healing area. This ovular shape of the CARP rings creates larger tissue surface area for the healing process to take place (Figure 14).

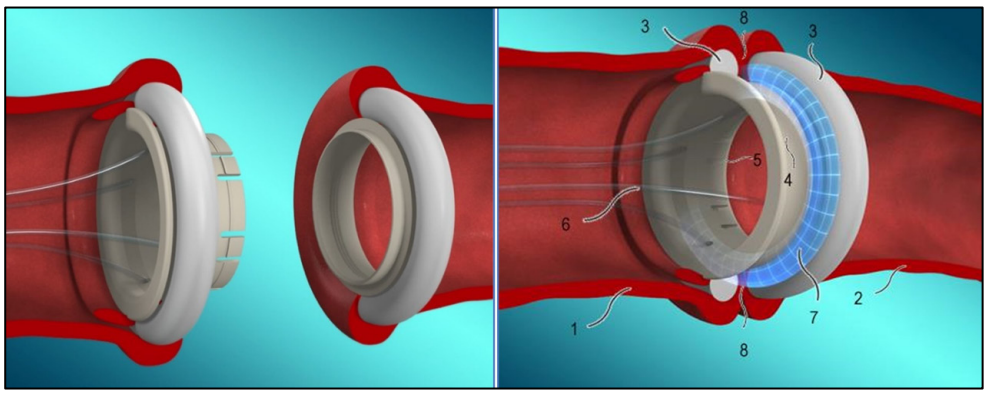

Figure 14: The ovular shape of the CARP anastomosis. The blue shaded area (7) illustrates the closed circular space in the anastomosis which is in connection with the catheters of the male ring (6). Distal part of the intestine (1). Proximal part of the intestine (2). The O-rings (3). The male anastomotic ring (4). The female anastomotic ring (5). Closed anastomotic space (8). Copyright by CarpoNovum, reproduced with permission.

The CARP device is composed of a male and a female plastic anastomotic ring and two elastic silicon rings (O-rings) that anchor and compress the wall of the intestinal ends to the anastomotic rings and make the rings more ovular in shape. The CARP rings were designed to have an adjustable width between the rings after they are locked in position with the captured bowel wall, to be able to adjust the desired anastomotic contact pressure. The male and female parts are then connected with a simple mechanical click function. The male anastomotic ring contains four built-in catheters designed to be placed through the distal end of the bowel, so that the catheters protrude through the anus.

After the CARP anastomosis has been formed, it conceals a ‘closed space’ located between the anastomotic rings and the healing site of the anastomotic line (Figure

14). This space is connected to the four catheters in the male ring, making it possible to perform measurements of the anastomotic contact pressure values between the intestinal ends in the anastomosis and to perform postoperative radiological examination of the anastomotic integrity.

The CARP device comes in three different sizes (29, 32 and 35 mm) and utilizes helping tools to aid the placement of the anastomotic rings into the intestinal ends which are to be anastomosed. The male assisting tool is equipped with a guide that assists the passage of the catheters through the intestine and out through the anus, minimizing the risk of bowel injuries.

C-REX compression anastomosis

The CARP device is limited to open abdominal surgery and for the creation of an intraperitoneal colorectal anastomosis. This is because the device is dependent on a manual click function when connecting the male and female parts of the device.

The CARP device has subsequently been modified and improved to be used during minimally invasive surgery and for the construction of anastomosis during rectal surgery through the transanal approach. This new device is called C-REX (Colorectal anastomosis; Re-join the intestine and validate the anastomosis; Extract samples for analysis; X-ray through connected catheters).

The C-REX method includes two anastomotic devices, one device adopted for intraabdominal anastomoses which is called LapAid, and one device adopted for transanal anastomoses which is called RectoAid (Figure 15). The RectoAid instrument is similar in appearance to the traditional circular staple instrument used to day with its curved design.

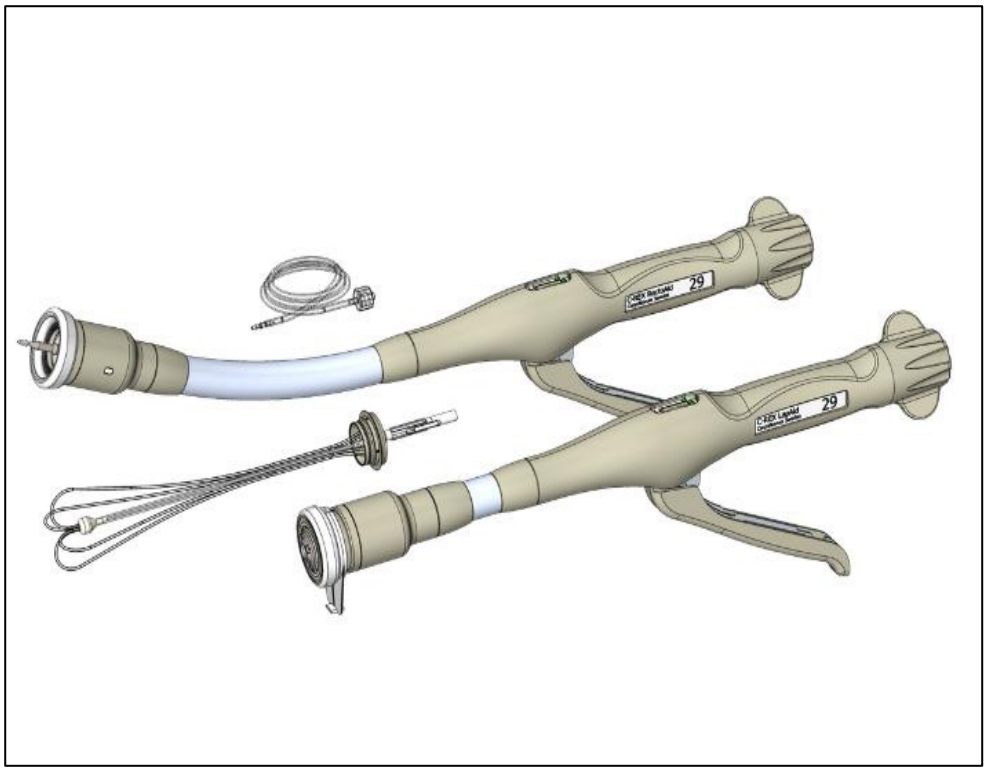

Figure 15: The LapAid and RectoAid devices of the C-REX method and their associated catheters. The RectoAid instrument is similar in appearance as the circular staple instrument with its curved design. Copyright by CarpoNovum, reproduced with permission.

The C-REX device is a refined CARP device which also contains the four built-in catheters in connection to the anastomotic space, running from the anastomotic line via the distal bowel segment and protruding through the anus (Figure 16). As with the CARP method, this C-REX catheter-based system enables intraoperative measurements of the ACP and postoperative radiological examination of the anastomotic integrity.

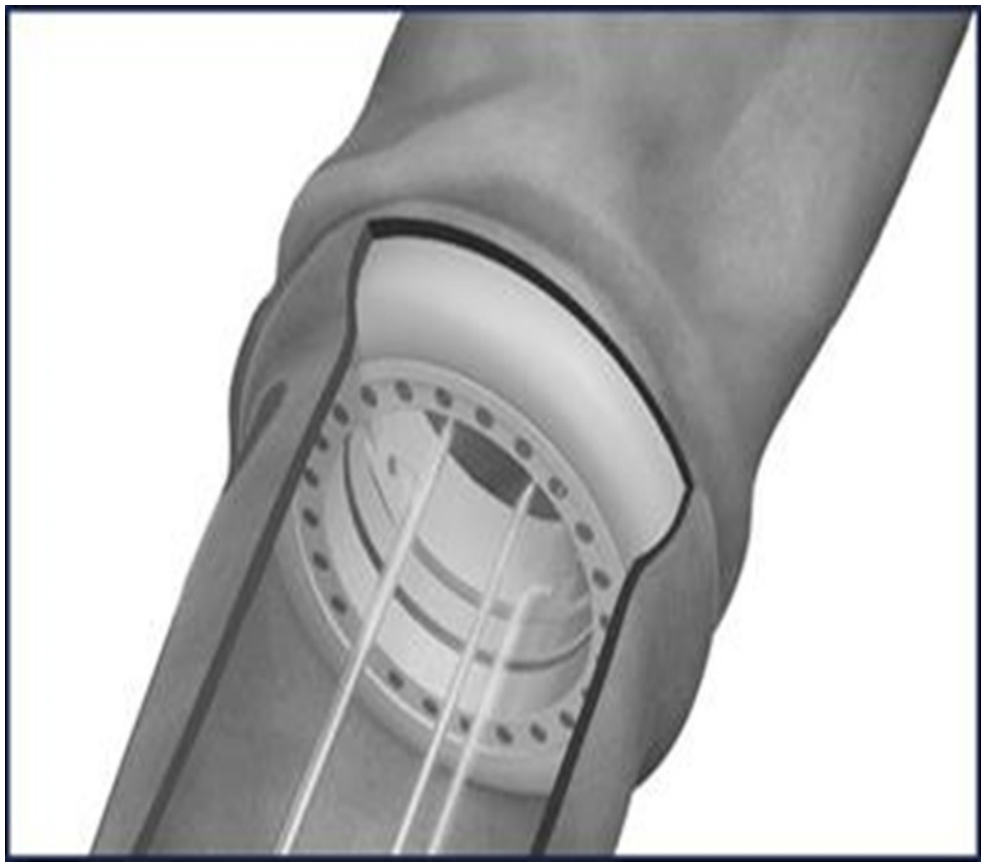

Figure 16: A C-REX end-to-end anastomosis with its built-in catheters in connection to the anastomotic space, running from the anastomotic line via the distal bowel segment and protruding through the anus. The intestinal wall is partially cut to allow visualisation of the anastomotic rings in the bowel lumen. Copyright by CarpoNovum, reproduced with permission.

The C-REX method has a specific testing device used for measuring the best suitable size of the C-REX rings (26, 29, and 32 mm) during the anastomotic construction (Figure 17). When measuring the best suitable ring size, the transected intestinal end is grabbed gently with two Babcock instruments on each side, and the testing device is gently intubated into the bowel end until it fits the diameter of the intestinal lumen. The head of the testing device is marked with the available ring sizes of the C-REX rings.

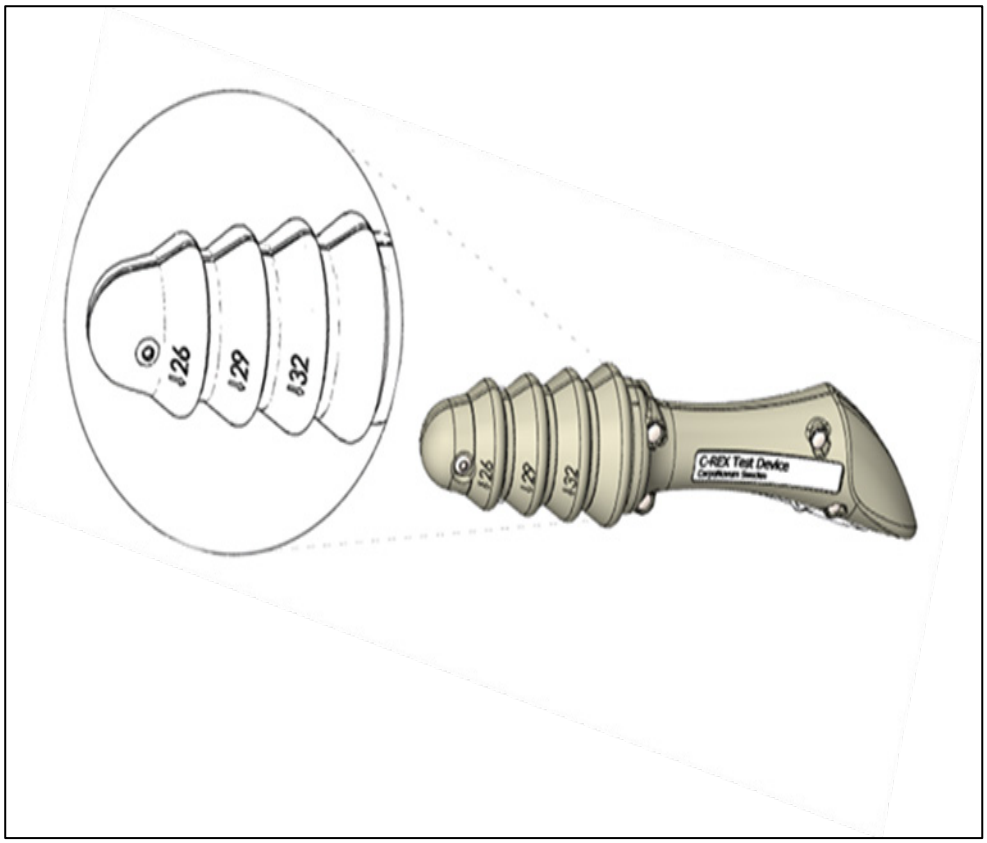

Figure 17: The specific testing device used for measuring the best suitable size of the C-REX rings. The head of the device is marked with the ring sizes of the available sizes of the C-REX rings. Copyright by CarpoNovum, reproduced with permission.

The C-REX anastomotic ring construction has a larger ovular shape than the CARP rings, separating even more efficiently the compression ischemic collar from the healing anastomotic area and creating larger tissue surface area for the healing process to occur (Figures 18 and 19).

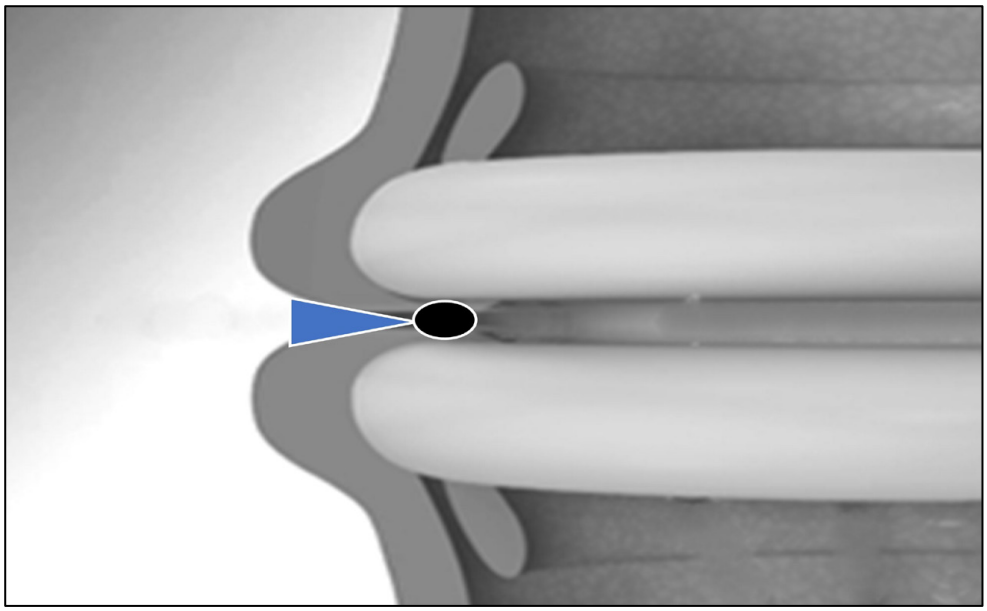

Figure 18: The C-REX anastomosis. The black circle demonstrates the area of maximum bowel wall pressure. The blue triangle demonstrates the healing area with gradually decreasing bowel wall pressure in the lateral direction. Copyright by author.

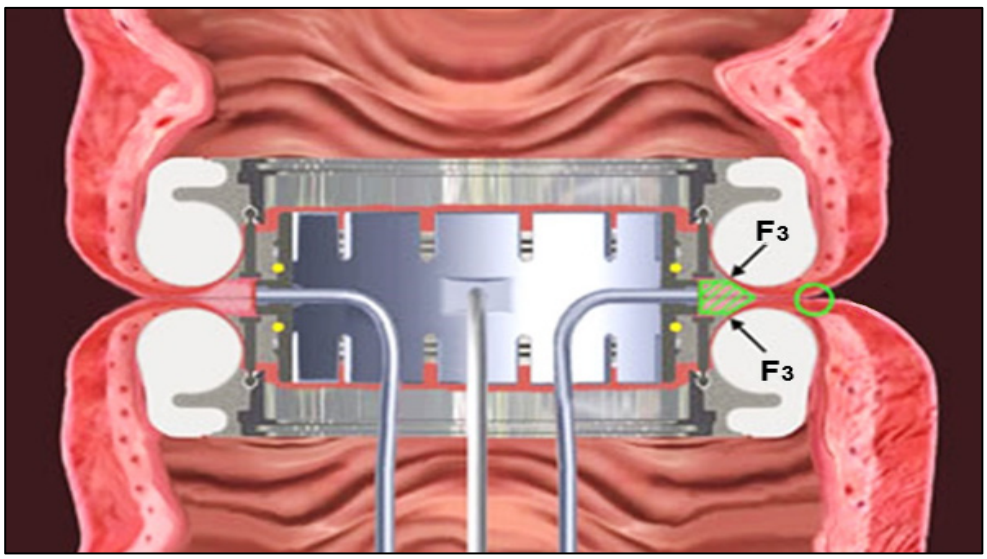

Figure 19: The C-REX anastomosis. The black arrows (F3) demonstrate the area of maximum bowel wall compression. The green circle demonstrates the healing area. Copyright by CarpoNovum, reproduced with permission.

Aims and objectives

The overall objective of this thesis was to evolve and assess the novel compression anastomotic devices CARP and C-REX, and evaluate their safety and efficacy in performing colorectal anastomoses.

Specific aims

Paper I

To assess the safety of the CARP device in performing colonic anastomoses following open resection of the sigmoid colon in an experimental model in pigs. To evaluate the efficacy of the catheter-based system of CARP in measuring the intraoperative ACP and evaluating the postoperative anastomotic integrity.

Paper II

To assess the safety of CARP in performing colonic anastomoses following open left-sided colonic resection in human settings and to evaluate CARP´s efficacy in determining intraoperative ACP and postoperative anastomotic integrity.

Paper III

To assess the safety and efficacy of the C-REX devices in performing rectal anastomoses in the upper third of the rectum following open and minimally invasive high anterior resection in human settings.

Paper IV

To evaluate the early postoperative mechanical strength of colorectal anastomoses in an experimental model in pigs, comparing bursting pressure of C-REX anastomoses and traditional circular stapled anastomoses following resection of the sigmoid colon.

Material and methods

Ethical principles and approvals

Both studies involving animal models presented in this thesis (*papers I and IV*) were conducted according to animal research ethics and welfare, addressing the "3Rs" of replacement, reduction, and refinement. Ethical approval was granted prior to each study from the regional ethical review board in Lund (*paper I*) and from the regional ethical review board in Gothenburg (*paper IV*).

Both clinical studies involving human subjects presented in this thesis (*papers II and III*) were conducted according to the 1975 Helsinki Declaration, addressing ethical principles for human medical research. Ethical approval was granted prior to each study from the regional ethical review board in Lund.

The CARP device

The CARP device is a compression anastomotic instrument that was designed for constructing colorectal anastomosis during open surgery. CARP is composed of male and female anastomotic rings, made of plastic polyether ether ketone. The device also includes two elastic silicon rings (O-rings) which anchor and compress the transected intestinal wall to the anastomotic rings (Figure 20). The male ring is designed to be inserted into the distal part of the transected intestinal end and the female ring into the proximal part. The male ring contains four built-in catheters intended to be placed through the distal bowel end, so the catheters can protrude through the anal verge.

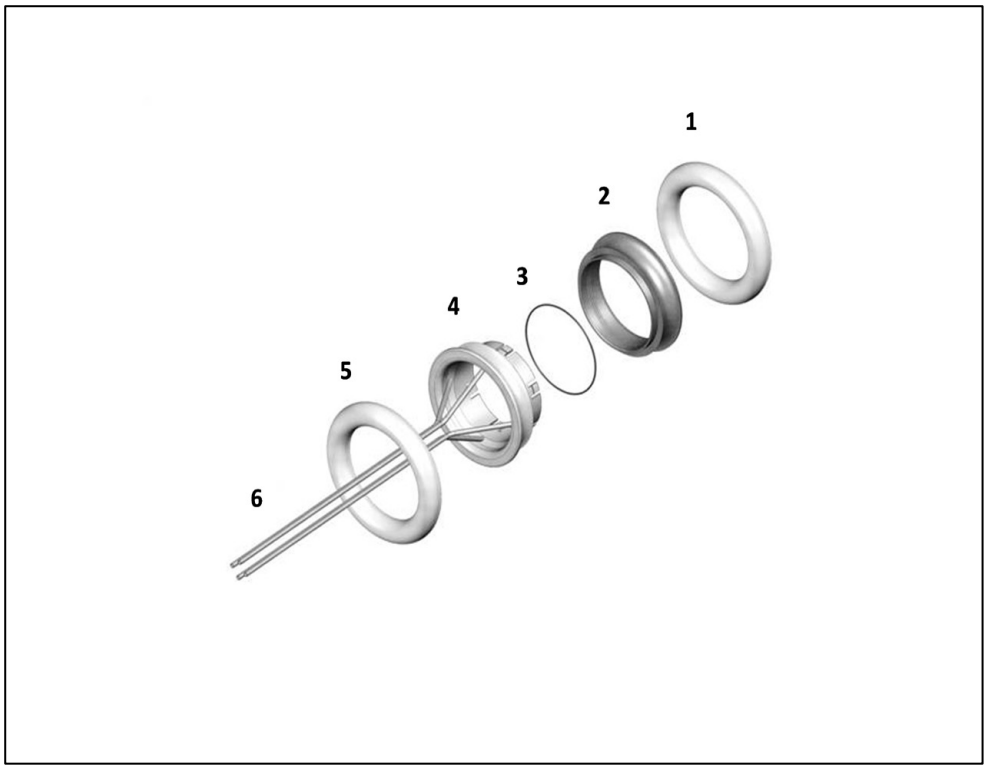

Figure 20: The CARP anastomotic rings. The O-rings (1 and 5), the female ring (2), the sealing ring (3), and the male ring with its four catheters (4). Copyright by Springer, reproduced with permission.

The CARP rings were made in three different sizes (29, 32, and 35 mm) and are provided with a sealing ring positioned between the male and female rings making the connection waterproof. There are specific helping tools for assisting the placement of these rings into the intestinal ends, where the male helping tool is equipped with a guide that assists the intubation of the four catheters through the distal bowel end and out through the anal verge (Figure 21). When both the male and female rings are in place, a coupling segment is connected to the male ring, and the CARP anastomosis is formed by connecting the male and female rings together with a simple mechanical click function. This clicking maneuver requires both hands of the surgeon to perform.

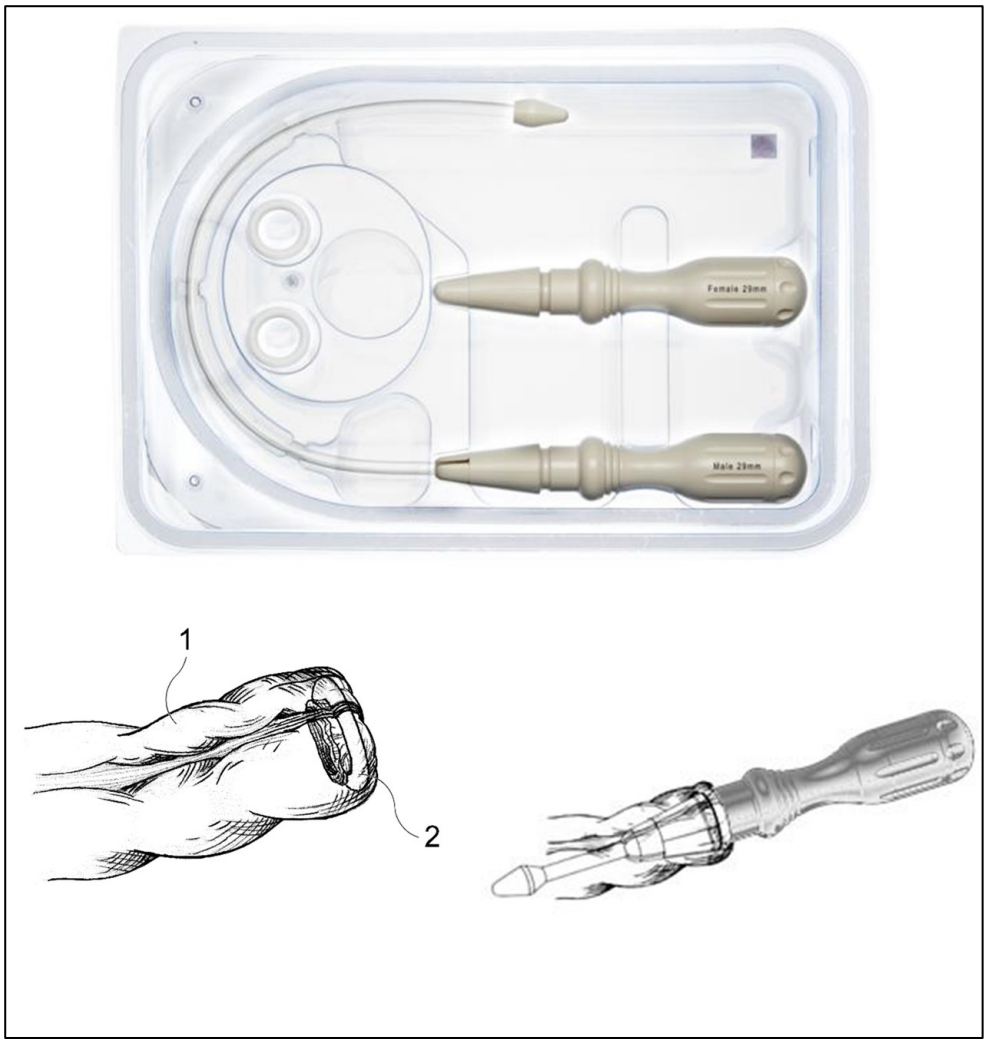

Figure 21: A sterile packaged male and female helping tools with associated O-rings (upper figure). The O-ring (2) is placed into the transected bowel end (1) and the intestinal wall is inverted around the ring (left lower figure). The male assisting tool equipped with its guide, assisting the placement of the male anastomotic CARP ring into the distal bowel end between the O-ring and the intestinal wall (right lower figure). Copyright by CarpoNovum, reproduced with permission.

After the formation of the CARP anastomosis, the anastomotic line conceals a closed space between the anastomotic rings and the healing area of the intestinal anastomosis. This closed space is in direct contact with the catheters of the male ring, enabling measurements of the anastomotic contact pressure through these catheters with the help of a manometer, and can also be used to perform postoperative radiological examination of the anastomotic integrity. The CARP device was designed to have an adjustable width between the male and female rings

after they are locked in position. This construction makes it possible to adjust the desired anastomotic contact pressure between the transected intestinal ends in the anastomosis.

The C-REX device

The C-REX device is a modified version of the CARP device. C-REX is a compression anastomotic instrument adapted to minimally invasive surgery and transanal construction of rectal anastomoses.

There are two specific C-REX instruments designed for placing the anastomotic rings into the transected intestinal ends and are used in pairs when constructing the anastomosis. The C-REX LapAid instrument inserts the anastomotic ring into the proximal bowel end. When performing an intraperitoneal anastomosis, another C-REX LapAid instrument inserts the other ring into the distal end. This is called the C-REX LapAid-LapAid technique (LL), and like the CARP method, the LL anastomosis is formed with a simple mechanical click function that requires both hands of the surgeon to perform. When constructing a rectal anastomosis with the transanal approach, the C-REX LapAid is used to insert the proximal ring and the C-REX RectoAid instrument is used for placing the distal ring and firing the anastomosis. This is called the C-REX LapAid-RectoAid technique (LR).

The C-REX rings were made in three different sizes (26, 29, and 32 mm), and there is a specific test device used intraoperatively for measuring the best suitable size of the rings. The sizes of the C-REX rings are not comparable to the traditional circular staples devices. For example, the 29 mm C-REX device creates an anastomosis with an inner diameter of 20 mm when the anastomotic rings are still in place but an outer diameter of 37 mm. Hence, a C-REX anastomosis with a mean diameter of 29 mm (Figure 22). When the anastomotic rings detach from the anastomosis, it theoretically gains an intestinal lumen corresponding closer to the outer diameter of the anastomotic rings.

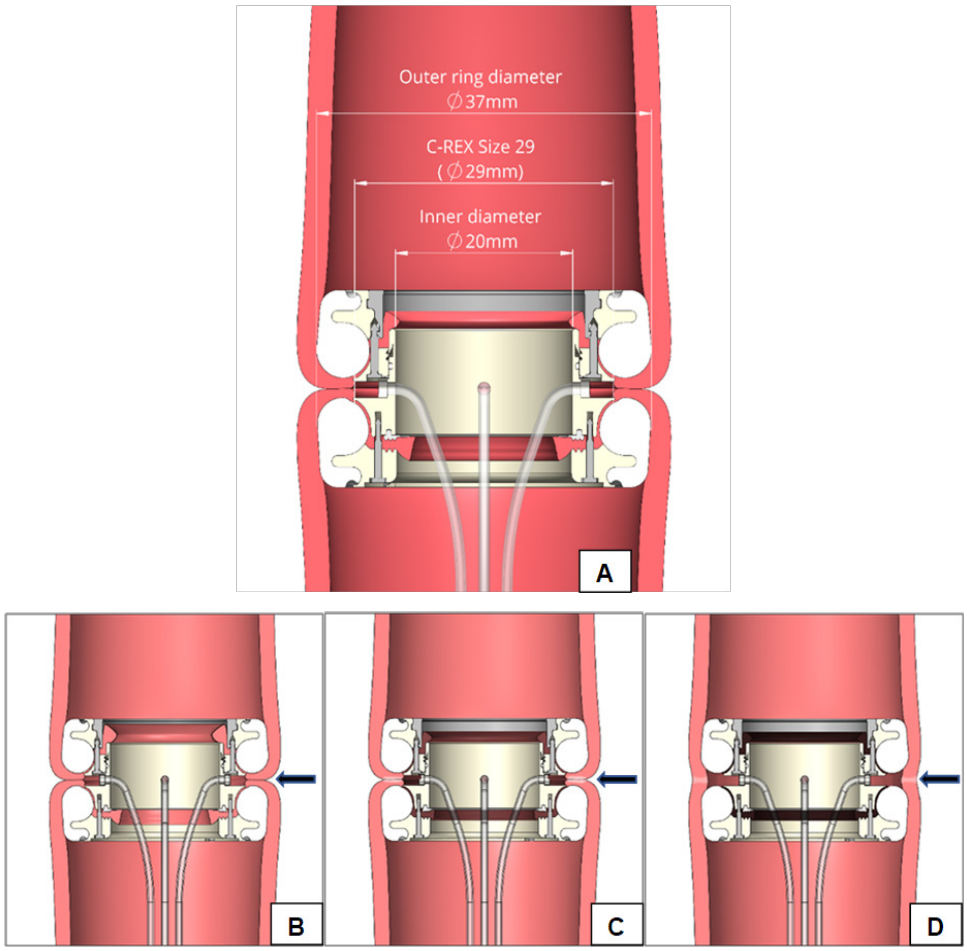

Figure 22: The 29-mm C-REX anastomosis has an inner diameter of 20 mm when the anastomotic rings are in place, but an outer diameter of 37 mm (A). When the anastomotic rings have detached, the anastomosis acquires a lumen corresponding closer to the outer diameter of the anastomotic rings (B-D). The black arrows in figures B-D point at the progressing necrosis of the compressed bowel wall during the healing process, resulting in the ring detachment from the anastomosis which are expelled through the natural route. Copyright by Springer, reproduced with permission.

Study design

Paper I

This was a proof-of-concept study evaluating the safety and efficacy of the CARP device in performing left-sided colonic anastomoses in an experimental model of 31 pigs. The pigs underwent midline incision and resection of the sigmoid colon under anesthesia with an end-to-end colonic anastomosis performed with the CARP device with different widths between the anastomotic rings. After the procedure, the animals were divided into two groups, a group of 19 pigs for analysis of short-term results and a group of 12 pigs for analysis of long-term results. The pigs in the long-term group were returned to the animal farm after the surgical recovery and re-operated 8-15 weeks after the previous procedure.

In the short-term group, CARP´s efficacy in measuring the intraoperative anastomotic contact pressure and the ability to monitor the postoperative anastomotic integrity was assessed. The anastomotic contact pressure was measured by infusing air into one of the four catheters through a system with a manometer while clamping the other three catheters with forceps. When the air pressure in the closed space in the anastomotic line exceeded the contact-induced closure of the anastomotic CARP rings, the pressure abruptly dropped due to air leakage between the rings and was defined as the anastomotic contact pressure.

The anastomotic contact pressure of the CARP anastomosis was evaluated in a subgroup of pigs using different widths between the anastomotic rings (0.5 mm, 1.0 mm, 1.5 mm, 2.0 mm, and 3.0 mm), as well as assessing the changes in the anastomotic contact pressure over time during the first five postoperative days in CARP anastomoses with 1.5 mm width between the rings. The integrity of CARP anastomoses was assessed during the first week after the procedure by injecting water-soluble contrast media through one of the catheters during X-ray fluoroscopy to indicate possible leakage of contrast medium from the closed space of the CARP anastomosis. Furthermore, the histological appearance of the CARP anastomotic healing process with a 1.5 mm width between the rings was evaluated at different time intervals during the first postoperative days with Hematoxylin-eosin and Sirius Red staining.

In the long-term group, besides measuring the intraoperative anastomotic contact pressure immediately after the anastomotic formation, the macroscopic appearance and elasticity of the CARP anastomoses were assessed for the presence of anastomotic stricture 8-15 weeks after the initial procedure. This was done by inflating a closed colon segment containing the CARP anastomosis with a saline solution. In addition, the mechanical strength of the anastomotic repair of the CARP anastomoses was measured with the bursting pressure method. This was done by inflating air with a continuous flow rate of 50 ml/min into the closed colon segment

containing the CARP anastomosis until a sudden drop in the intraluminal pressure with associated air leakage was noted and registered with a manometer.

Statistical methods:

Due to the small sample size of the subgroups of animals used in this study, non-parametric statistical methods and concepts were used, presented with medians, ranges and quartiles.

Paper II

This was a non-randomized prospective clinical safety study on 25 patients (13 men and 12 women) undergoing open left-sided hemicolectomy or resection of the sigmoid colon due to cancer in the descending- or sigmoid colon. An end-to-end anastomosis was performed with the CARP device (1.5 mm width between the rings) above or at the level of the sacral promontory. The patients were included after informed consent.

Inclusion criteria were patients between 18 and 90 years of age having the cognitive ability to understand written and oral information and accept participation in the study. Exclusion criteria were ASA-score ≥ 4, concurrent IBD, albumin levels $<$ 25g/L, and treatment with immunosuppressive medication less than one month before the procedure.

Patient demographics were recorded. The anastomotic contact pressure was measured intraoperatively. Intraoperative data regarding operation time and size of the CARP rings used were registered. Postoperative data was recorded regarding the time to first stool, time of elimination of the CARP rings, and occurrence of adverse events. Daily blood samples were taken, including CRP and white blood cell count. The integrity of CARP anastomoses was assessed in five patients by injecting water-soluble contrast media through the CARP catheter system during X-ray fluoroscopy a few days after the procedure.

Statistical methods:

Non-parametric statistical methods and concepts were used, presented with medians, ranges and quartiles.

Paper III

This was a non-randomized prospective clinical safety study on 21 patients (16 men and 5 women) undergoing open or laparoscopic high anterior resection due to cancer in the distal part of the sigmoid colon or upper rectum. An end-to-end colorectal anastomosis was performed with the C-REX device 10-12 cm above the anal verge. The patients were included after informed consent.

Inclusion criteria were patients' ≥ 18 years of age having the cognitive ability to understand written and oral information and accept participation in the study.

Exclusion criteria were ASA score ≥ 4, stage IV colorectal cancer, concurrent IBD, the need for emergency procedure, albumin level < 25g/L, and treatment with immunosuppressive medication less than one month prior to the procedure.

Patient demographics were recorded. The anastomotic contact pressure was measured intraoperatively. Intraoperative data regarding the operation time and size of the C-REX rings used were registered. Postoperative data was recorded regarding the time to first stool, time for elimination of the C-REX rings, and occurrence of adverse events. Daily blood samples were taken, including CRP and white blood cell count.

Statistical methods:

Non-parametric statistical methods and concepts were used, presented with medians, ranges and quartiles.

Paper IV

This was an experimental comparative study in 48 pigs where the early mechanical anastomotic strength was compared between C-REX LapAid anastomoses and circular stapled anastomoses using bursting pressure.

All pigs underwent a midline incision with resection of the sigmoid colon under anesthesia with an end-to-end anastomosis at the level of the sacral promontory, with 27 anastomoses performed with C-REX LapAid device and 21 with circular staple device.

Bursting pressure was measured with a manometer at set time intervals, *i.e.* after 1 hour, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, and 7 days after the surgical procedure. This was done in vivo through an anal plug where the upper border of the anastomosed bowel segment was closed with a bowel clamp. The main focus of this study was on the mechanical anastomotic strength during the first 24 hours.

The early histological appearance of the C-REX LapAid anastomoses was assessed with microscopy 6 and 24 hours after the formation of the anastomoses with vascular endothelial CD31 and collagen Masson´s Trichrome staining.

Statistical methods:

Due to the small sample size of the subgroups of animals used in this study, non-parametric statistical methods and concepts were used, presented with medians, ranges and quartiles. The Mann-Whitney U test was used to compare the difference between the bursting pressure in the stapled anastomotic group and the C-REX LapAid anastomotic group.

Results

Paper I

31 pigs were operated with resection of the sigmoid colon and an end-to-end colocolic anastomosis performed with the CARP device with different widths between the anastomotic rings.

The anastomotic contact pressure was measured in all animals directly after the anastomosis formation, where after the 31 pigs where divided into different groups. The anastomotic contact pressure was indirectly proportional to the increasing width between the anastomotic rings (Figure 23).

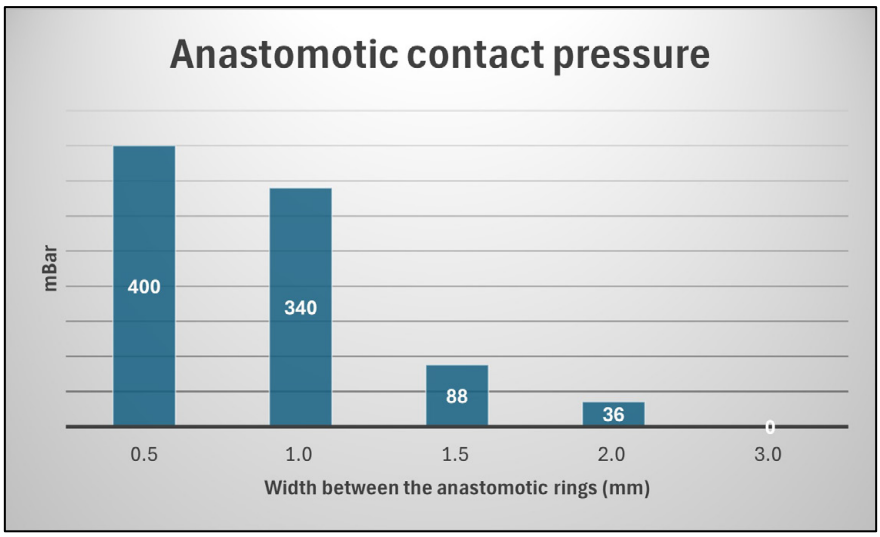

Figure 23: The median anastomotic contact pressure (mBar) immediately after the anastomotic formation depending on the different widths between the CARP anastomotic rings (mm). Copyright by author.

Short-term results

After the first operation, 19 pigs were used for analysis of short-term results. No anastomotic leakage was observed in this group and no animal had to be sacrificed prematurely.

The animals were reoperated 1-5 days after the initial procedure and the anastomotic contact pressure was measured again. The anastomotic contact pressure was relatively unchanged during the first 48 hours after the anastomosis formation, thereafter followed by a sharp increase in anastomotic contact pressure after 72 hours (Figure 24), which continued up to 96 hours after the anastomosis formation.

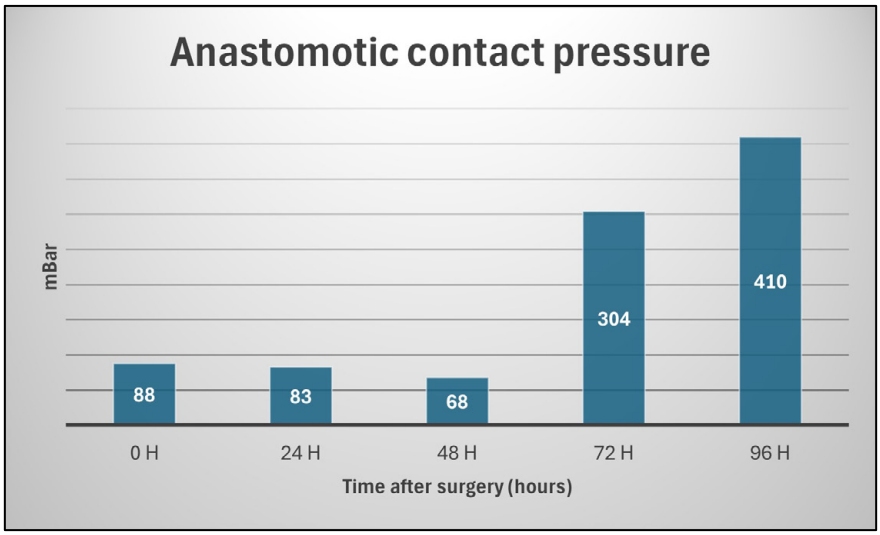

Figure 24: The changes in anastomotic contact pressure over time after anastomoses formation with 1.5 mm width between the rings, demonstrating pronounce pressure increase 72 hours after the procedure. The same pattern was seen with other anastomotic widths. Copyright by author.

The anastomotic integrity of the CARP anastomoses in this group was assessed by injecting water-soluble contrast media through the catheter system of the CARP rings during X-ray fluoroscopy, and was without signs of contrast medium leakage from the closed space between the CARP rings and the anastomotic line in all animals (Figure 25).

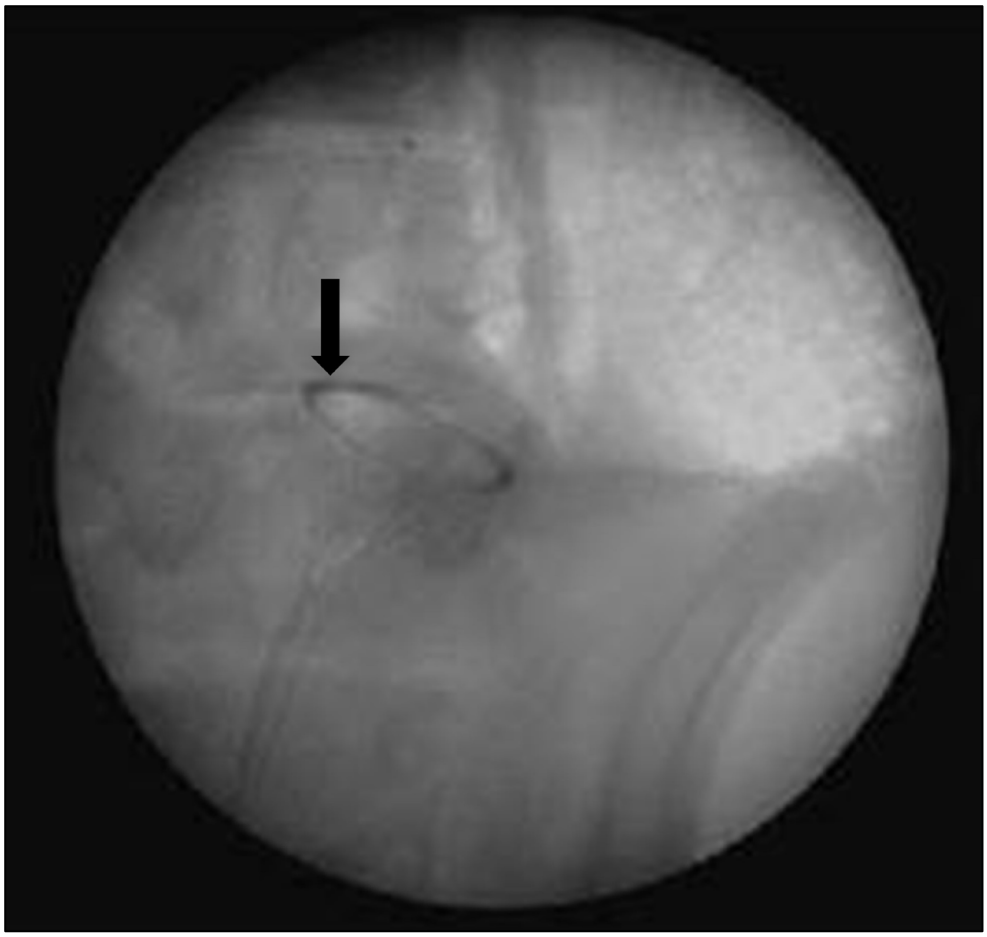

Figure 25: A CARP anastomosis after injecting water-soluble contrast media through the catheter system of the CARP device during X-ray fluoroscopy. The figure demonstrates the contrast medium in the closed space between the CARP rings and the anastomotic line (black arrow), without signs of contrast leakage. Copyright by KARGER, reproduced with permission.

Microscopic examination of the CARP anastomotic tissue with 1.5 mm ring width demonstrated the formation of granulation tissue three days after the anastomosis formation, bridging the anastomosis with a triangular healing area between the anastomotic rings and the newly formed serosa (Figure 26).

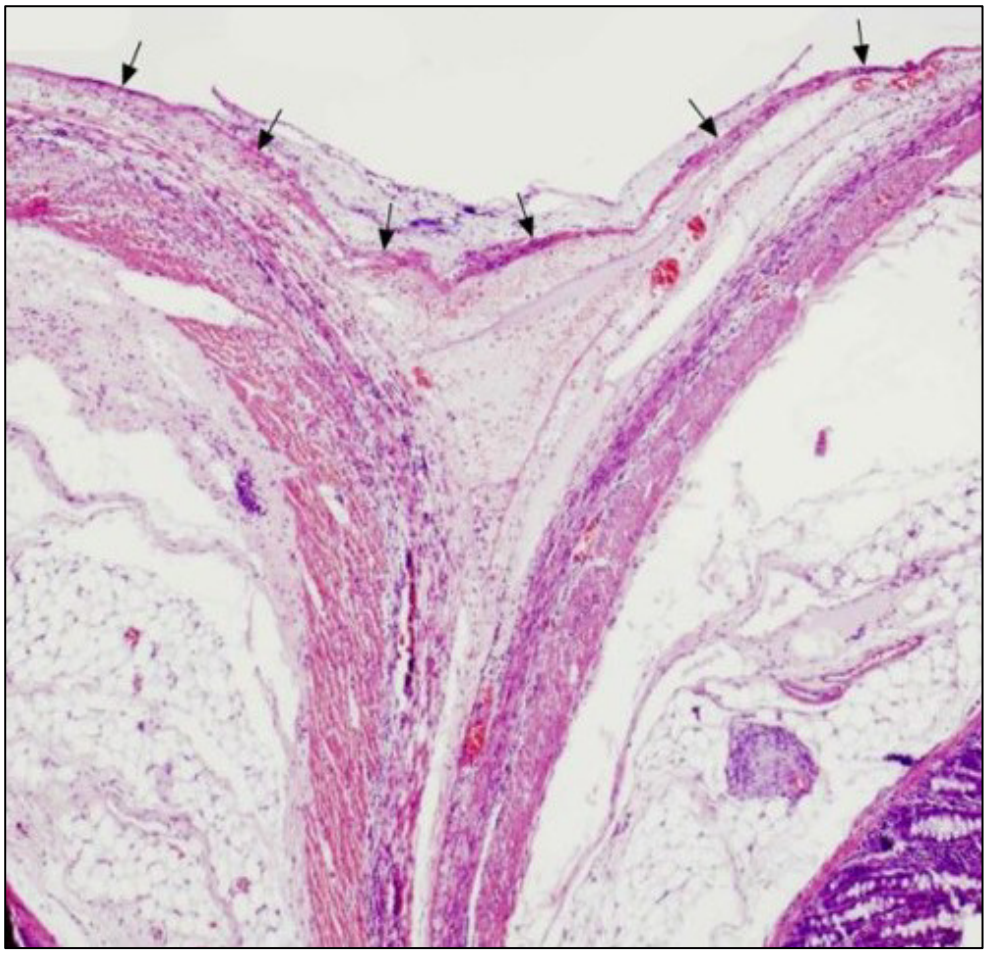

Figure 26: Hematoxylin-eosin staining of the anastomotic area showing the triangular-shaped healing tissue of the CARP anastomosis under the newly formed serosa (black arrows) 72 hours after the anastomotic formation. Copyright by CarpoNovum, reproduced with permission.

Sirius Red staining of the CARP anastomotic tissue, highlighting collagen, demonstrated collagen in the submucosa of the intestinal ends, but was not visible in the triangular healing granulation tissue 72 hours after the anastomotic formation (Figure 27).

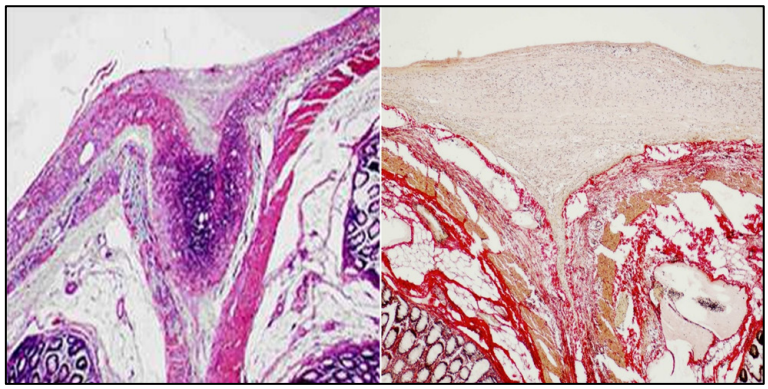

Figure 27: Hematoxylin-eosin staining of the anastomotic area showing the triangular healing process of the CARP anastomosis 72 hours after the anastomotic formation (left figure). Sirius Red staining highlighting collagen in the submucosa (red color) and not in the triangular healing granulation tissue after 72 hours (right figure). Copyright by CarpoNovum, reproduced with permission.

Long-term results

12 pigs were used for analysis of long-term results, and all animals recovered without adverse events after the initial surgical procedure. No anastomotic leakage was observed in this group, and no animal had to be sacrificed prematurely. The CARP rings were expelled through the natural route within 6 days after the anastomotic formation (range 4–6).

The animals in this group were sacrificed and set time points to be able to evaluate the macroscopic appearance and elasticity of the CARP anastomoses. This was done by inflating the colon segment containing the anastomosis with a saline solution 8-15 weeks after the procedure. All CARP anastomoses healed without signs of anastomotic stricture and the colon segments containing the anastomoses had good bowel wall compliance and elasticity (Figure 28).

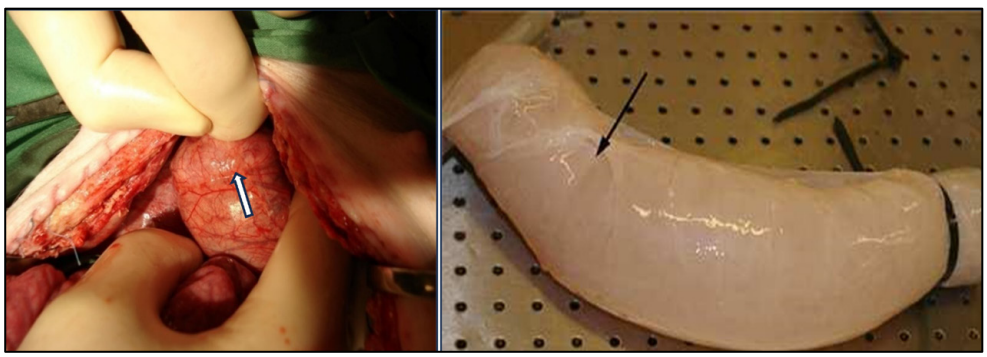

Figure 28: A CARP anastomosis 8 weeks after the surgical procedure. The left figure shows the anastomosis in situ (white arrow) and the right figure demonstrates the CARP anastomosis (black arrow) without signs of stricture. Copyright by KARGER, reproduced with permission.

The mechanical strength of the CARP anastomoses was measured 8-15 weeks after the surgical procedure. This was done by inflating air into the colon segment containing the anastomosis until a sudden drop in the intraluminal pressure was observed with associated air leakage from the bursting pressure-induced intestinal rupture. The median bursting pressure was 166 mBar (range 120-235), and the rupture site was located outside the anastomotic scar tissue in 10 out of 12 CARP anastomoses (Figure 29).

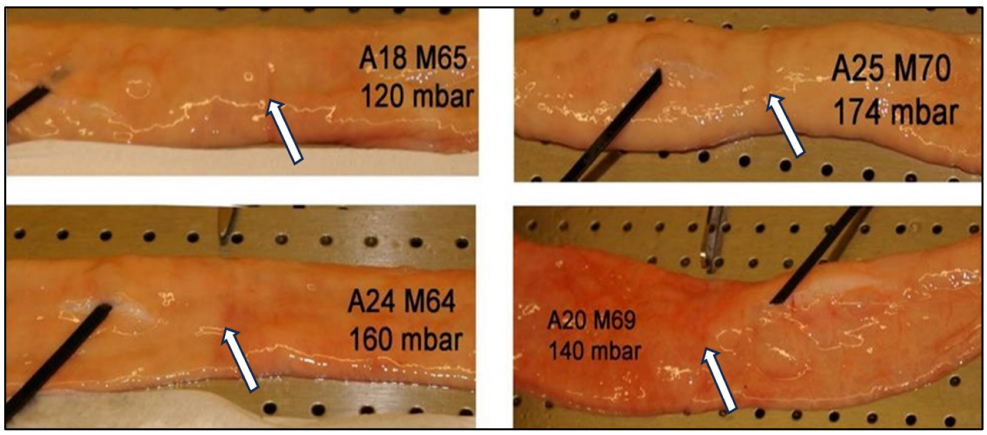

Figure 29: Four CARP anastomoses demonstrating intestinal rupture outside the anastomotic scar tissue. The white arrows point at the anastomotic line and the black forceps point at the rupture side. Copyright by author.

Paper II

Twenty-five patients were included and operated with either an open left-sided hemicolectomy or a sigmoid colon resection. The CARP device with a 1.5 mm width between the anastomotic rings was used in this study.

Two patients received a terminal colostomy instead of an anastomosis following the resection due to advanced tumor disease. Of the remaining 23 patients, only 14 patients received end-to-end CARP anastomosis (Figures 30 and 31). This was because the intestinal lumen in nine patients was too narrow for the smallest CARP rings (29 mm). In these nine patients, including seven women, a hand-sewn end-to-end anastomosis was constructed instead.

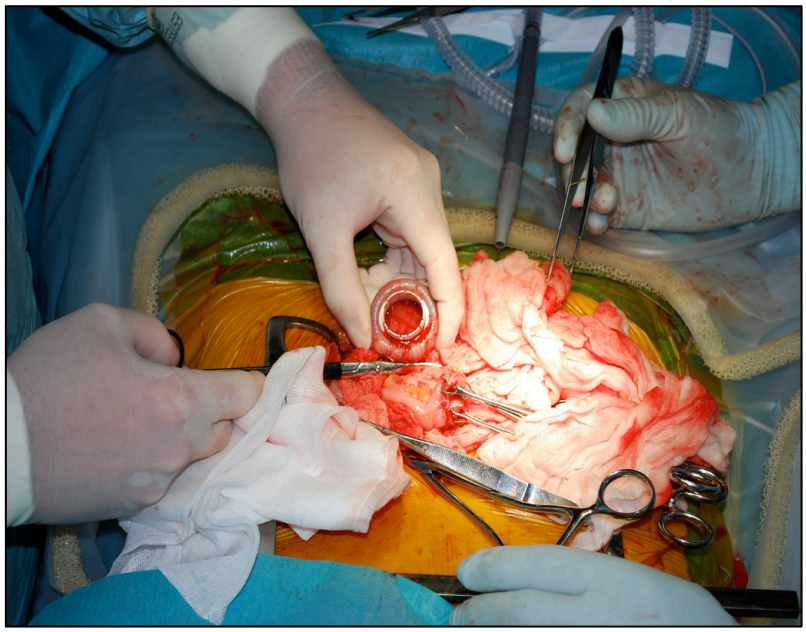

Figure 30: The CARP anastomotic male ring in place in the distal intestinal end with the coupling segment connected. Copyright by author.

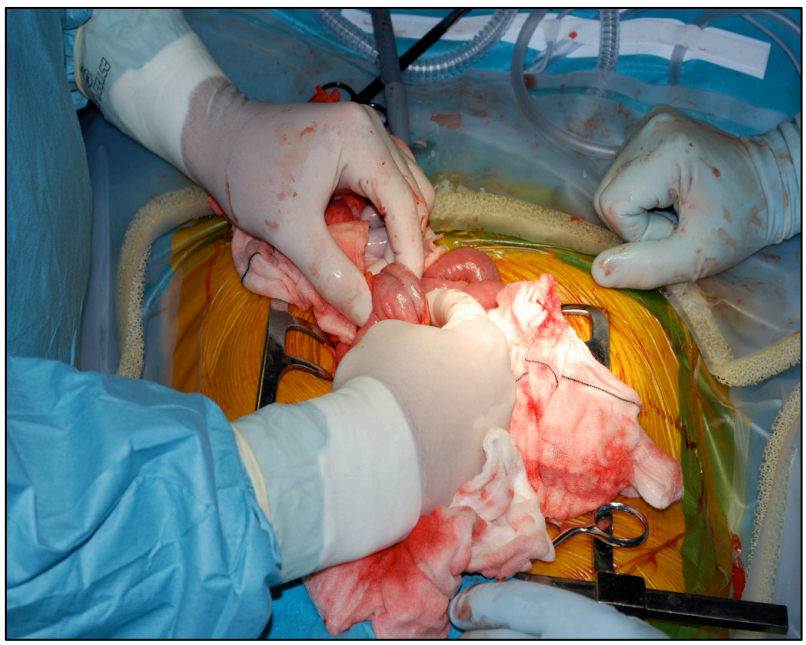

Figure 31: When both the male and female rings are in place, the CARP anastomosis is formed by connecting the rings together with a simple mechanical click function. This clicking maneuver requires both hands of the surgeon to perform. Copyright by author.

The age of the CARP patients ranged between 54-89 years (median 74) and BMI 22-37 (median 25). Ten patients had an ASA score of 2 and four patients had a score of 3 (Table 3). The duration of the surgical procedures ranged between 128-246 minutes (median 175). The 29 mm CARP rings were used in nine patients, 32 mm rings in five patients, and the 35 mm anastomotic ring was not used.

Table 3: Characteristics of the CARP patients

Patient	ASA score	BMI	Comorbidities
1	2	23	Hypothyroidism
2	2	22	HTN
3	2	23	DM, PVD
4	3	27	CM, AF, TIA
5	3	25	HTN, MI, CVI
6	2	29	HTN
7	2	37	RA, obesity
8	2	29	DM, HTN
9	2	22	DM, HTN, CVI
10	3	25	HTN, AF
11	2	25	None
12	3	30	AF
13	2	25	None
14	2	31	DM, HTN

ASA American Society of Anesthesiologists, *BMI* body mass index, *HTN* hypertension, *DM* diabetes mellitus, *PVD* peripheral vascular disease, *CM* cardiomyopathy, *AF* atrial fibrillation, *TIA* transient ischemic attack, *MI* myocardial infarction, *CVI* cerebrovascular insult, *RA* rheumatic arthritis.

No signs of anastomotic leakage or postoperative bowel obstruction were observed in the CARP anastomoses or the hand-sewn anastomoses. The anastomotic rings were expelled through the natural route in all CARP patients 7-14 days after the procedure (median 10).

The CRP levels in the CARP patients showed the highest values on the second postoperative day, with a median CRP at 223 mg/L (Figure 32). No patient demonstrated a biphasic postoperative CRP curve.

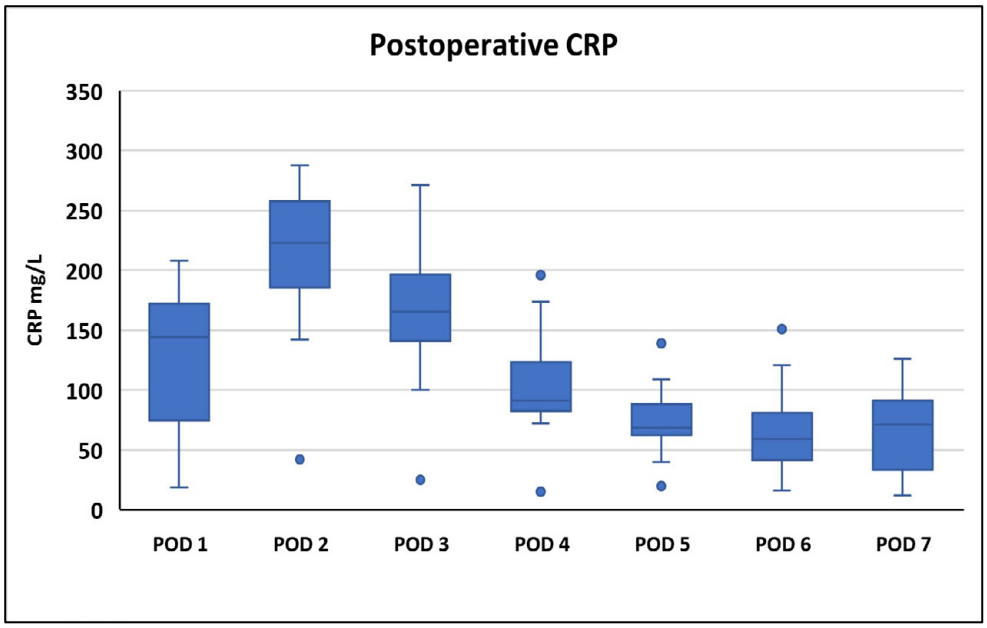

Figure 32: CRP following open resection of the descending- or sigmoid colon with an end-to-end CARP anastomosis. The CRP distribution is shown with boxes and whiskers plots. The boxes show the 25-75% range and whiskers the total range. The horizontal line in the box is the median value. High and low outliers are shown as dots and there are no extreme values. *POD* postoperative day. Copyright by author.

One patient was reoperated due to wound dehiscence and his postoperative CRP curve was comparative to patients without adverse events. Three cases of minor adverse events were observed, one case of pneumonia and two events of superficial wound infection without the need for surgical intervention. These three patients had a slower CRP decline postoperative days 3-5. All fourteen CARP patients were without gastrointestinal symptoms during the outpatient follow-up 4-6 weeks after the surgical procedure.

The integrity of four CARP anastomoses was examined on the second or third postoperative day with water-soluble contrast media through the catheter system of the CARP anastomotic rings during X-ray fluoroscopy. All four anastomoses were without anastomotic contrast leakage (Figure 33).

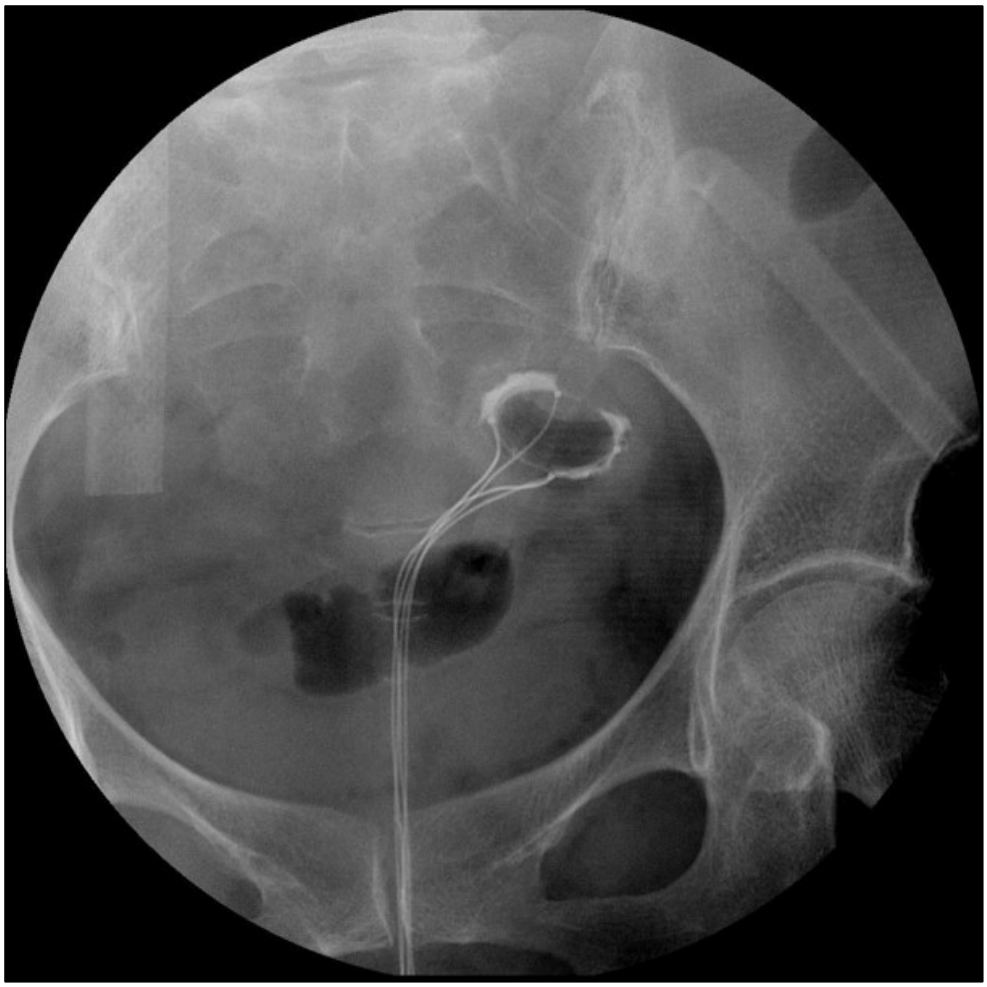

Figure 33: A CARP anastomosis after injecting water-soluble contrast media through the catheter system of the CARP rings during X-ray fluoroscopy. The figure shows the contrast medium in the closed space between the rings and the anastomotic line, without signs of contrast leakage. Copyright by Springer, reproduced with permission.

The postoperative macroscopic appearance of the CARP anastomoses was evaluated with a flexible sigmoidoscopy in twelve of the fourteen patients 8-12 weeks after the surgical procedure, and in one patient two years after the operation. In all cases, the endoscopy examination showed well-healed anastomoses without signs of inflammation or stenosis formation (Figure 34).

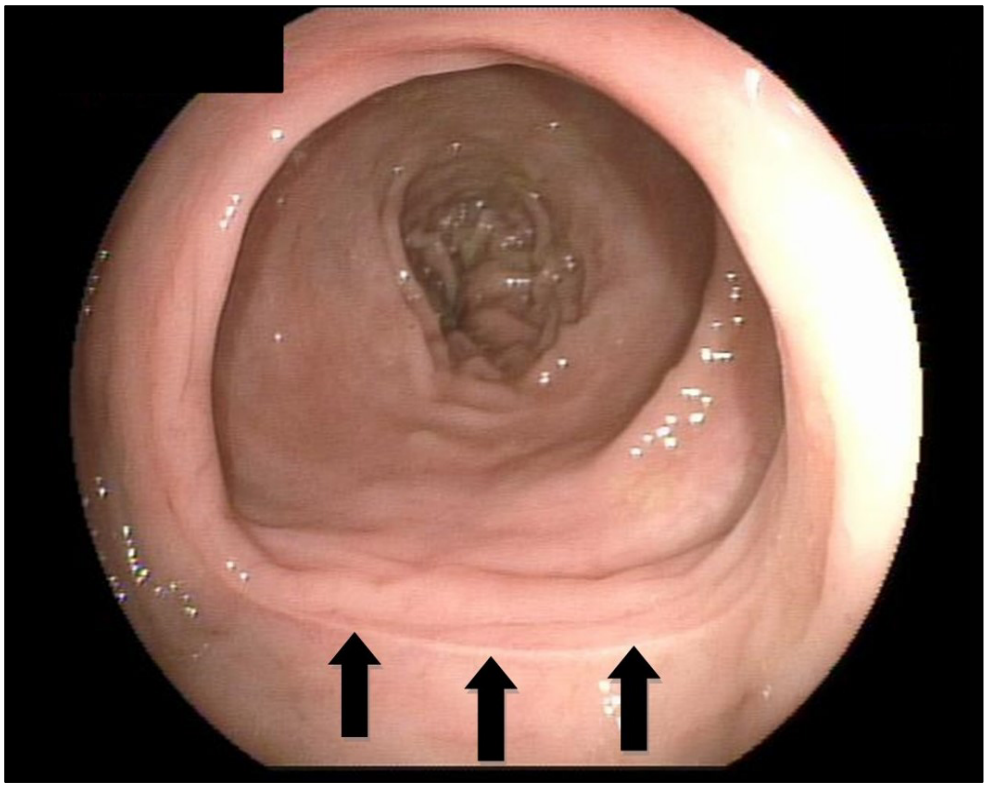

Figure 34: A CARP anastomosis 8 weeks after the anastomotic formation, showing well-healed anastomosis without macroscopic inflammation or stricture formation. Black arrows point at the anastomotic line. Copyright by Springer, reproduced with permission.

Paper III

Twenty-one patients were included and operated with a high anterior resection and an end-to-end colorectal anastomosis constructed 10-12 cm above the anal verge with the C-REX device. A partial mesorectal excision (PME) was performed in all patients, and the inferior mesenteric artery was transected below the left colic branch.

The first 11 patients were operated on with an open technique, and the following 10 patients with a minimally invasive technique, where one laparoscopic procedure was converted early to an open approach due to extensive abdominal adhesions.

The first six patients operated on with the open approach were anastomosed intraabdominally with the LL technique of the C-REX method. The remaining 15 patients were anastomosed with the transanal LR technique, during both open and minimally invasive approaches.

The age of the C-REX patients ranged between 46-85 years (median 72) and BMI 18-30 (median 26). Three patients had an ASA score of 1, fourteen had a score of 2, and four patients had a score of 3 (Table 4). The duration of the surgical procedures ranged between 152 and 300 minutes (median 209). The 26 mm C-REX rings were used in fourteen patients, the 29 mm rings in six patients, and the 32 mm anastomotic rings in one patient.

Table 4: Characteristics of the C-REX patients

Patient	ASA score	BMI	Comorbidities
1	2	22	HTN, DM
2	1	18	Hypothyroidism
3	2	26	None
4	2	29	HTN, AF, TIA
5	3	28	AF, CVI
6	2	30	HTN
7	2	26	DM, TIA
8	2	29	None
9	3	25	HTN, AF
10	2	27	HTN
11	2	28	DM
12	2	25	Hypothyroidism
13	2	28	HTN
14	2	22	Temporal arteritis
15	2	27	None
16	1	24	None
17	3	24	HTN, AF, TIA
18	2	26	HTN, DM, single kidney
19	1	27	None
20	2	25	HTN
21	3	22	AF

ASA American Society of Anesthesiologists, *BMI* body mass index, *HTN* hypertension, *DM* diabetes mellitus, *AF* atrial fibrillation, *TIA* transient ischemic attack, *CVI* cerebrovascular insult.

The six patients who were operated on with the LL technique of the C-REX method had an intraoperative ACP ranging between 50-180 mBar (median 95). The first patient operated on with this approach had an intraoperative ACP of 50 mBar and developed an anastomotic leakage. During his reoperation, which was performed on the sixth postoperative day, it was noted that the C-REX anastomotic rings were not fully closed (Figure 35).

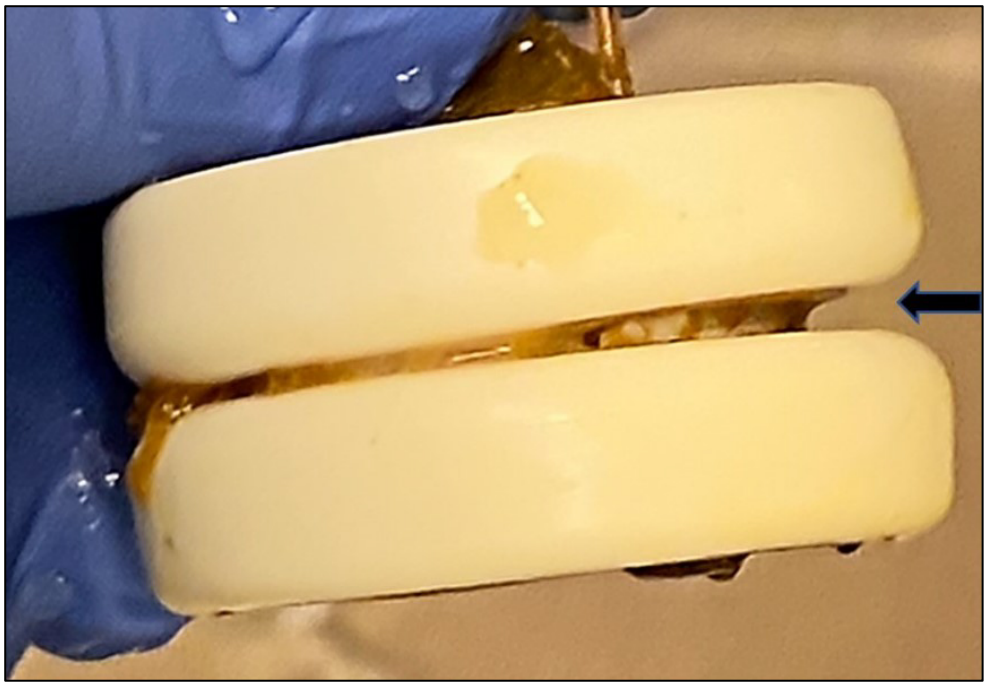

Figure 35: The C-REX rings were not symmetrically closed, as the width between the anastomotic rings was broader on one side (the black arrow) compared to the other. Copyright by Springer, reproduced with permission.

After this anastomotic adverse event, two additional patients operated with the LL approach with an intraoperative ACP of 50 and 60 mBar were converted to conventional circular stapled anastomoses after the C-REX anastomoses were removed.

None of the 15 patients operated with the C-REX transanal LR technique developed anastomotic leakage, and their intraoperative ACP ranged between 145-300 mBar (median 250) (Figure 36). One patient operated on with a minimally invasive approach was reoperated on POD 7 due to small bowel herniation at one of the laparoscopic port sites, where the herniation was managed through the port hole opening without the need for bowel resection. Both the stapled anastomoses healed without clinical signs of anastomotic leakage.

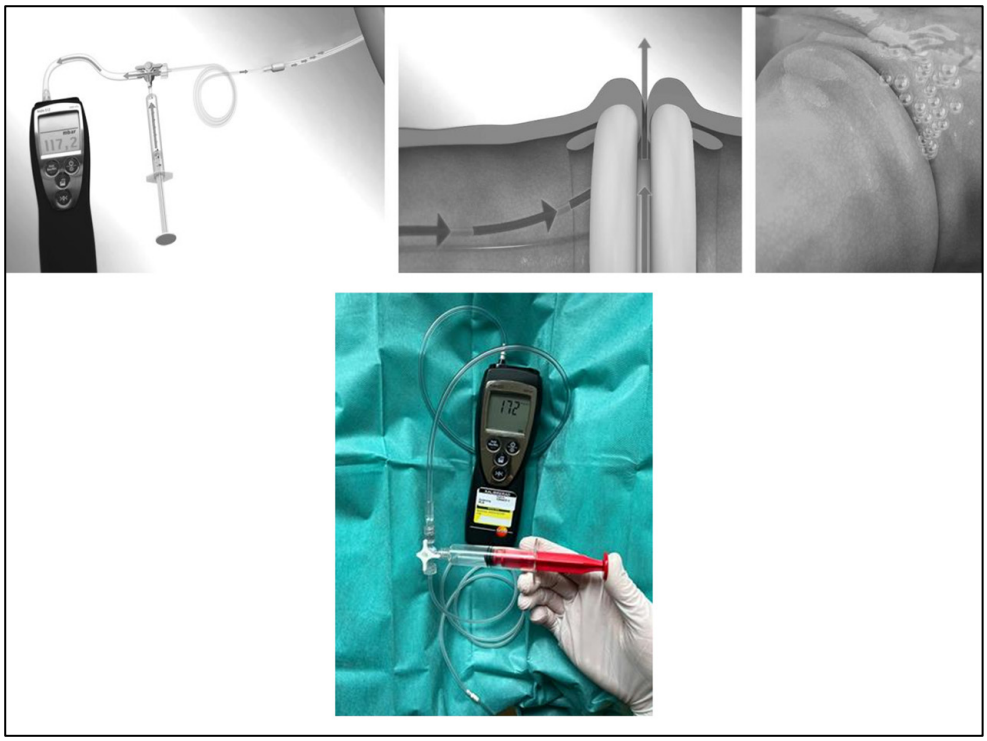

Figure 36: The figure demonstrates measurements of the anastomotic contact pressure with a manometer. It was performed by infusing air through the C-REX catheter system and into the closed space between the anastomotic rings and the anastomotic line. The appearance of air bubbles from the submerged C-REX anastomosis during the test was defined as the anastomotic contact pressure. Copyright by CarpoNovum, reproduced with permission.

The anastomotic rings were expelled through the natural route in all C-REX patients 7-19 days after the procedure (median 10). All eighteen C-REX patients were without gastrointestinal symptoms during the outpatient follow-up 4-6 weeks after the surgical procedure.

The level of CRP values peaked on the second postoperative day following both the open and laparoscopic procedures. The CRP peaks were higher following open surgery compared to the minimally invasive approach, with a median CRP of 187 mg/L compared to a median CRP of 69 mg/L respectively (Figure 37). The patients who developed adverse events showed biphasic CRP curves compared with the uniphasic CRP curves in the patients without adverse events.

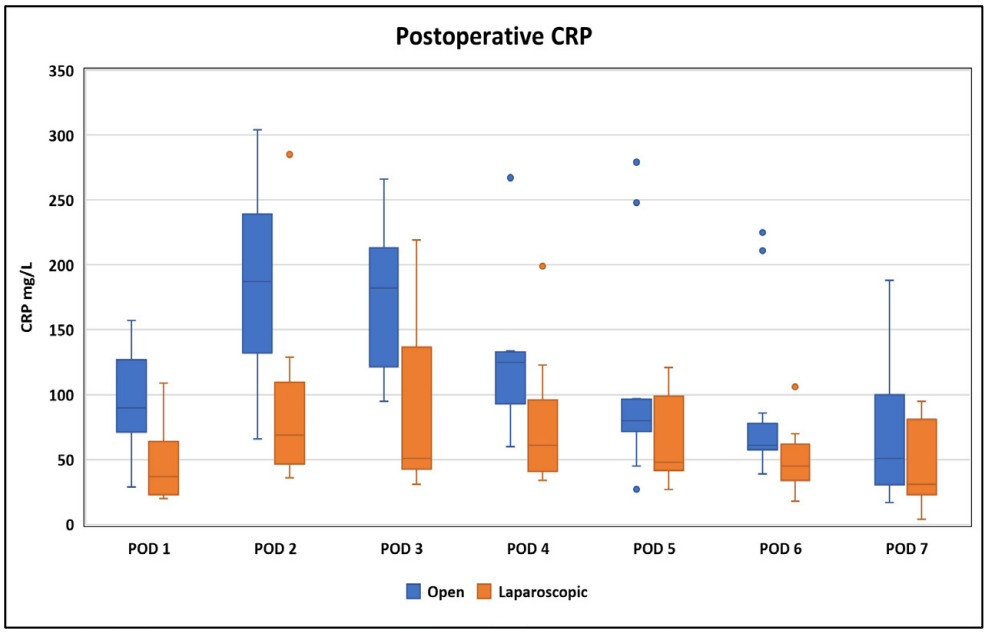

Figure 37: CRP following open and minimally invasive high anterior resections with C-REX end-to-end colorectal anastomoses. The CRP distribution is shown with boxes and whiskers plots. The boxes show the 25-75% range and whiskers the total range. The horizontal line in the box is the median value. High and low outliers are shown as dots and there are no extreme values. *POD* postoperative day. Copyright by Springer, reproduced with permission.

The postoperative macroscopic appearance of the C-REX anastomoses was evaluated with a flexible sigmoidoscopy in all eighteen C-REX patients 4-25 weeks after the surgical procedure (median 12). The endoscopy examination showed well-healed anastomoses without signs of pathological inflammation or stenosis in seventeen patients (Figure 38), whereas one patient had a moderate stricture without any clinical symptoms (Figure 39).

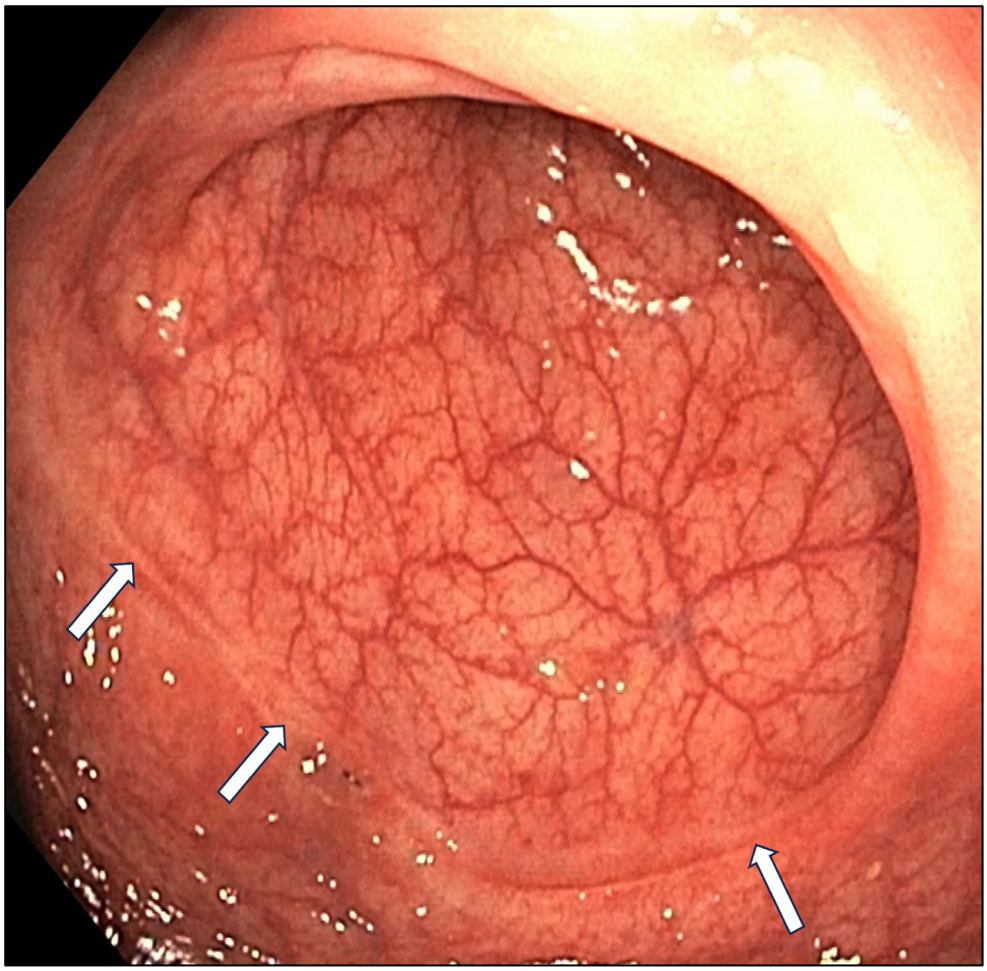

Figure 38: The macroscopic appearance of a C-REX anastomosis 8 weeks after the surgical procedure, showing a well-healed anastomosis without signs of inflammation or stenosis. The white arrows point at the anastomotic line. Copyright by Springer, reproduced with permission.

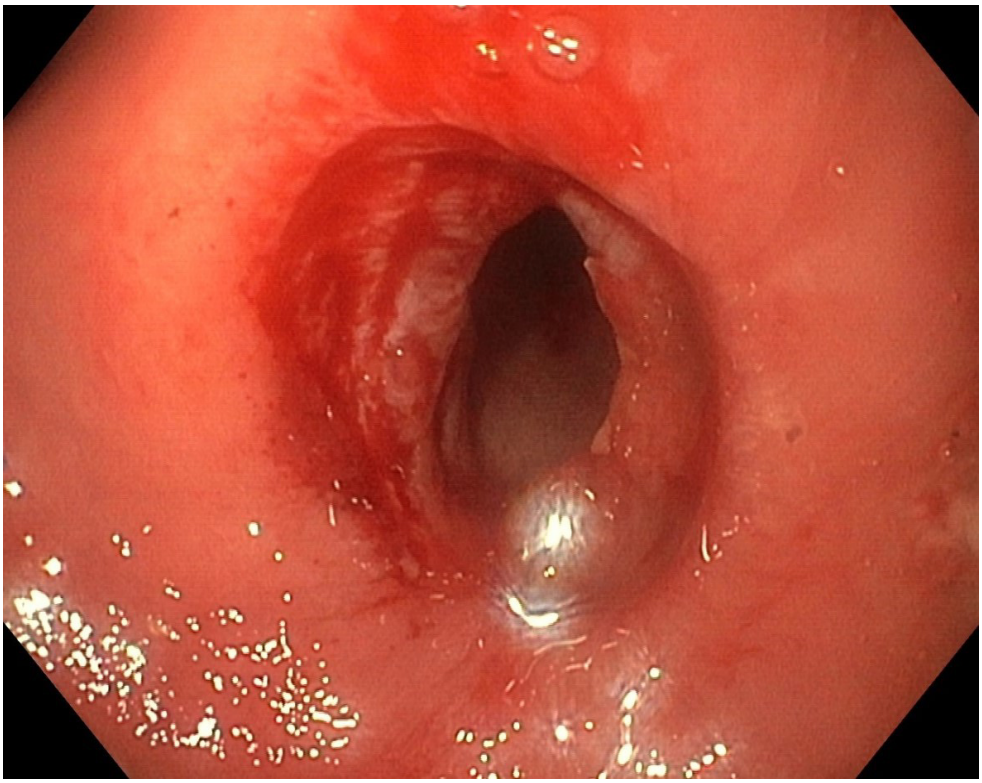

Figure 39: The macroscopic appearance of a C-REX anastomosis 8 weeks after the surgical procedure, demonstrating a moderate stricture where the patient was without any clinical symptoms. Copyright by author.

Paper IV

48 pigs were operated with a resection of the sigmoid colon, and an end-to-end anastomosis was constructed 15 cm above the anal verge, where 27 pigs were anastomosed with the C-REX LapAid device and 21 pigs with traditional circular staplers.

All the 48 end-to-end colorectal anastomoses were easy to perform (Figure 40). All pigs recovered uneventfully following the procedure, and no animal was excluded due to adverse events. The pigs were sacrificed after the initial surgical procedure at set time intervals, and an examination of the anastomoses revealed intact anastomoses without signs of pathological inflammation, adhesions, or stenosis in both groups.

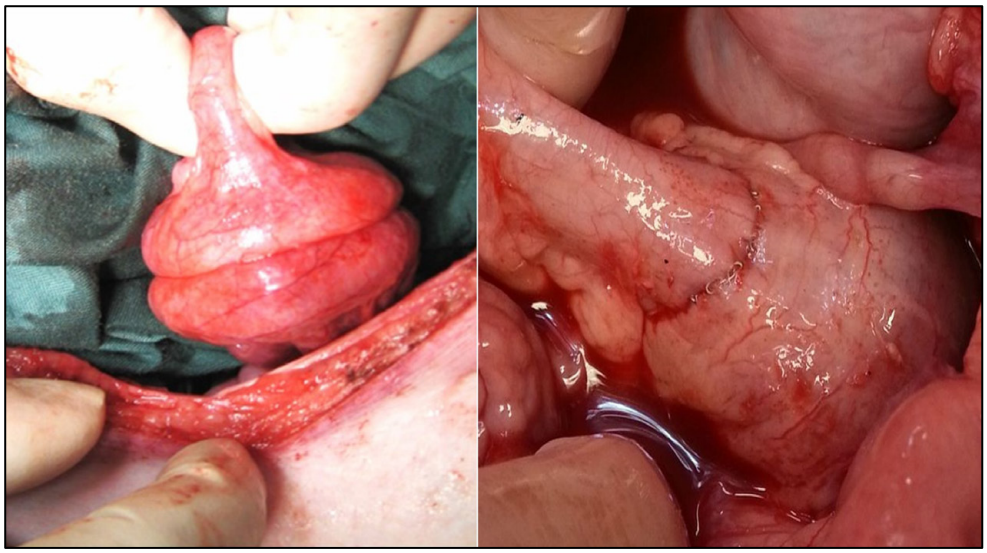

Figure 40: A C-REX LapAid anastomosis (left figure) and a circular stapled anastomosis (right figure) directly after their formation. Copyright by author (submitted).

The bursting pressure was measured in all anastomoses at set time intervals (Figures 41 and 42). The median bursting pressure value 1 hour after the surgical procedure was 195 mBar in the C-REX LapAid group (range 180-240) compared to 36 mBar (range 28-64) in the circular stapled anastomotic group ($p<0.001$). After 6-12 hours, the median bursting pressure value was 180 mBar in the C-REX LapAid group (range 160-220) compared to 77 mBar (range 43-185) in the circular stapled anastomotic group ($p=0.044$). The median bursting pressure value was 225 mBar in the C-REX LapAid group (range 160-260) compared to 215 mBar (range 190-240) in the circular stapled anastomotic group ($p=0.558$) 1 -7 days after the surgical procedure.

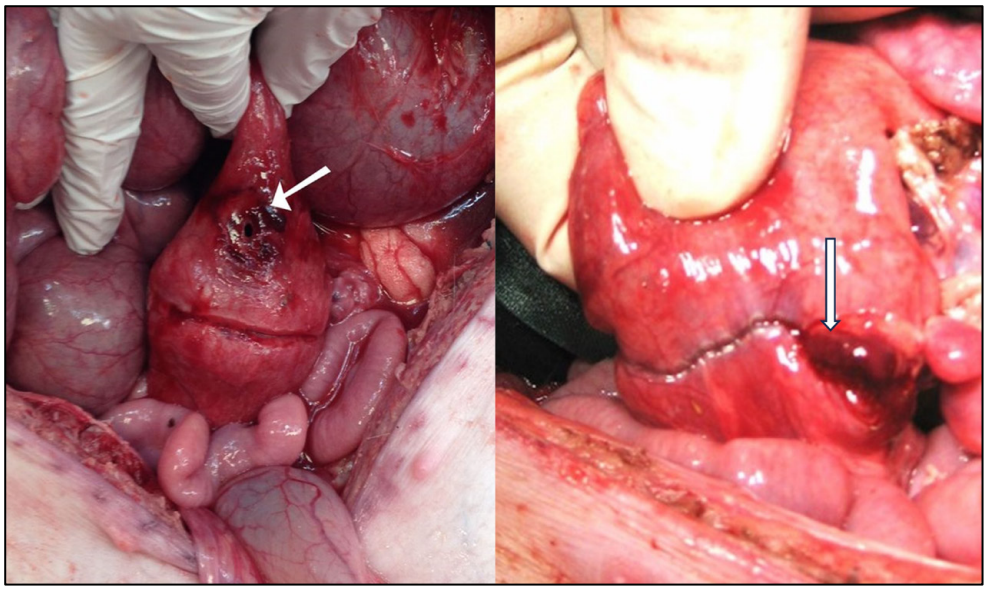

Figure 41: Bursting pressure-induced rupture of the bowel wall containing the anastomoses (white arrows). A C-REX LapAid anastomosis with the intestinal rupture proximal to the anastomosis (left figure), and a circular stapled anastomosis with the intestinal rupture in the anastomotic line (right figure). Copyright by author (submitted).

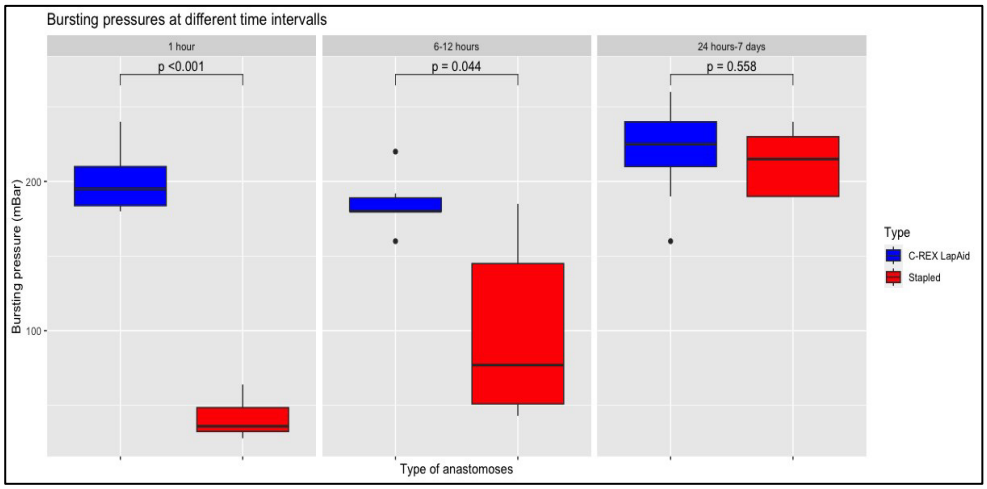

Figure 42: Bursting pressure distribution in circular stapled versus C-REX LapAid anastomoses 1 hour, 6-12 hours, and 24 hours – 7 days after the surgical procedure. The boxes show the 25-75% range and whiskers the total range. High and low outliers are shown as dots and there are now extreme values. Copyright author (submitted).

The early microscopic appearance of the C-REX LapAid anastomoses was assessed with vascular CD31 and collagen Masson Trichrome staining 6 and 24 hours after the anastomotic formation. This staining method demonstrated increasing granulation tissue bridging the anastomotic gap between the anastomotic rings with time (Figure 43). There were only minor changes in the vascularization of the healing tissue during this early time period. Collagen was located in the submucosa and serosa of the intestinal wall and not in the anastomotic gap.

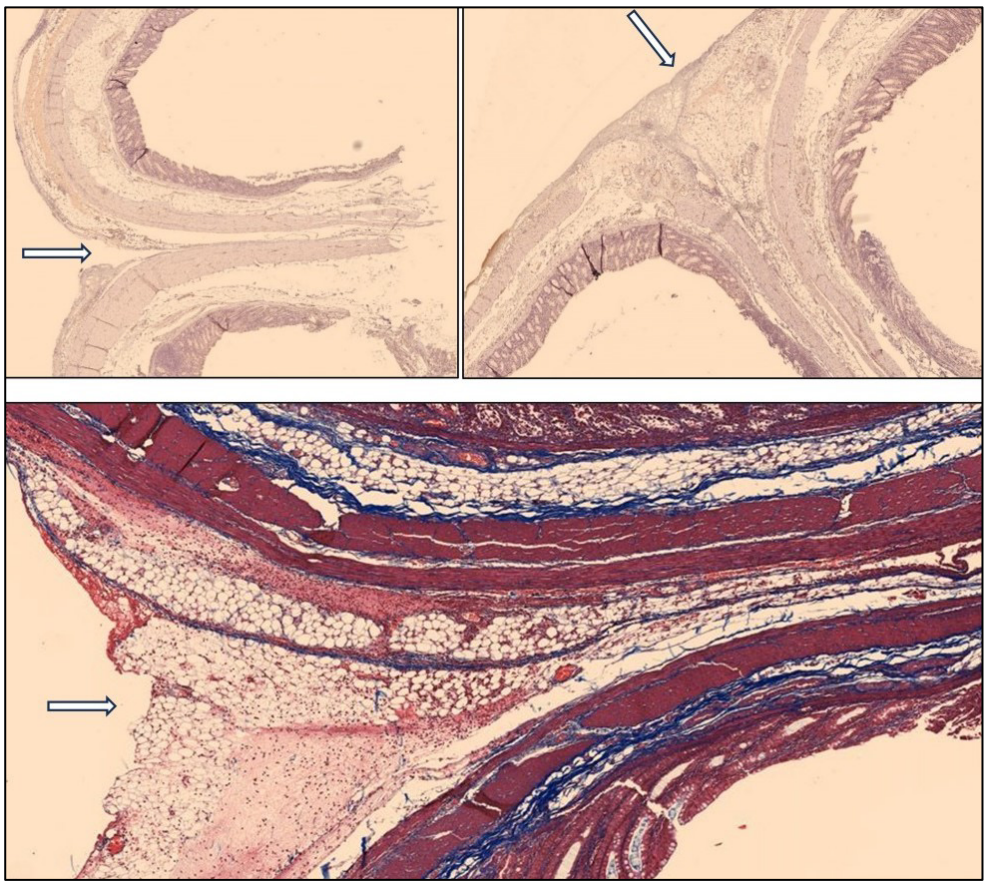

Figure 43: Microscopic appearance of the C-REX LapAid anastomoses. Vascular CD31 staining 6 hours (upper left figure) and 24 hours (upper right figure) after anastomosis formation, with increased granulation tissue by time bridging the anastomotic gap (white arrows). A Masson´s Trichrome staining 24 hours after anastomosis formation (lower figure) demonstrated collagen in the intestinal submucosa and serosa (highlighted in blue color), but without the presence of collagen in the anastomotic gap. Copyright by author (submitted).

Discussion

Further development is needed

Anastomotic leakage is a feared surgical complication in colorectal surgery and is a major cause of postoperative morbidity and mortality[3, 4, 152]. Anastomotic leakage causes significant patient suffering, extended hospital stay, and increased healthcare costs[2, 3, 153]. Furthermore, anastomotic leakage following colorectal cancer surgery might have a negative impact on overall survival, cancer-specific survival, local recurrence, and overall recurrence[154, 155].

Although traditional handsewn and stapled colorectal anastomoses are similar in terms of clinical safety and efficiency, they are not without risks and numerous patients are affected by anastomotic leakage. The reported leakage rate of colorectal anastomoses is 2-20%, with the highest leakage rate in low rectal anastomoses[156-158].

The technique of compression anastomosis in colorectal surgery was first described in the 19th century. Since then, anastomotic rings like Murphy's button, AKA-2, Valtrac BAR and NiTi have been used without being accepted as routine anastomotic methods[159]. There are several theoretical advantages with the technique of compression anastomoses, such as a more symmetrical distribution of tensile forces in the anastomotic line and the absence of foreign material in the anastomosis after its formation. Previous studies of compression anastomoses have though reported a frequency of postoperative bowel obstruction between 2-16 %[21].

Considering these facts above, further development of anastomotic techniques and devices is of paramount importance, to improve anastomotic healing and reduce the risk of this demanding anastomotic adverse event.

The development of a new anastomotic device

The development of a new anastomotic device takes a long time and the instrument testing itself requires both experimental- and clinical trials with meticulous study protocols. Clinical trials that evaluate the performance of a novel surgical device can cause harm to human participants and require testing in animal models as a proof-of-concept, before initiating human testing in the form of a safety study. When designing a human safety study, ethical considerations are of paramount importance

due to the delicate balance between the potential benefits of the new method and the risk of harm to the participants.

A human safety study has an inherent sample size limitation and is not designed to evaluate the incidence of anastomotic leakage. This is because it is important to assess the potential occurrence of serious adverse events of the new method in a smaller group of participants before testing is considered safe and can be allowed to be performed in studies with a larger sample size. The purpose of this thesis was to conduct both experimental proof-of-concept and clinical safety studies to evaluate if the CARP and C-REX methods were safe and efficient enough to be able to continue to the next level of testing in a larger human sample size for evaluation of the incidence of anastomotic leakage.

Both the CARP and C-REX devices have a CE-marking (Conformité Européenne). This indicates that the devices have been assessed and meet the general safety and performance requirements of the legal framework of MDR 2017/745 (Medical Device Regulation), signifying compliance with the regulations of medical devices in the European Union.

The CARP device was developed during the past two decades and was designed to construct colorectal anastomoses in open abdominal surgery. The first experimental and clinical studies of CARP included in this thesis (*Papers I and II*) were carried out during a time of changing surgical techniques, with the start of minimally invasive colorectal procedures. This new surgical era called for further development of the CARP device by adjusting it to this new surgical method, and an improved version of the instrument was created. This novel and refined CARP device is called C-REX and is adapted for usage in minimally invasive colorectal surgery and for performing rectal anastomosis through the transanal approach. The development of the novel C-REX instrument called for additional experimental and clinical trials, evaluating the safety and efficacy of this new device in performing colorectal anastomoses (*Papers III and IV*).

The CARP and C-REX anastomotic rings are more ovular in shape compared to other anastomotic compression rings and were created with the intention to separate the necrotic tissue area of the compression anastomotic technique more effectively from the healing tissue area in the anastomoses. The C-REX method separates these two tissue processes even further by gradually decreasing bowel wall pressure from the point of maximum pressure with tissue necrosis and towards the area of the anastomotic healing process. By doing so, this new anastomotic C-REX method creates both an area of compression and an area of adaptation, where the area of adaptation triggers the healing process. The C-REX anastomotic device is, therefore, not a pure compression anastomotic method and could be defined as an adaptive anastomotic technique instead.

The CARP method

CARP´s safety and efficacy in performing left-sided colorectal anastomoses were evaluated in both experimental (*Paper I*) and clinical (*Paper II*) studies.

Paper I

No anastomotic leakage was observed clinically or radiologically in this study and no animal had to be sacrificed prematurely due to other complications. A subgroup of pigs was followed up to three and a half months after the construction of the anastomoses without signs of anastomotic stricture formation in all animals. Stricture formation is a well-recognized anastomotic complication in stapled anastomoses[160, 161].

Previous animal studies have used bursting pressure to assess mechanical anastomotic strength by inflating the intestinal segment containing the anastomosis with air. Besides that, in *Paper I* we measured also the contact pressure of the CARP anastomosis by infusing air directly into the anastomotic line instead. This was done by using the catheters-based system of the CARP device, enabling measurement of the contact-induced closure strength of the anastomotic rings, which hold the intestinal anastomosis together. This is the first time that an anastomotic method has been utilized to quantify the contact pressure in an anastomosis, thus giving the surgeon direct feedback on the mechanical strength of the connection between the intestinal ends in an anastomosis.

After the formation of the CARP anastomoses, the anastomotic contact pressure decreased slightly with time in conjunction with the acute inflammatory phase, and then increased again on the third and fourth postoperative days. This pattern was independent of the width between the anastomotic rings (not shown in this thesis). The microscopic architecture of the CARP anastomoses on the third and fourth postoperative day, showed granulation tissue with bridging serosa over the anastomotic gap and signs of submucosal fusion between the intestinal ends, which can explain this finding and reflects the beginning of the proliferative phase of the anastomotic healing process.

The placement of the anastomotic CARP rings into the transected colonic bowel ends was uncomplicated and straightforward. The study demonstrated that the catheter-based system of the CARP rings allowed measurement of the anastomotic contact pressure as well as postoperative radiological monitoring of the anastomotic line up to 4-5 days after the initial surgical procedure, after which the anastomotic rings probably had started detaching from the anastomotic line.

The CARP device with a 1.5 mm width between the rings had a median anastomotic contact pressure of 88 mBar, which was theoretically suitable. This width of 1.5 mm between the rings was in line with the results of other study groups on compression

anastomoses devices, using ring width between 1.5-2.5 mm[21, 140], and was chosen for further testing in a human safety study (*Paper II*).

Paper II

No anastomotic leakage was observed clinically or radiologically in this study and there were no clinical signs of bowel obstruction in the CARP patients.

Only 14 patients out of the 25 included in the study received the CARP anastomosis. The ring sizes of the CARP instrument were suboptimal in this human setting, as the smallest rings (29 mm) were most often used, whereas the largest CARP rings (32 mm) were not used at all. These ring sizes were determined during testing on human cadavers, which was not optimal as it turned out. During the study period, a 26 mm CARP ring was created and used in two additional female patients with favorable results. The 26 mm ring size was also more compatible with the smallest ring sizes of other anastomotic compression devices and conventional staple instruments.

As in our earlier experimental study, the catheter system of the CARP device allowed intraoperative measurement of the anastomotic contact pressure as well as postoperative radiological monitoring of the anastomotic integrity in human settings. The integrity of the CARP anastomoses was examined in four CARP patients 48-72 hours after their formation without signs of anastomotic contrast leakage. During this examination, the pressure used to infuse the contrast media into the catheter system of the CARP rings was kept under the recorded intraoperative anastomotic contact pressure in these patients to avoid the possible risk of iatrogenic anastomotic leakage.

Flexible endoscopy was performed in twelve out of fourteen patients 8-12 weeks after the surgical procedure. The patient who was reoperated on due to wound dehiscence was not examined with endoscopy until 2 years later because of a protocol miss. All endoscopic examinations showed well-healed anastomoses without macroscopic inflammation or stenosis formation. One CARP patient died just over three months after the surgical procedure due to cardiovascular insult and was not examined endoscopically. This patient was without gastrointestinal symptoms at the postoperative follow-up six weeks after the surgical procedure.

The median CRP levels peaked on the second postoperative day in the CARP patients. No biphasic CRP curves were observed and the patient who was reoperated due to fascia dehiscence had a similar postoperative CRP curve as patients without adverse events. The CARP patient who suffered from postoperative pneumonia had the highest CRP values during the first four postoperative days.

The C-REX method

The C-REX´s safety and efficacy in performing colorectal anastomoses in open and minimally invasive colorectal surgery were evaluated in experimental (*Paper IV*) and clinical studies (*Paper III*).

Paper III

The 21 included patients in this study were operated on with a high anterior resection and anastomosed with two different C-REX methods, 6 patients with the LL technique and 15 patients with the transanal LR technique.

No clinical anastomotic leakage was observed in the fifteen LR patients, or other anastomosis-related complications such as abscesses or fistulas. This transanal anastomotic method was easy to use in both male and female patients, independent of the surgical approach. The RectoAid instrument completed the anastomosis during instrument firing, without the need of the click maneuver.

On the other hand, the LL method was technically difficult in constructing colorectal anastomosis below the sacral promontory. This is because the LL method, like the CARP method, involves manual completion of the anastomosis by connecting both anastomotic rings together with a click maneuver, which requires both hands of the surgeon. This method was easier to use in the two female patients operated on with this technique due to their larger pelvic area, contrary to the small pelvis of the four male patients who were operated on with the LL technique.

The first patient in the study, a male patient anastomosed with the LL method, was reoperated on the sixth postoperative day due to anastomotic leakage. He was doing well the first five postoperative days and had a low CRP curve. The reoperation revealed that the C-REX anastomotic rings were not symmetrically closed, and a small part of the wall of the intestinal end had released from this gaping ring area causing a small fistula. The patient was managed with an anastomotic take down and a permanent stoma. He recovered quickly and was discharged 16 days after the initial procedure and received his recommended adjuvant oncological treatment in time.

After this anastomotic adverse event and considering the previous CARP study results demonstrating no anastomotic leakage with the lowest recorded ACP value of 85 mBar, two subsequent male patients anastomosed with the LL method with an ACP of 50 and 60 mBar respectively, were converted to circular stapled end-to-end anastomosis instead. All three remaining LL patients, and both the stapled patients, recovered without signs of anastomotic leakage.

Due to this operator-dependent anastomotic closure of the LL technique, the author does not recommend this method being used in performing anastomoses below the

sacral promontory and should instead be limited to colonic anastomoses. On the other hand, the transanal LR technique of the C-REX method was efficient in constructing anastomoses below the sacral promontory, as the RectoAid device resembles traditional circular staplers both in design and handling.

As with the CARP method, the C-REX methods enabled intraoperative measurements of the ACP, giving the surgeon immediate feedback on the contact pressure of the anastomoses. The LR method resulted in a higher median ACP compared to the anastomoses performed with the LL method, 250 mBar versus 95 mBar respectively, demonstrating that the LR method is more optimal in constructing colorectal anastomoses below the sacral promontory.

An endoscopic examination of the C-REX anastomoses showed well-healed anastomoses without macroscopic inflammation in 17 patients, whereas one patient had a moderate anastomotic stricture without clinical symptoms. As with the CARP technique, the C-REX method induced an adequate anastomotic healing.

The median CRP value peaked on the second postoperative day following both open- and minimally invasive approach, with a higher median CRP peak after open surgery (187 mg/L) compared to laparoscopic approach (69 mg/L). This is reasonable as the minimally invasive approach involves lesser tissue trauma compared to the open technique. The C-REX patients with adverse events had biphasic CRP curves compared with uniphasic CRP curves in patient without.

Paper IV

All pigs recovered uneventfully following the initial surgical procedure, and none were excluded due to adverse events. During the planned reoperation, a macroscopic examination of the colorectal anastomoses revealed no signs of anastomotic leakage or stenosis in neither the C-REX LapAid nor the stapled groups.

The primary focus of this study was on the early anastomotic strength. This was done because a higher mechanical strength in the early phase of the anastomotic healing process might entail an advantage by improved anastomotic integrity and possibly preventing an early subclinical leakage with extraluminal pericolic inflammation with subsequent risk of future anastomotic leakage. The traditional sutured- and stapled anastomoses do not theoretically provide an immediate "sealed" anastomosis, possibly risking an early subclinical leakage that might lead to disturbances during the acute inflammatory phase of the anastomotic healing process with subsequent anastomotic leakage.

The median bursting pressure in the C-REX LapAid anastomoses was more than 5-fold higher one hour after the surgical procedures compared to the traditional circular stapled anastomoses and more than 2-fold higher after 6-12 hours. The median bursting pressure of the C-REX LapAid anastomoses remained high

throughout the study period of one week, but after 24 hours, there was no significant difference between the groups.

The number of pigs examined more than 24 hours after the procedure was low, as the main purpose of this study was to assess the anastomotic strength during the first 24 hours. However, it may be added that our previous unpublished results in using the C-REX RectoAid device in a porcine model following resection of the sigmoid colon demonstrated a high median anastomotic bursting pressure throughout the first postoperative week. The median bursting pressure after 24 hours was 200 mBar (range 180-220), 220 mBar after 48 hours (range 210-240), 220 mBar after 72 hours (range 180-240), and 220 mBar after seven days (range 200-230 mBar).

The early histological architecture of the C-REX LapAid anastomoses 6 -24 hours after the procedure demonstrated minimal anastomotic tissue reaction of vascular CD31 and collagen. This is in line with the acute inflammatory phase, producing mainly provisional granulation tissue in the anastomotic gap so early in the healing phase of the anastomotic wound. This early histological appearance of the anastomoses suggests the importance of the mechanical properties of the anastomotic method in the early phase of the healing process, where the anastomoses are most vulnerable.

Methodological considerations

All the studies in this thesis are either experimental proof-of-concept studies (*Papers I and IV*) or human safety studies (*Papers II and III*). These types of studies have inherent sample size limitations, as mentioned earlier. This thesis is therefore, not designed to evaluate the incidence of anastomotic leakage. Instead, the purpose of this thesis was to evaluate the safety and efficiency of the novel CARP and C-REX methods and assess if they are considered safe for the next step of testing in a larger human sample size.

Because of the small sample sizes and non-parametric nature, data was presented with medians, ranges, and quartiles (*Papers I-IV*). The Mann-Whitney U test was used in *Paper IV* to compare the difference between the bursting pressure values in the circular stapled anastomotic group and the C-REX LapAid anastomotic group. This statistical test was chosen because of its ability to compare the difference between two independent and not normally distributed effects, where the sample sizes in each group are small (< 30). The Mann-Whitney U test is the non-parametric equivalent to the two-sample independent parametric t-test. A p-value of < 0.05 was considered significant.

Clinical implications

Intestinal anastomotic leakage is commonly detected by clinical signs, imaging, and laboratory parameters. The diagnosis is challenging, as these clinical symptoms are sometimes difficult to detect at an early postoperative stage and often become first apparent when patients have developed signs of sepsis. It is therefore of paramount importance to be able to diagnose anastomotic leakage as early as possible and reduce morbidity- and mortality rates associated with this anastomotic complication. Furthermore, early detection of anastomotic leakage could possibly increase the chance of preserving the anastomosis, instead of performing anastomotic take down.

In this thesis, we demonstrated that the injection of contrast medium into the catheter-based system of this novel anastomotic method could be used to delineate the anastomoses during the early postoperative period. Theoretically, this method enables the diagnosis of anastomotic leakage in the early postoperative phase before the appearance of clinical symptoms. This is the first time that an anastomotic method has been used to monitor the integrity of an anastomosis after its formation, with further trials needed to investigate this potential application. This novel method offers opportunities to monitor the anastomotic healing process both radiologically and biochemically, theoretically enabling faster intervention in disturbed anastomotic healing and reducing the necessity for routine defunctioning ileostomies during low anterior resections.

It is important to note that this contrast monitoring method only assesses the integrity of the anastomosis and not the surrounding intestinal segment. That means that if an anastomotic leakage is suspected, and the anastomotic line does not show leakage of contrast medium, this postoperative radiological monitoring method does not exclude intraluminal leakage from the surrounding bowel area, such as from a minor bowel wall damage arising from intraoperative bowel management with surgical instruments like Babcock forceps. It is also important to note that the significance of an early and small radiological contrast leakage is unknown, as contrast leakage during the early postoperative phase might not always develop into clinical anastomotic leakage. Hence, further trials are needed to investigate this potential application.

The catheter-based system of this novel anastomotic method enables fluid sample collections from the anastomotic line to investigate the dynamic aspects of the anastomotic healing process, such as assessing the local inflammatory response in the anastomotic wound related to cytokines, neutrophil activity, and growth factors. In addition, this anastomotic access could, in theory, be used for modulating the anastomotic healing process by local administration of specific medical compounds directly into the anastomosis, such as selective MMP inhibitors. This catheter-based access to the healing anastomosis could theoretically allow a gentle intermittent

suction to maintain a fixed adaptive pressure of the anastomotic tissue in the first postoperative days during the acute inflammatory phase.

In *Paper III*, we demonstrated that this catheter-based system could be used to quantify the intraoperative ACP and to evaluate and select the optimal intraoperative anastomotic strategy where two patients operated with the LL technique of the C-REX method with a low ACP were converted to circular stapled anastomosis instead. At present, surgeons are limited to assess rectal anastomoses by using air leak tests and morphological inspections only[162].

The larger ovular ring structure of the CARP and C-REX rings creates a broader surface area for wound healing in the anastomotic gap. This might benefit the anastomotic healing process and possibly lead to increased anastomotic strength.

Both intestinal staple lines must be removed to construct a staple-free anastomosis when using the transanal LR technique of the C-REX method. This is achieved in the proximal transected bowel end by removing the staple line before placing the anastomotic ring with the C-REX LapAid device. On the other hand, the distal staple line in the transected rectal end is gently invaginated into the distal anastomotic C-REX RectoAid ring if possible and removed when firing the RectoAid instrument and completing the LR anastomosis. In *Paper III*, we managed to remove the rectal staple line in all but two patients, where only a small portion of the staple line was left behind in these patients. The C-REX anastomotic technique is theoretically optimal when using the surgical method of transanal total mesorectal excision (TaTME), where the rectal stump is transected without the need for surgical staple devices.

As mentioned earlier, this thesis was not designed to evaluate the incidence of intestinal anastomotic leakage. We have now operated on 35 patients with resection of the descending colon, the sigmoid colon, or high anterior resection of the rectum with CARP and C-REX anastomoses with only one event of anastomotic leakage (2.9%), which is promising results for low colo-colic and high rectal anastomoses. These preliminary results might indicate that this novel method could lead to lower anastomotic healing complications, though the design of this thesis does not allow such conclusions.

The development and assessment of the CARP and C-REX devices have been time-consuming. During this journey, both structural- and mechanical changes have been performed to adapt and adjust these instruments to clinical practice. We have assessed both devices in experimental- and clinical settings and gathered significant experience a long the way, such as suboptimal ring sizes and difficulties in using the LL method in the pelvis. This long process has led to the development of the novel C-REX RectoAid device, which needs to be evaluated in low anterior resection.

Conclusions

This thesis evaluated the novel CARP and C-REX devices performing colorectal anastomoses in experimental- and clinical settings. The major conclusions are:

- The CARP method was safe and efficient in performing end-to-end colonic anastomoses following open resection of the sigmoid colon in a pig model. The catheter system of CARP could be used to measure intraoperative anastomotic contact pressure and assess the postoperative integrity of the anastomoses.
- The CARP method was safe and efficient in performing end-to-end colonic anastomoses following open resection of the descending- or sigmoid colon in human settings. As in study I, CARP´s catheter-based system could be used to measure intraoperative anastomotic contact pressure and assess the postoperative integrity of the anastomoses. The sizes of the CARP anastomotic rings were not optimal in this human safety study.
- The transanal LR technique of the C-REX method was safe and efficient in performing end-to-end colorectal anastomoses following open and minimally invasive high anterior resection in clinical settings. On the other hand, the LL technique of the C-REX method was cumbersome to use below the sacral promontory and should be limited to colonic anastomoses above the pelvic area.
- The C-REX LapAid device was safe and efficient in performing colorectal anastomoses 15 cm above the anal verge following open resection of the sigmoid colon in a pig model and demonstrated a high mechanical strength during the first postoperative week. The median bursting pressure in the C-REX LapAid anastomoses was more than 2-5 folds higher during the early phase of the anastomotic healing when compared to the traditional circular stapled anastomoses.

Future perspectives

This thesis evaluated the safety and usability of novel compression anastomotic devices in performing colorectal anastomoses in experimental- and clinical trials.

To proceed, a new human safety study is needed. This study should evaluate the safety and efficacy of the C-REX RectoAid device in performing colorectal anastomoses following low anterior resection of the rectum where the frequency of anastomotic leakage is the highest. The implementation of such a safety study is necessary before proceeding with a larger national and international randomized trial aiming at comparing the incidence of anastomotic leakage associated with this new method and the traditional circular stapling method used today.

This safety study is already in the pipeline. The study will evaluate the transanal LR method in constructing end-to-end colorectal anastomosis in 20 patients following open or minimally invasive low anterior resection of the rectum without a defunctioning loop ileostomy. Inclusion criteria are patients' ≥ 18 years of age scheduled for elective low anterior resection due to rectal cancer, having the cognitive ability to understand written and oral information, and accepting participation in the study. Exclusion criteria are ASA score ≥ 4, stage IV colorectal cancer, concurrent IBD, the need for an emergency procedure, albumin < 25g/L, current smoking, diabetes type 1 and 2, treatment with immunosuppressive medication less than one month before the procedure, and neoadjuvant oncological treatment prior to the surgery.

Besides assessing anastomotic adverse events, the local inflammatory response in the anastomotic wound (*e.g.* pro-inflammatory cytokines, neutrophil activity, and growth factors) will be analysed by collecting fluid samples directly from the anastomotic gap through the catheter-based system of the C-REX RectoAid during the first seven postoperative days. In addition, the fluid from the anastomotic wound will be sent for bacterial cultures during the third postoperative day, mapping the bacterial flora located in the anastomotic line at that specific time point.

This human safety study has already been approved by the Swedish Medical Products Agency, fulfilling the requirements of regulation 2017/745 of the European Parliament concerning CE-marked medical devices according to MDR Article 82. We are also awaiting ethical approval from the Swedish Ethical Review Authority.

If this clinical safety study demonstrates promising results, we will continue with a larger national and international randomized trial aiming at comparing the incidence of anastomotic leakage associated with the C-REX LR method and the traditional circular stapling method, in construction low rectal anastomoses.

The C-REX LR method was designed with the intention to reduce the anastomotic adverse events associated with rectal anastomoses. If this novel anastomotic method should demonstrate superiority in creating low rectal anastomoses compared to the traditional circular stapled method, it will have a large international impact for patients affected by rectal cancer, who can be treated with a low anterior resection. Decreased incidence of anastomotic leakage will reduce the rate of postoperative morbidity and mortality associated with this dreaded anastomotic adverse event and the necessity for defunctioning ileostomy.

Populärvetenskaplig sammanfattning

Introduktion

Tarmskarvsläckage är en av de mest fruktade komplikationerna vid tjocktarms- och ändtarmskirurgi. Läckage förekommer i 3–20% av ingreppen, med högre läckagefrekvens från tarmskarvar utförda i ändtarmen. Läckaget leder till påtagligt patientlidande, långdragen återhämtning, ökade vårdkostnader och är förenat med hög sjuklighet samt dödlighet som följd av infektionen som uppstår vid läckaget från tarmen. Tarmskarvsläckage som uppstår efter operation av tjocktarms- eller ändtarmscancer riskerar även att påverka den onkologiska behandlingen efter ingreppet och är möjligen associerad med högre risk för återfall av tumörsjukdomen och sämre långtidsöverlevnad.

I dag används två olika metoder för att skarva ihop tarmen efter att en del av tarmen blivit borttagen. Dels den handsydda- och dels den staplade (hophäftande) metoden. Trots att bägge metoderna anses vara säkra, är frekvensen av tarmskarvsläckage alldeles för hög. Det finns därför ett behov av att utveckla nya tarmskarvsmetoder för att försöka att åstadkomma en säkrare tarmskarv vid tjocktarms- och ändtarmskirurgi.

Målet med denna avhandling var att utveckla och utvärdera nya instrument, som kallas CARP och C-REX, som tillämpar en annan teknik för att skarva ihop tarmändar än de som används som standard i dag.

Arbete 1

I arbete 1 undersöktes CARP instrumentet vid hopkoppling av tarmen efter borttagning av sista delen av tjocktarmen, i en experimentell modell på gris. CARP-instrumentet var effektivt och säkert vid hopkoppling av tjocktarmen vid öppen teknik, där inga djur utvecklade tarmskarvsläckage. CARP metoden påvisade fin tarmskarvsläkning vid mikroskopisk undersökning. Metoden kunde användas för att mäta och kvantifiera kontakttrycket i tarmskarven direkt efter att den var utförd, och även användas för att bedöma tarmskarvens integritet med röntgengenomlysning upp till 4-5 dagar efter operationen.

Arbete 2

I arbete 2 undersöktes CARP instrumentet vid hopkoppling av tarmen efter borttagning av sista delen av tjocktarmen hos patienter med tjocktarmscancer. CARP instrumentet var effektivt och säkert vid utförande av en tarmskarv i tjocktarmen vid öppen teknik och inga patienter utvecklade tarmskarvsläckage. Storlekarna på CARP-ringarna var inte optimala, vilket resulterade i att 9 av 25 patienter inte gick att operera med tekniken på grund av att ringarana var för stora. CARP metoden påvisade fin tarmskarvsläkning vid koloskopi (kamera undersökning av tjocktarmen).

Arbete 3

I arbete 3 undersöktes C-REX instrumenten (LapAid och RectoAid) vid hopkoppling av tarmen efter borttagning av sista delen av tjocktarmen och övre delen av ändtarmen hos patienter med cancer i övergången mellan tjocktarmen och ändtarmen. Ingreppen utfördes med både öppen- och titthålsteknik och med två olika C-REX metoder (LL samt LR). LR metoden var effektiv och säker vid utförande av en tarmskarv i övre ändtarmen och inga patienter som opererades med LR metoden utvecklade tarmskarvsläckage.

Däremot var LL metoden svårare att utföra i bäckenet och ledde till ett tarmskarvsläckage av de sex opererade patienterna med tekniken. LL metoden rekommenderas därför inte för att utföra tarmskarvar i bäckenet. LL och LR metoderna påvisade fin tarmskarvsläkning vid koloskopisk undersökning av tjocktarmen i alla fall utom ett, som uppvisade en måttlig förträngning i tarmskarven, men utan kliniska tarmsymptom.

Arbete 4

I arbete 4 undersöktes C-REX LapAid instrumentet vid hopkoppling av tarmen efter borttagning av sista delen av tjocktarmen i en experimentell modell på gris, och jämfördes med tarmskarvar utförda med cirkulär staplad tarmskarvsteknik. Metoderna jämfördes avseende den tidiga mekaniska tarmskarvsstyrkan med så kallat ”bursting pressure” test. Undersökningen visade att C-REX LapAid tarmskarvarna hade högre mekanisk styrka i den tidiga fasen av läkningsprocessen jämfört med den staplade metoden.

Slutsatser

I denna avhandling undersöktes CARP och C-REX instrumenten vid hopkoppling av den sista delen av tjocktarmen och respektive övre delen av ändtarmen både i en experimentell modell på gris och på patienter. CARP ingreppen utfördes med öppen teknik och C-REX ingreppen med både öppen- och titthålsteknik.

CARP instrumentet var effektivt och säkert vid hopkoppling av tjocktarmen, men är begränsat till öppen kirurgisk teknik och till tarmskarvar ovanför bäckeningången.

C-REX RectoAid instrumentet (LR metoden) var effektivt och säkert vid hopkoppling av övre ändtarmen, och kunde användas vid både öppen- och titthålsteknik. LL metoden var tekniskt svårt att utföra i bäckenet och bör begränsas till tarmskarvar ovanför bäckenet, liksom CARP metoden.

C-REX metoden medför högre mekanisk styrka i hopkopplingen i den tidiga fasen jämfört med nu använda rutinmetoder, som kan gynna läkningen i tarmskarven.

Innan C-REX metoden kan utvärderas i en större national och international randomiserat klinisk studie, som jämför LR tarmskarvstekningen med den staplade tekniken, behöver en ytterligare säkerhetsstudie utföras som utvärderar C-REX LR metoden i att utföra låga ändtarmskarvar, där risken är som högst för tarmskarvsläckage. Denna säkerhetsstudie är redan på gång.

Vísindaleg samantekt á íslensku

Inngangur

Leki á hægðainnihaldi frá samtengingu þarma er mjög alvarlegur fylgikvilli sem getur komið upp í kjölfar þarmaaðgerða. Leki á sér stað í 3–20% slíkra skurðaðgerða, þar sem hæsta lekatíðnin er frá samtengingum í endaþarmi. Leki frá þarmatengingu leiðir til lífhimnubólgu með verulegri vanlíðan sjúklinga og töluverðri aukningu á veikinda- og dánartíðni. Þessi fylgikvilli hefur þannig áhrif á lengd sjúkrahússlegu og eykur töluvert heilbrigðiskostnað. Leki á hægðarinnihaldi frá þarmatengingum í kjölfar skurðaðgerða vegna krabbameins í ristli og endaþarmi getur einnig komið í veg fyrir að sjúklingar fái áætlaða krabbameinslyfjameðferð og er sennilegast tengdur aukinni áhættu á endurkomu æxlissins ásamt verri langtímalifun.

Í dag eru tvær mismunandi aðferðir notaðar við þarmatengingu eftir að búið er að fjarlægja þarmahluta, svokölluð handsaumuð og heftuð aðferð. Jafnvel þó að báðar þessar aðferðir séu taldar öruggar, þá er lekatíðnin allt of há. Það er því mikil þörf á að þróa nýjar aðferðir við samtengingu þarma og þannig gera þarmatengingar í kjölfar ristil- og endaþarmsaðgerða öruggari. Markmið þessarar doktorsritgerðar var að þróa og meta ný lækningatæki og aðferðir við samtengingu þarma, kölluð CARP og C-REX, sem beita allt annarri tækni við að samtengingu þarma en þær aðferðir sem notaðar eru í dag.

Grein 1

Í fyrstu greininni var CARP aðferðin metin í að samtengja þarma í kjölfar opins brottnáms bugaristils í svínamódeli. CARP aðferðin reyndist áhrifarík og örugg í að samtengja ristilendana og ekkert dýr fékk hægðaleka frá samtengingunni. CARP aðferðin sýndi fram á góðan gróanda í þarmatengingunni við vefjafræðilega smásjárskoðun. Aðferðina var hægt að nota til að mæla tengiþrýstinginn sem heldur þarmatengingunni saman beint eftir að hún var framkvæmd og heilleika þarmatengingarinnar með röntgenmyndatöku allt að 4-5 dögum eftir aðgerðina.

Grein 2

Í annarri greininni var CARP aðferðin metin í að samtengja þarma í kjölfar opins brottnáms á vinstri hluta ristils eða bugaristli hjá sjúklingum með ristilkrabbamein. CARP aðferðin reyndist áhrifarík og örugg í að samtengja ristilendana og enginn sjúklingur fékk hægðaleka frá tengingunni. Stærðir CARP hringjanna voru ekki ákjósanlegir, sem leiddi til að hjá 9 af 25 sjúklingum var ekki hægt að samtengja ristilendanna með CARP aðferðinni þar sem minnstu hringirnir voru of stórir til að passa inn í ristilinn. CARP sýndi fram á góðan gróanda í þarmatengingunni við ristilspeglun.

Grein 3

Í þriðju greininni voru C-REX lækningatækin LapAid og RectoAid metin í að samtengja þarma í kjölfar brottnáms bugaristils og efri hluta endaþarms hjá sjúklingum með krabbamein í neðsta hluta bugaristils eða efri hluta endaþarms. Aðgerðirnar voru framkvæmdar með opinni aðferð eða kviðsjártækni, með tveimur mismunandi C-REX aðferðum (LL og LR). LR aðferðin var áhrifarík og örugg við samtengingu þarma í efri hlutar endaþarms í grindarholinu og enginn LR sjúklingur fékk hægðaleka frá tengingunni.

Erfiðara var að útfæra LL-aðferðina í grindarholinu, sem leiddi til hægðaleka frá tengingunni hjá einum af sex sjúklingum sem voru meðhöndlaðir með þessari aðferð. Við mælum því ekki með að LL-aðferðin sé notuð við að samtengja þarma í grindarholi og eigi aðeins að nota í kviðarholi. Bæði LL og LR aðferðirnar sýndu fram á góðan gróanda í tengingunni í efri hluta endaþarms við ristilspeglun hjá öllum sjúklingum nema einum. Sá sjúklingur var með miðlungs þrengingu í samtengingunni, en án klínískra einkenna.

Grein 4

Í fjórðu greininni var C-REX LapAid aðferðin metin í að samtengja þarmaenda í kjölfar brottnáms bugaristils í svínamódeli og borin saman við samtengingar sem framkvæmdar voru með hefti-aðferð. Hinn snemmbúni mekaníski styrkur í tengingunni var borinn saman á milli C-REX LapAid samtengingar og hefti samtengingar, með svokölluðu „bursting pressure“ prófi. Rannsóknin sýndi fram á að C-REX LapAid tengingar höfðu hærri mekaníska styrk á fyrsta stigi gróandans í samtengingunni borið saman við heftuðu aðferðina.

Niðurstöður

Í þessari doktorsritgerð voru CARP og C-REX aðferðirnar metnar í að framkvæma samtengingar þarma í neðsta hluta ristils og efri hluta endaþarms í svínamódeli og hjá manneskjum. CARP samtengingarnar voru framkvæmdar með opinni tækni og C-REX samtengingarnar með ýmist opinni aðgerð eða með kviðsjártækni.

CARP aðferðin var áhrifarík og örugg í að útfæra samtengingar í ristli, en er takmörkuð við opna skurðaðgerð og við framkvæmd samtengingar þarma ofan grindarholsins.

C-REX RectoAid lækningatækið (LR aðferðin) var áhrifaríkt og öruggt við útfærslu þarmatenginga í efri hluta endaþarms í grindarholinu og var hægt að nota aðferðina við bæði opna aðgerð og í kviðsjártækni. Aftur á móti var LL aðferðin tæknilega erfið við framkvæmd þarmatenginga í grindarholinu og ætti að takmarkast við framkvæmd samtengingu þarma ofan grindarholsis, eins og á við um CARP aðferðina.

C-REX aðferðin leiðir til hærri snemmbúins mekanísk styrks í þarmatengingunni borið saman við heftuðu aðferðina, sem gæti stuðlað að betri gróanda í samtengingunni.

Áður en hægt er að meta C-REX LR aðferðina í stærri innlendri og alþjóðlegri slembivalsaðri klínískri rannsókn, sem ber hana saman við hefti-aðferðina í að samtengja þarminn eftir hlutabrottnám endaþarms, þarf að framkvæma aðra minni klíníska rannsókn sem metur C-REX LR aðferðina í að útfæra samtengingar í neðri hluta endaþarms, þar sem hættan á hægðaleka frá samtengingu er sem hæst. Sú rannsókn er í bígerð.

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Reference

1. Healy MA, Mullard AJ, Campbell DA Jr., Dimick JB. Hospital and payer costs associated with surgical complications. JAMA Surg. 2016;151:823–830. doi: 10.1001/jamasurg.2016.0773.
2. Vonlanthen R, Slankamenac K, Breitenstein S, Puhan MA, Muller MK, Hahnloser D, Hauri D, Graf R, Clavien P-A. The impact of complications on costs of major surgical procedures: a cost analysis of 1200 patients. Ann Surg. 2011;254:907–913. doi: 10.1097/SLA.0b013e31821d4a43.
3. Turrentine FE, Denlinger CE, Simpson VB, Garwood RA, Guerlain S, Agrawal A, Friel CM, LaPar DJ, Stukenborg GJ, Jones RS. Morbidity, mortality, cost, and survival estimates of gastrointestinal anastomotic leaks. J Am Coll Surg. 2015;220:195–206. doi: 10.1016/j.jamcollsurg.2014.11.002.
4. Branagan G, Finnis D; Wessex Colorectal Cancer Audit Working Group. Prognosis after anastomotic leakage in colorectal surgery. Dis Colon Rectum. 2005;48:1021–1026. doi: 10.1007/s10350-004-0869-4.
5. Krarup P-M, Nordholm-Carstensen A, Jorgensen LN, Harling H. Anastomotic leak increases distant recurrence and long-term mortality after curative resection for colonic cancer: a nationwide cohort study. Ann Surg. 2014;259:930–938. doi: 10.1097/SLA.0b013e3182a6f2fc.
6. Jang JH, Kim HC, Huh JW, Park YA, Cho YB, Yun SH, Lee WY, Yu JI, Park HC, Park YS, Park JO. Anastomotic leak does not impact oncologic outcomes after preoperative chemoradiotherapy and resection for rectal cancer. Ann Surg 2019;269:678–685. doi: 10.1097/SLA.0000000000002582.
7. Senn N. Enterorrhaphy; its history, technique and present status. JAMA. 1893;21:215-235.
8. Dietz U A, Debus E-S. Intestinal anastomoses prior to 1882; a legacy of ingenuity, persistence, and research form a foundation for modern gastrointestinal surgery. World J Surg. 2005;29(03):396-401. doi: 10.1007/s00268-004-7720-x.
9. Travers B. An inquiry into the process of nature in repairing injuries of the intestines; illustrating the treatment of penetrating wounds, and strangulated hernia. London. 1812.
10. Abramowitz HB, Butcher Jr HJ. Everting and inverting anastomoses. An experimental study of comparative safety. Am J Surg. 1971;121(1):52-56. doi: 10.1016/0002-9610(71)90077-8.
11. Goligher JC, Morris C, McAdam WA, De Dombal FT, Johnston D. A controlled trial of inverting versus everting intestinal suture in clinical large-bowel surgery. Br J Surg. 1970;57:817-822. doi: 10.1002/bjs.1800571106.

12. Lister J. On the antiseptic principle in the practice of surgery. BMJ. 1867;2:246-248. doi: 10.1136/bmj.2.351.246.
13. Gaidry AD, Tremblay L, Nakayama D, Ignacio RC Jr. The history of surgical staplers: a combination of Hungarian, Russian, and American Innovation. Am Surg. 2019;85:563–566. doi: 10.1177/000313481908500617.
14. Akopov A, Artioukh DY, Molnar TF: Surgical Staplers: The History of Conception and Adoption. Ann Thorac Surg. 2021;112:1716-21. doi: 10.1016/j.athoracsur.2021.03.107.
15. Enoch S, Leaper DJ. Basic science of wound healing. Surgery. 2008:26(2):31-37. doi: 10.1016/j.mpsur.2007.11.005.
16. Thompson SK, Chang EY, Jobe BA. Clinical review: healing in gastrointestinal anastomoses, part I. Microsurgery. 2006;26:131-136. doi: 10.1002/micr.20197.
17. Lam A, Fleischer B, Alverdy J. The biology of anastomotic healing-the unknown overwhelms the known. Gastrointestinal Surgery. 2020;24:2160-2166. doi: 10.1007/s11605-020-04680-w.
18. Thornton FJ, Barbul A. Healing in the gastrointestinal tract. Surg Clin North Am. 1997;77:549-573. doi: 10.1016/s0039-6109(05)70568-5.
19. Martens MF, Hendriks T. Postoperative changes in collagen synthesis in intestinal anastomoses of the rat: differences between small and large bowel. Gut. 1991;32:1482-1487. doi: 10.1136/gut.32.12.1482.
20. Chen C. The art of bowel anastomosis. Scand J Surg. 2012;101:238-240. doi: 10.1177/145749691210100403.
21. Ho YH, Ashour MAT. Techniques for colorectal anastomosis. World J Gastroenterol. 2010;16:1610-1621. doi: 10.3748/wjg.v16.i13.1610.
22. Page-McCaw A, Ewald AJ, Werb Z. Matrix metalloproteinases and the regulation of tissue remodelling. Nat Rev Mol Cell Biol. 2007;8:221-233. doi: 10.1038/nrm2125.
23. Steffensen B, Häkkinen L, Larjava H. Proteolytic events of wound-healing--coordinated interactions among matrix metalloproteinases (MMPs), integrins, and extracellular matrix molecules. Crit Rev Oral Biol Med. 2001;12:373-398. doi: 10.1177/10454411010120050201.
24. Salo AM, Myllyharju J. Prolyl and lysyl hydroxylases in collagen synthesis. Exp Dermatol. 2021;30:38-49. doi: 10.1111/exd.14197.
25. Hendriks T, Mastboom WJ. Healing of experimental intestinal anastomoses. Parameters for repair. Dis Colon Rectum. 1990;33:891-901. doi: 10.1007/BF02051930.
26. Månsson P, Zhang X W, Jeppson B, Thorlacius H. Anastomotic healing in rat colon: Comparison between a radiological method, breaking strength and bursting pressure. Int J Colorectal Dis. 2002;17:420-425. doi: 10.1007/s00384-002-0392-9.
27. Jönsson K, Jiborn H, Zederfeldt B. Changes in collagen content of the small intestinal wall following anastomosis. Am J Surg. 1984;150:315-317. doi: 10.1016/0002-9610(85)90068-6.
28. Jönsson K, Jiborn H, Zederfeldt B. Collagen metabolism in small intestinal anastomosis. Am J Surg. 1987;154:288-291. doi: 10.1016/0002-9610(89)90612-0.

29. Törnqvist A, Blomquist P, Jiborn H, Zederfeldt B. Anastomotic healing after resection of a left-colon stenosis. Effect on collagen metabolism and anastomotic strength. Dis Col & Rect. 1990;33:217-221. doi: 10.1007/BF02134183.

30. Bosmans JWAM, Jongen ACHM, Bouvy ND, Derikx JPM. Colorectal anastomotic healing: why the biological processes that lead to anastomotic leakage should be revealed prior to conducting intervention studies. BMC Gastroenterol. 2015;15:180. doi: 10.1186/s12876-015-0410-3.

31. de Hingh IHJT, van Goor H, de Man BM, Lomme RMLM, Bleichrodt RP, Hendriks T. Selective cyclo-oxygenase 2 inhibition affects ileal but not colonic anastomotic healing in the early postoperative period. Br J Surg. 2006;93:489-497. doi: 10.1002/bjs.5288.

32. da Costa MAR, Campos ACL, Coelho JCU, de Barros AM, Matsumoto HM. Oral glutamine and the healing of colonic anastomoses in rats. JPEN J Parenter Enteral Nutr. 2003;27:182-185. doi: 10.1177/0148607103027003182.

33. Högström H, Haglund U. Postoperative decrease in suture holding capacity in laparotomy wounds and anastomoses. Acta Chir Scand. 1985; 151:533-535.

34. Högström H, Haglund U, Zederfeldt B. Suture technique and early strength of intestinal anastomoses and laparotomy wounds. Acta Chir Scand. 1985;151:441-443.

35. Weiber S, Jiborn H, Zederfeldt B. Preoperative irradiation and colonic healing. Eur J Surg. 1994;160:47-51.

36. Jiborn H, Ahonen J, Zederfeldt B. Healing of experimental colonic anastomoses. I. Bursting strength of the colon after left colon resection and anastomosis. Am J Surg. 1978;136:587-594. doi: 10.1016/0002-9610(78)90315-x.

37. Nelsen TS, Anders CJ. Dynamic aspects of small intestinal rupture with special consideration of anastomotic strength. Arch Surg 1966;93:309-14. doi: 10.1001/archsurg.1966.01330020101016.

38. Bruce J, Krukowski ZH, Al-Khairy G, Russel EM, Park KG. Systematic review of the definition and measurement of anastomotic leak after gastrointestinal surgery. Br J Surg 2001;88:1157-1168. doi: 10.1046/j.0007-1323.2001.01829.x.

39. Rahbari NN, Weitz J, Hohenberger W, Heald RJ, Moran B, Ulrich A, Holm T, Wong WD, Tiret E, Moriya Y, Laurberg S, den Dulk M, van de Velde C, Büchler MW. Definition and grading of anastomotic leakage following anterior resection of the rectum: a proposal by the International Study Group of Rectal Cancer. Surgery. 2010;147:339-351. doi: 10.1016/j.surg.2009.10.012.

40. Ellis CT, Maykel JA. Defining anastomotic leak and the clinical relevance of leaks. Clin Colon Rectal Surg. 2021;34:359-365. doi: 10.1055/s-0041-1735265.

41. McKenna NP, Bews KA, Cima RR, Crowson CS, Habermann EB. Development of a risk score to predict anastomotic leak after left-sided colectomy: which patients warrant diversion? J Gastrointest Surg. 2020;24:132–143. doi: 10.1007/s11605-019-04293-y.

42. Matthiessen P, Hallböök O, Rutegård J, Simert G, Sjödahl R. Defunctioning stoma reduces symptomatic anastomotic leakage after low anterior resection of the rectum for cancer: a randomized multicenter trial. Ann Surg. 2007;246:207-214. doi: 10.1097/SLA.0b013e3180603024.

43. Bostrom P, Haapamäki MM, Rutegård J, Matthiessen P, Rutegård M. Population-based cohort study of the impact on postoperative mortality of anastomotic leakage after anterior resection for rectal cancer. BJS Open. 2019;3:106-111. doi: 10.1002/bjs5.50106.

44. Bakker IS, Grossmann I, Henneman D, Havenga K, Wiggers T. Risk factors for anastomotic leakage and leak-related mortality after colonic cancer surgery in a nationwide audit. Br J Surg. 2014;101:424–432;discussion 432. doi: 10.1002/bjs.9395.

45. Krarup P-M, Jorgensen LN, Andreasen AH, Harling H; Danish Colorectal Cancer Group. A nation-wide study on anastomotic leakage after colonic cancer surgery. Color Dis. 2012;14:e661–e667. doi: 10.1111/j.1463-1318.2012.03079.x.

46. Eriksen MT, Wibe A, Norstein J, Haffner J, Wiig JN; Norwegian Rectal Cancer Group. Anastomotic leakage following routine mesorectal excision for rectal cancer in a national cohort of patients. Colorectal Dis. 2005;7:51–57. doi: 10.1111/j.1463-1318.2004.00700.x.

47. Choi H-K, Law W-L, Ho JWC. Leakage after resection and intraperitoneal anastomosis for colorectal malignancy: analysis of risk factors. Dis Colon rectum. 2006;49:1719–1725. doi: 10.1007/s10350-006-0703-2.

48. Gessler B, Eriksson O, Angenete E. Diagnosis, treatment, and consequences of anastomotic leakage in colorectal surgery. Int J Colorectal Dis. 2017;32:549-556. doi: 10.1007/s00384-016-2744-x.

49. El-Ahmar M, Koch F, Ristig M, Lehmann K, Ritz JP. Reconstruction, oversewing, or taking the anastomosis down – which surgical intervention is most potent in the treatment of anastomotic leaks following colorectal resections? Langenbecks Arch Surg. 2023;408:266. doi: 10.1007/s00423-023-02986-2.

50. Hernandez PT, Paspulati RM, Shanmugan S. Diagnosis of anastomotic leak. Clin Colon Rectal Surg. 2021;34:391-399. doi: 10.1055/s-0041-1735270.

51. Lim S-B, Yu CS, Kim CW, Yoon YS, Park IJ, Kim JC. Late anastomotic leakage after low anterior resection in rectal cancer patients: clinical characteristics and predisposing factors. Colorectal Dis. 2016;18:O135-140. doi: 10.1111/codi.13300.

52. Thornton M, Joshi H, Vimalachandran C, Heath R, Carter P, Gur U, Rooney P. Management and outcome of colorectal anastomotic leaks. Int J Colorectal Dis. 2011; 26:313–320. doi: 10.1007/s00384-010-1094-3.

53. Hyman N, Manchester TL, Osler T, Burns B, Cataldo PA. Anastomotic leaks after intestinal anastomosis: it's later than you think. Ann Surg 2007;245(02):254–258. doi: 10.1097/01.sla.0000225083.27182.85.

54. Jutesten H, Draus J, Frey J, Neovius G, Lindmark G, Buchwald P, Lydrup ML. Late leakage after anterior resection: a defunctioning stoma alters the clinical course of anastomotic leakage. Colorectal Dis. 2018;20:150-159. doi: 10.1111/codi.13914.

55. Matthiessen P, Lindgren R, Hallböök O, Rutegård J, Sjödahl R. Symptomatic anastomotic leakage diagnosed after hospital discharge following low anterior resection for cancer. Colorectal Dis. 2010; 12(7 online):e82-87. doi: 10.1111/j.1463-1318.2009.01938.x.

56. Macarthur DC, Nixon SJ, Aitken RJ. Avoidable deaths still occur after large bowel surgery. Scottish audit of surgical mortality, Royal College of Surgeons of Edinburgh. Br J Surg. 1998;85:80-83. doi: 10.1046/j.1365-2168.1998.00554.x.

57. Alves A, Panis Y, Trancart D, Regimbeau J-M, Pocard M, Valleur P. Factors associated with clinical significant anastomotic leakage after large bowel resection: multivariate analysis of 707 patients. World J Surg. 2002;26:499-502. doi: 10.1007/s00268-001-0256-4.

58. Kushner I. The phenomenon of the acute phase response: Ann N Y Acad Sci. 1982;389:39-48. doi: 10.1111/j.1749-6632.1982.tb22124.x.

59. Simons JP, Loeffler JM, Al-Shawi R, Ellmerich S, Hutchinson WL, Tennent GA, Petrie A, Raynes JG, de Souza JB, Lawrence RA, Read KD, Pepys MB. C-reactive protein is essential for innate resistance to pneumococcal infection. Immunology. 2014;142:414-20. doi: 10.1111/imm.12266.

60. Welsch T, Müller SA, Ulrich A, Kischlat A, Hinz U, Kienle P, Büchler MW, Schmidt J, Schmied BM. C-reactive protein as early predicator for infectious postoperative complications in rectal surgery. Int J Colorectal Dis. 2007;22:1499-1507. doi: 10.1007/s00384-007-0354-3.

61. Kørner H, Nielsen HJ, Søreide JA, Nedrebø BS, Søreide K, Knapp JC. Diagnostic accuracy of C-reactive protein for intraabdominal infections after colorectal resections. J Gastrointest Surg. 2009;13:1599-1606. doi: 10.1007/s11605-009-0928-1.

62. Daams F, Wu Z, Lahaye MJ, Jeekel J, Lange JF. Prediction and diagnosis of colorectal anastomotic leakage: a systematic review of literature. World J Gastrointest Surg. 2014;6:14–26. doi: 10.4240/wjgs.v6.i2.14.

63. Hirst NA, Tiernan JP, Millner PA, Jayne DG. Systematic review of methods to predict and detect anastomotic leakage in colorectal surgery. Colorectal Dis. 2014;16:95–109. doi: 10.1111/codi.12411.

64. Kornmann VNN, Treskes N, Hoonhout LHF, Bollen TL, van Ramshorst B, Boerma D. Systematic review on the value of CT scanning in the diagnosis of anastomotic leakage after colorectal surgery. Int J Colorectal Dis. 2013;28:437-445. doi: 10.1007/s00384-012-1623-3.

65. Marres CCM, van de Ven AWH, Leijssen LGJ, Verbeek PCM, Bemelman WA, Buskens CJ. Colorectal anastomotic leak: delay in reintervention after false-negative computed tomography scan is a reason for concern. Tech Coloproctol. 2017;21:709-714. doi: 10.1007/s10151-017-1689-6.

66. Eckmann C, Kujath P, Schiedeck THK, Shekarriz H, Bruch H-P. Anastomotic leakage following low anterior resection: result of a standardized diagnostic and therapeutic approach. Int J Colorectal Dis. 2004;19:128-133. doi: 10.1007/s00384-003-0498-8.

67. Lindner S, Eitelbuss S, Hetjens S, Gawlitza J, Hardt J, Seyfried S, Galata C, Reissfelder C, Sandra-Petrescu F, Herrle F. Less is more – the best test for anastomotic leaks in rectal cancer patients prior to ileostomy reversal. Int J Colorectal Dis. 2021;36:2387-2398. doi: 10.1007/s00384-021-03963-1.

68. Fang AH, Chao W, Ecker M. Review of colonic anastomotic leakage and prevention methods. J Clin Med. 2020;9:4061. doi: 10.3390/jcm9124061.

69. Thomas MS, Margolin DA. Management of colorectal anastomotic leak. Clin Colon Rectal Surg. 2016;29:138-144. doi: 10.1055/s-0036-1580630.

70. Clifford, RE, Fowler H, Govindarajah N, Vimalachandran D, Sutton PA. Early anastomotic complications in colorectal surgery: A systematic review of techniques for endoscopic salvage. Surg Endosc. 2019;33:1049-1065. doi: 10.1007/s00464-019-06670-9.

71. Boyce SA, Harris C, Stevenson A, Lumley J, Clark D. Management of low colorectal anastomotic leakage in the laparoscopic era: more than a decade of experience. Dis Colon Rectum. 2017;60:807-814. doi: 10.1097/DCR.0000000000000822.

72. Krarup P-M, Jorgensen LN, Harling H; Danish Colorectal Cancer Group. Management of anastomotic leakage in a nationwide cohort of colonic cancer patients. J Am Coll Surg. 2014:218:940-949. doi: 10.1016/j.jamcollsurg.2014.01.051.

73. Law WL, Choi HK, Lee YM, Ho JWC, Seto CL. Anastomotic leakage is associated with poor long-term outcome in patients after curative colorectal resection for malignancy. J. Gastrointest Surg. 2007;11:8-15. doi: 10.1007/s11605-006-0049-z.

74. Mirnezami A, Mirnezami R, Chandrakumaran K, Sasapu K, Sagar P, Finan P. Increased local recurrence and reduced survival from colorectal cancer following anastomotic leak: systematic review and meta-analysis. Ann Surg. 2011;253:890-899. doi: 10.1097/SLA.0b013e3182128929.

75. Wang S, Liu J, Wang S, Zhao H, Ge S, Wang W. Adverse Effects of Anastomotic Leakage on Local Recurrence and Survival After Curative Anterior Resection for Rectal Cancer: A Systematic Review and Meta-analysis. World J Surg. 2017;41:277-84. doi: 10.1007/s00268-016-3761-1.

76. Yang J, Chen Q, Jindou L, Cheng Y. The influence of anastomotic leakage for rectal cancer oncologic outcome: A systematic review and meta-analysis. J Surg Oncol. 2020;121(8):1283-97. doi: 10.1002/jso.25921.

77. Koedam TWA, Bootsma BT, Deijen CL, van de Brug T, Kazemier G, Cuesta MA, Fürst A, Lacy AM, Haglind E, Tuynman JB, Daams F, Bonjer HJ. Oncological Outcomes After Anastomotic Leakage After Surgery for Colon or Rectal Cancer: Increased Risk of Local Recurrence. Ann Surg. 2022;275(2):e420-e427. doi: 10.1097/SLA.0000000000003889.

78. Phatak UR, Kao LS, You YN, Rodriguez-Bigas MA, Skibber JM, Feig BW, Nguyen S, Cantor SB, Chang GJ. The impact of ileostomy-related complications on the multidisciplinary treatment of rectal cancer. Ann Surg Oncol. 2014;21:507-512. doi: 10.1245/s10434-013-3287-9.

79. Chow A, Tilney HS, Paraskevas P, Jeyarajah S, Zacharakis E, Purkayastha S. The morbidity surrounding reversal of defunctioning ileostomies: a systematic review of 48 studies including 6107 cases. Int J Colorectal Dis. 2009;24:711-723. doi: 10.1007/s00384-009-0660-z.

80. Rutegård M, Häggström J, Back E, Holmgren K, Wixner J, Rutegård J, Matthiessen P, Sjöström O. Defunctioning loop ileostomy in anterior resection for rectal cancer and subsequent renal failure: nationwide population-based study. BJS Open. 2023;7:zrad010. doi: 10.1093/bjsopen/zrad010.

81. Bax TW, McNevin MS. The value of diverting loop ileostomy on the high-risk colon and rectal anastomosis. Am J Surg. 2007;193:585-588. doi: 10.1016/j.amjsurg.2007.01.005.

82. Messaris E, Sehgal R, Deiling S, Koltun WA, Stewart D, McKenna K, Poritz LS. Dehydration is the most common indication for readmission after diverting ileostomy creation. Dis Colon Rectum. 2012;55:175-180. doi: 10.1097/DCR.0b013e31823d0ec5.

83. Jutesten H, Draus J, Frey J, Neovius G, Lindmark G, Buchwald P, Lydrup ML. High risk of permanent stoma after anastomotic leakage in anterior resection for rectal cancer. Colorectal Dis. 2019;21:174-182. doi: 10.1111/codi.14469.

84. Davis B, Rivadeneira DE. Complications of colorectal anastomoses: leaks, strictures and bleeding. Surg Clin North Am. 2013;93:61-87. doi: 10.1016/j.suc.2012.09.014.

85. Park JS, Choi G-S, Kim SH, Kim HR, Kim NK, Lee KY, Kang SB, Kim JY, Lee KY, Kim BC, Bae BN, Son GM, Lee SI, Kang H. Multicenter analysis of risk factors for anastomotic leakage after laparoscopic rectal cancer excision: the Korean laparoscopic colorectal surgery study group. Ann Surg. 2013;257:665-671. doi: 10.1097/SLA.0b013e31827b8ed9.

86. Kim JS, Cho SY, Min BS, Kim NK. Risk factors for anastomotic leakage after laparoscopic intracorporal colorectal anastomosis with a double stapling technique. J Am Coll Surg. 2009;209:694-701. doi: 10.1016/j.jamcollsurg.2009.09.021.

87. Kawada K, Hasegawa S, Hida K, Hirai K, Okoshi K, Nomura A, Kawamura J, Nagayama S, Sakai Y. Risk factors for anastomotic leakage after laparoscopic low anterior resection with DST anastomosis. Surg Endosc. 2014;28:2988-2995. doi: 10.1007/s00464-014-3564-0.

88. Katsuno H, Shiomi A, Ito M, Koide Y, Maeda K, Yatsuoka T, Hase K, Komori K, Minami K, Sakamoto K, Saida Y, Saito N. Comparison of symptomatic anastomotic leakage following laparoscopic and open low anterior resection for rectal cancer: a propensity score matching analysis of 1014 consecutive patients. Surg Endosc. 2016;30:2848-2856. doi: 10.1007/s00464-015-4566-2.

89. Kim CW, Baek SJ, Hur H, Min BS, Baik SH, Kim NK. Anastomotic leakage after low anterior resection for rectal cancer is different between minimally invasive surgery and open surgery. Ann Surg. 2016;263:130-137. doi: 10.1097/SLA.0000000000001157.

90. Qu H, Liu Y, Bi D-S. Clinical risk factors for anastomotic leakage after laparoscopic anterior resection for rectal cancer: a systematic review and meta-analysis. Surg Endosc. 2015;29:3608-3617. doi: 10.1007/s00464-015-4117-x.

91. Egorov VI, Schastlivtsev V, Turusov RA, Baranov AO. Participation of the intestinal layers in supplying of the mechanical strength of the intact and sutured gut. Eur Surg Res. 2002;34:425-431. doi: 10.1159/000065712.

92. Fielding LP, Stewart-Brown S, Blesovsky L, Kearney G. Anastomotic integrity after operations for large-bowel cancer: a multicenter study. Br Med J. 1980;281:411-414. doi: 10.1136/bmj.281.6237.411.

93. Favuzza J. Risk Factors for Anastomotic Leak, Consideration for Proximal Diversion, and Appropriate Use of Drains. Clin Colon Rectal Surg. 2021;34:366-370. doi: 10.1055/s-0041-1735266.

94. McDermott FD, Heeney A, Kelly ME, Steele RJ, Carlson GL, Winter DC. Systematic review of preoperative, intraoperative and postoperative risk factors for colorectal anastomotic leaks. Br J Surg. 2015;102:462-479. doi: 10.1002/bjs.9697.

95. Kang CY, Halabi WJ, Chaudhry OO, Nguyen V, Pigazzi A, Carmichael JC, Mills S, Stamos MJ. Risk factors for anastomotic leakage after anterior resection for rectal cancer. JAMA Surg. 2013;148:65-71. doi: 10.1001/2013.jamasurg.2.

96. Peeters KCMJ, Tollenaar RAEM, Marijnen CAM, Kranenbarg EK, Steup WH, Wiggers T, Rutten HJ, van de Velde CJH; Dutch Colorectal cancer Group. Risk factors for anastomotic failure after total mesorectal excision of rectal cancer. Br J Surg. 2005;92:211-216. doi: 10.1002/bjs.4806.

97. Matthiessen P, Hallböök O, Andersson M, Rutegård J, Sjödahl R. Risk factors for anastomotic leakage after anterior resection of the rectum. Colorectal Dis. 2004;6:462-469. doi: 10.1111/j.1463-1318.2004.00657.x.

98. Bertelsen CA, Andreasen AH, Jørgensen T, Harling H; Danish Colorectal Cancer Group. Anastomotic leakage after curative anterior resection for rectal cancer: short and long-term outcome. Colorectal Dis. 2010;12:37-43. doi: 10.1111/j.1463-1318.2008.01711.x.

99. Sørensen LT, Jørgensen T, Kirkeby LT, Skovdal J, Vennits B, Wille-Jørgensen P. Smoking and alcohol abuse are major risk factors for anastomotic leakage in colorectal surgery. Br J Surg. 1999;86:927-931. doi: 10.1046/j.1365-2168.1999.01165.x.

100. Richards CH, Campbell V, Ho C, Hayes J, Elliott T, Thompson-Fawcett M. Smoking is a major risk factor for anastomotic leak in patients undergoing low anterior resection. Colorectal Dis. 2012;14:628-633. doi: 10.1111/j.1463-1318.2011.02718.x.

101. Golub R, Golub RW, Cantu Jr R, Stein HD. A multivariate analysis of factors contributing to leakage of intestinal anastomoses. J Am Coll Surg. 1997;184:364-372.

102. Krysa J, Patel V, Taylor J, Williams AB, Carapeti E, George ML. Outcome of patients on renal replacement therapy after colorectal surgery. Dis Colon Rectum. 2008;51:961–96. doi: 10.1007/s10350-008-9225-4.

103. Jestin P, Påhlman L, Gunnarsson U. Risk factors for anastomotic leakage after rectal cancer surgery: a case-control study. Colorectal Dis. 2008;10:715-721. doi: 10.1111/j.1463-1318.2007.01466.x.

104. Cohn Jr I, Rivers JD. Antibiotic protection of colon anastomoses. Ann Surg. 1955;141:707-717. doi: 10.1097/00000658-195505000-00016.

105. Anderson DI, Keskey R, Ackerman MT, Zaborina O, Hyman N, Alverdy JC, Shogan BD. Enterococcus faecalis is associated with anastomotic leak in patients undergoing colorectal surgery. Surg Infect (Larchmt). 2021;22:1047-1051. doi: 10.1089/sur.2021.147.

106. Williamson AJ, Alverdy JC. Influence of the Microbiome on Anastomotic Leak. Clin Colon Rectal Surg. 2021;34:439-446. doi: 10.1055/s-0041-1735276.

107. Hernández-González PI, Barquín J, Ortega-Ferrete A, Patón V, Ponce-Alonso M, Romero-Hernández B, Ocaña J, Caminoa A, Conde-Moreno E, Galeano J, Campo RD, García-Pérez JC. Anastomotic leak in colorectal cancer surgery: Contribution of gut microbiota and prediction approaches. Colorectal Dis. 2023;25:2187-2197. doi: 10.1111/codi.16733.

108. Johansson MEV, Sjövall H, Hansson GC. The gastrointestinal mucus system in health and disease. Nat Rev Gastroenterol Hepatol. 2013;10:352-361. doi: 10.1038/nrgastro.2013.35.

109. Bosmans JWAM, Jongen ACHM, Birchenough GMH, Nyström EEL, Gijbels MJJ, Derikx JPM, Bouvy ND, Hansson GC. Functional mucous layer and healing of proximal colonic anastomoses in an experimental model. Br J Surg. 2017;104:619-630. doi: 10.1002/bjs.10456.

110. Yeh CY, Changchien CR, Wang J-Y, Chen J-S, Chen HH, Chiang J-M, Tang R. Pelvic drainage and other risk factors for leakage after elective anterior resection in rectal cancer patients: a prospective study of 978 patients. Ann Surg. 2005;241:9-13. doi: 10.1097/01.sla.0000150067.99651.6a.

111. Jung SH, Yu CS, Choi PW, Kim DD, Park IJ, Kim HC, Kim JC. Risk factors and oncologic impact of anastomotic leakage after rectal cancer surgery. Dis Colon Rectum. 2008;51:902-908. doi: 10.1007/s10350-008-9272-x.

112. Eriksen TF, Lassen CB, Gögenur I. Treatment with corticosteroids and the risk of anastomotic leakage following lower gastrointestinal surgery: a literature survey. Colorectal Dis. 2014;16:O154-160. doi: 10.1111/codi.12490.

113. Lipska MA, Bissett IP, Parry BR, Merrie AEH. Anastomotic leakage after lower gastrointestinal anastomosis: men are at a higher risk. ANZ J Surg. 2006;76:579–585. doi: 10.1111/j.1445-2197.2006.03780.x.

114. Jouvin I, Lefevre JH, Creavin B, Pitel S, Chafai N, Tiret E, Beaugerie L, Parc Y; Saint-Antoine IBD Network. Postoperative morbidity risks following ileocolic resection for Crohn's disease treated with anti-TNF alpha therapy: a retrospective study of 360 patients. Inflamm Bowel Dis. 2018;24:422–432. doi: 10.1093/ibd/izx036.

115. Krarup P-M, Rehn M, Sand-Dejmek J, Ehrnström R, Ågren MS, Syk I. Rapid morphological changes and loss of collagen following experimental acute colonic obstruction. Int J Colorectal Dis. 2013;28:341-347. doi: 10.1007/s00384-012-1548-x.

116. Syk I, Mirastschijski U, Jeppson BW, Ågren MS. Experimental colonic obstruction increases collagen degradation by matrix metalloproteinases in the bowel wall. Dis Colon Rectum. 2003;46:1251-1259. doi: 10.1007/s10350-004-6723-x.

117. Rehn M, Ågren MS, Syk I. Collagen levels are normalized after decompression of experimentally obstructed colon. Colorectal Dis. 2011;13:e165-e169. doi: 10.1111/j.1463-1318-2011.02633.x.

118. Agren MS, Andersen TL, Mirastschijski U, Syk I, Schiødt CB, Surve V, Lindebjerg J, Delaissé J-M. Action of matrix metalloproteinases at restricted sites in colon anastomosis repair: an immunohistochemical and biochemical study. Surgery. 2006;140:72-82. doi: 10.1016/j.surg.2005.12.013.

119. de Hingh IH, de Man BM, Lomme RM, van Goor H, Hendriks T. Colonic anastomotic strength and matrix metalloproteinase activity in an experimental model of bacterial peritonitis. Br J Surg. 2003;90:981-988. doi: 10.1002/bjs.4146.

120. Ballantyne GH. The experimental basis of intestinal suturing. Effects of surgical technique, inflammation, and infection on enteric wound healing. Dis Colon Rectum. 1984;27:61-71. doi: 10.1007/BF02554084.

121. Lim CBB, Goldin RD, Darzi A, Hanna GB. Characterization of materials eliciting foreign body reaction in stapled human gastrointestinal anastomoses. Br J Surg. 2008;95:1044-1050. doi: 10.1002/bjs.6071.

122. Berho M, Wexner SD, Botero-Anug A-M, Pelled D, Fleshman JW. Histopathologic advantages of compression ring anastomosis healing as compared with stapled anastomosis in a porcine model: a blinded comparative study. Dis Colon Rectum. 2014;57:506-513. doi: 10.1097/DCR.0000000000000009.

123. Koruda MJ, Rolandelli RH. Experimental studies on the healing of colonic anastomoses. J Surg Res. 1990;48:504-515. doi: 10.1016/0022-4804(90)90021-s.

124. Senagore A, Milsom JW, Walshaw RK, Dunston R, Chaudry IH. Direct comparison between Czerny-Lembert and circular-stapled anastomotic techniques in colorectal anastomosis: a similar pattern of healing for both. Dis Colon Rectum. 1992;35:862-869. doi: 10.1007/BF02047874.

125. Neutzling CB, Lustosa SAS, Proenca IM, da Silva EMK, Matos D. Stapled versus handsewn methods for colorectal anastomosis surgery. Cochrane Database Syst Rev. 2012;15:CD003144. doi: 10.1002/14651858.CD003144.pub2.

126. MacRae HM, McLeod RS. Handsewn vs. stapled anastomoses in colon and rectal surgery: a meta-analysis. Dis Colon Rectum. 1998;41:180-189. doi: 10.1007/BF02238246.

127. Shikata S, Yamagishi H, Taji Y, Shimada T, Noguchi Y. Single- versus two-layer intestinal anastomosis: a meta-analysis of randomized controlled trials. BMC Surg. 2006;6:2. doi: 10.1186/1471-2482-6-2.

128. Kar S, Mohapatra V, Singh S, Rath PK, Behera TR. Single layered versus double layered intestinal anastomosis: a randomized controlled trial. J Clin Diagn Res. 2017;11:PC01-PC04. doi: 10.7860/JCDR/2017/24817.9983.

129. Sajid MS, Siddiqui MRS, Baig MK. Single layer versus double layer suture anastomosis of the gastrointestinal trac. Cochrane Database of Systematic Reviews. 2012;1:CD005477. doi: 10.1002/14651858.CD005477.pub4.

130. Ricciardi R, Roberts PL, Marcello PW, Hall JF, Read TE, Schoetz DJ. Anastomotic leak testing after colorectal resection. What are the data? Arch Surg. 2009;144:407-411. doi: 10.1001/archsurg.2009.43.

131. Zbar AP, Nir Y, Weizman A, Rabau M, Senagore A. Compression anastomoses in colorectal surgery: a review. Tech Coloproctol. 2012;16:187-199. doi: 10.1007/s10151-012-0825-6.

132. Kaidar-Person O, Rosenthal RJ, Wexner SD, Szomstein S, Person B. Compression anastomosis: history and clinical consideration. Am J Surg. 2008;195:818-826. doi: 10.1016/j.amjsurg.2007.10.006.

133. Aggarwal R, Darzi A. Compression anastomoses revisited. J Am Coll Surg. 2005;201:965-971. doi: 10.1016/j.jamcollsurg.2005.06.255.

134. Ghitulescu GA, Morin N, Jetty P, Belliveau P. Revisiting the biofragmentable anastomotic ring: is it safe in colonic surgery? Can J Surg. 2003;46:92-98.

135. Påhlman L, Ejerblad S, Graf W, Kader F, Kressner U, Lindmark G, Raab Y. Randomized trial of a biofragmentable bowel anastomosis ring in high-risk colonic resection. Br J Surg. 1997;84:1291-1294. doi: 10.1046/j.1365-2168.1997.02771.x.

136. Chen S, Yang B, He J-H, Zhang Y-C, Lai D-M. Randomized trial on the application of biofragmentable anastomosis ring in intestinal anastomosis. Chin Med J (Engl). 2009;122:1755-1758.

137. Forde KA, Goodell KH, DellaBadia M. A 10-year single-institutional study of the biofragmentable anastomosis ring. Am J Surg. 2006;191:483-487. doi: 10.1016/j.amjsurg.2006.01.005.

138. Choi HJ, Kim HH, Jung GJ, Kim SS. Intestinal anastomosis by use of the biofragmentable anastomotic ring: is it safe and efficacious in emergency operations as well? Dis Colon Rectum. 1998;41:1281-1286. doi: 10.1007/BF02258229.

139. Corman ML, Prager ED, Hardy Jr TG, Bubrick MP. Comparison of the Valtrac biofragmentable anastomosis ring with conventional suture and stapled anastomosis in colon surgery. Results of a prospective, randomized clinical trial. Dis Colon Rectum. 1989;32:183-187. doi: 10.1007/BF02554523.

140. Kim S-H, Choi H-J, Park K-J, Kim J-M, Kim K-H, Kim M-C, Kim Y-H, Cho S-H, Jung G-J. Sutureless intestinal anastomosis with the biofragmentable anastomosis ring: experience of 632 anastomoses in a single institute. Dis Colon Rectum. 2005;48:2127-2132. doi: 10.1007/s10350-005-0144-3.

141. Cahill CJ, Betzler M, Gruwez JA, Jeekel J, Patel JC, Zederfeldt B. Sutureless large bowel anastomosis: European experience with the biofragmentable anastomosis ring. Br J Surg. 1989;76:344-347. doi: 10.1002/bjs.1800760409.

142. Galizia G, Lieto E, Castellano P, Pelosio L, Imperatore V, Canfora F, Pignatelli C. Comparison between the biofragmentable anastomosis ring and stapled anastomoses in the extraperitoneal rectum: a prospective, randomized study. Int J Colorectal Dis. 1999;14:286-290. doi: 10.1007/s003840050230.

143. Nudelman IL, Fuko VV, Morgenstern S, Giler S, Lelcuk S. Gastrointestinal anastomosis with the nickel-titanium double ring. World J Surg. 2000;24:874-877. doi: 10.1007/s002680010140.

144. Nudelman IL, Fuko V, Greif F, Lelcuk S. Colonic anastomosis with the nickel-titanium temperature-dependent memory-shape device. Am J Surg. 2002;183:697-701. doi: 10.1016/s0002-9610(02)00857-7.

145. Nudelman I, Fuko V, Rubin M, Lelcuk S. A nickel-titanium memory-shape device for colonic anastomosis in laparoscopic surgery. Surg Endosc. 2004;18:1085-1089. doi: 10.1007/s00464-003-9064-2.

146. Nudelman I, Fuko V, Waserberg N, Niv Y, Rubin M, Szold A, Lelcuk S. Colonic anastomosis performed with a memory-shaped device. Am J Surg. 2005;190:434-438. doi: 10.1016/j.amjsurg.2004.11.037.

147. Avgoustou C, Penlidis P, Tsakpini A, Sioros C, Giannousis D. Compression anastomoses in colon and rectal surgery with the NiTi ColonRing™. Tech Coloproctol. 2012;16:29-35. doi: 10.1007/s10151-011-0794-1.

148. Buchberg BS, Masoomi H, Bergman H, Mills SD, Stamos MJ. The use of a compression device as an alternative to hand-sewn and stapled colorectal anastomoses: is three a crowd? J Gastrointest Surg. 2011;15:304-310. doi: 10.1007/s11605-010-1376-7.

149. Rosati R, Rebuffat C, Pezzuoli G. A new mechanical device for circular compression anastomosis. Ann Surg. 1988;207:245-252. doi: 10.1097/00000658-198803000-00004.

150. Stewart D, Hunt S, Pierce R, Mao D, Frisella M, Cook K, Starcher B, Fleshman J. Validation of the NITI endoluminal compression anastomosis ring (EndoCAR) device and comparison to the traditional circular stapled colorectal anastomosis in a porcine model. Surg Innov. 2007;14:252-260. doi: 10.1177/1553350607312241.

151. Slesser AAP, Pellino G, Shariq O, Cocker D, Kontovounisios C, Rasheed S, Tekkis PP. Compression versus hand-sewn and stapled anastomosis in colorectal surgery: a systematic review and meta-analysis of randomized controlled trials. Tech Coloproctol. 2016;20:667-676. doi: 10.1007/s10151-016-1521-8.

152. Zarnescu EC, Zarnescu NO, Costea R. Updates of risk factors for anastomotic leakage after colorectal surgery. Diagnostics (Basel). 2021;11:2382. doi: 10.3390/diagnostics11122382.

153. Badia JM, Casey AL, Petrosillo N, Hudson PM, Mitchell SA, Crosby C. Impact of surgical site infection on healthcare costs and patient outcomes: a systematic review in six European countries. J Hosp Infect. 2017;96:1-15. doi: 10.1016/j.jhin.2017.03.004.

154. Lawler J, Choynowski M, Bailey K, Bucholc M, Johnston A, Sugrue M. Meta-analysis of the impact of postoperative infective complications on oncological outcomes in colorectal cancer surgery. BJS Open. 2020;4:737-747. doi: 10.1002/bjs5.50302.

155. Ha GW, Kim JH, Lee MR. Oncologic impact of anastomotic leakage following colorectal cancer surgery: a systematic review and meta-analysis. Ann Surg Oncol. 2017;24:3289-3299. doi: 10.1245/s10434-017-5881-8.

156. Saikaly E, Saad MK. Anastomotic leak in colorectal surgery: a comprehensive review. Surg Clin J. 2020;2:65-75.

157. Phitayakorn R, Delaney CP, Reynolds HL, Champagne BJ, Heriot AG, Neary P, Senagore AJ; International anastomotic leak study group. Standardized algorithms for management of anastomotic leaks and related abdominal and pelvic abscesses after colorectal surgery. World J Surg. 2008;32:1147-1156. doi: 10.1007/s00268-008-9468-1.

158. Vardhan S, Deshpande SG, Singh A, Kumar CA, Bisen YT, Dighe OR. Techniques for diagnosing anastomotic leaks intraoperatively in colorectal surgeries: a review. Cureus. 2023;15:e34168. doi: 10.7759/cureus.34168.

159. Steger J, Jell A, Ficht S, Ostler D, Eblenkamp M, Mela P, Wilhelm D. Systematic Review and meta-analysis on colorectal anastomotic techniques. Ther Clin Risk Manag. 2022;18:523-539. doi: 10.2147/TCRM.S335102.

160. Allen W, Wells CI, Greenslade M, Bissett IP, O´Grady G. Association between circular stapler diameter and stricture rates following gastrointestinal anastomosis: systematic review and meta-analysis. World J Surg. 2018;42:3097-3105. doi: 10.1007/s00268-018-4606-x.

161. Lee K-Y, Lee J, Park YY, Kim H-J, Oh ST. The effect of circular stapler size on anastomotic stricture formation in colorectal surgery: a propensity score matched study. PLoS One. 2023;18:e0287595. doi: 10.1371/journal.pone.0287595.

162. Sell NM, Francone TD. Anastomotic troubleshooting. Clin Colon Rectal Surg. 2021;34:385-390. doi: 10.1055/s-0041-1735269.