Surgery for Rectal Cancer: The Impact of Perioperative Factors

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The king of rectal cancer surgery? Pelvis Impressley
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The dissertation studies

This dissertation is based on the following original studies, referred to in the text by their Roman numerals.

I. Oncologic impact of high vascular tie after surgery for rectal cancer: a nationwide cohort study
   Boström P*, Kverneng Hultberg D*, Häggström J, Haapamäki MM, Matthiessen P, Rutegård J, Rutegård M.

II. Level of vascular tie and its effect on functional outcome 2 years after anterior resection for rectal cancer
    Kverneng Hultberg D, Afshar AA, Rutegård J, Lange M, Haapamäki MM, Matthiessen P, Rutegård M.

III. The impact of anastomotic leakage on functional outcome two years after anterior resection for rectal cancer
     Diseases of the Colon & Rectum: Accepted for publication

IV. Nonsteroidal anti-inflammatory drugs and the risk of anastomotic leakage after anterior resection for rectal cancer
    Kverneng Hultberg D, Angenete E, Lydrup M-L, Rutegård J, Matthiessen P, Rutegård M.

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*Both authors contributed equally to the manuscript
Related publications

Non-Steroidal Anti-Inflammatory Drug Use and Risk of Anastomotic Leakage after Anterior Resection: A Protocol-Based Study
Rutegård M, Westermark S, Kverneng Hultberg D, Haapamäki M, Matthiessen P, Rutegård J.

Substantial underreporting of anastomotic leakage after anterior resection for rectal cancer in the Swedish Colorectal Cancer Registry
Rutegård M, Kverneng Hultberg D, Angenete E, Lydrup ML.
Acta Oncol 2017;56:1741-5.

High stoma prevalence and stoma reversal complications following anterior resection for rectal cancer: a population-based multicentre study
Holmgren K, Kverneng Hultberg D, Haapamäki MM, Matthiessen P, Rutegård J, Rutegård M.
Abstract

Rectal cancer is one of the most common and deadly cancer forms worldwide. A large proportion of rectal cancer patients are surgically treated with curative intention, with anterior resection being the most frequently used method today. During surgery, the inferior mesenteric artery is either ligated proximal (high tie) or distal to the left colic artery (low tie). It is not known whether the tie level affects the oncologic nor the functional outcome. Postoperatively, about one in ten patients develop an anastomotic leakage. It is unclear whether treatment with nonsteroidal anti-inflammatory drugs (NSAIDs) affects the risk of leakage, or whether having a leakage influences the functional outcome.

The general aims of this dissertation were to increase the knowledge of intra- and postoperative treatment for rectal cancer, with the goal of improving the oncologic and functional outcomes, as well as reducing postoperative complications. National registers, predominantly the Swedish Colorectal Cancer Registry, were used in all of the dissertation's four retrospective cohort studies to identify and retrieve information regarding patients. Various statistical methods have been used in all studies with the aim of eliminating bias, including confounding.

In Study I, high tie slightly increased the total number of harvested lymph nodes in the included 8287 patients, as compared with low tie, while the primary outcome cancer-specific survival, as well as secondary oncologic outcomes, were not affected. This indicates that the oncologic outcome does not have to be considered when the surgeon determines the level of tie.

In Study II, investigating the effect of tie level on the functional outcome, the outcome was any defecatory or urogenital symptoms two years after anterior resection, assessed with a mailed questionnaire. With a response rate of 86%, 805 patients were included. High tie did not, except for increasing the need of defecation at night, influence the risk of major dysfunction. Again, this would facilitate the choice of tie level.

Study III used the same outcome, and in part the same study population, as Study II, but instead with the exposure anastomotic leakage. With a response rate of 82%, 1180 patients were included. We found that anastomotic leakage increased the risk of reduced sexual activity and increased the use of aid products for fecal incontinence after anterior resection, while the risk of urinary incontinence was unexpectedly decreased. Other outcomes were not clearly affected.
In Study IV, in addition to the register, information was gathered from patient records. In the included 1495 patients who had undergone anterior resection, postoperative NSAID treatment was not found to increase the risk of symptomatic anastomotic leakage. There were no differences between non-selective and COX-2 selective NSAIDs. This study does not support that NSAID treatment increases the risk of anastomotic leakage after such surgery.

**Keywords:** Rectal cancer, abdominal surgery, anastomotic leakage, anastomotic dehiscence, postoperative complications, vascular tie, ligation level, oncologic outcome, surgical oncology, survival, recurrence, functional outcome, urogenital, anorectal, incontinence, NSAID, COX
# Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>AL</td>
<td>Anastomotic leakage</td>
</tr>
<tr>
<td>ASA</td>
<td>American Society of Anesthesiologists</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>COX</td>
<td>Cyclooxygenase</td>
</tr>
<tr>
<td>DAG</td>
<td>Directed acyclic graph</td>
</tr>
<tr>
<td>Gy</td>
<td>Gray (SI unit of absorbed radiation)</td>
</tr>
<tr>
<td>HR</td>
<td>Hazard ratio</td>
</tr>
<tr>
<td>ICD-10</td>
<td>International Statistical Classification of Diseases and Related Health problems, 10th Revision</td>
</tr>
<tr>
<td>IQR</td>
<td>Interquartile range</td>
</tr>
<tr>
<td>ISREC</td>
<td>International Study Group of Rectal Cancer</td>
</tr>
<tr>
<td>LARS</td>
<td>Low anterior resection syndrome</td>
</tr>
<tr>
<td>N</td>
<td>Number</td>
</tr>
<tr>
<td>NSAID</td>
<td>Nonsteroidal anti-inflammatory drug</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>SCRCR</td>
<td>Swedish Colorectal Cancer Registry</td>
</tr>
<tr>
<td>TNM</td>
<td>TNM classification (Tumor, Node, Metastasis)</td>
</tr>
</tbody>
</table>
Populärvetenskaplig sammanfattning på svenska (summary in Swedish)

Bakgrund

Varje år insjuknar kring 700 000 personer världen över i den åttonde vanligaste cancerformen, ändtarmscancer, varav omkring 2 000 i Sverige. Merparten av dessa patienter genomgår bukkirurgi i botande syfte, där valet av operationsmetod bland annat baseras på tumörens allvarlighetsgrad, patientens hälsostatus samt hur nära ändtarmsöppningen tumören är belägen. Gemensamt för alla operationsmetoder är att kirurgen måste bestämma huruvida det blodkärl som försörjer den drabbade delen av tarmen ska delas antingen högt upp i en så kallad hög ligatur (avknytning) nära den stora kroppspulsådern (aorta), eller något längre ned på kärlträdet i en låg ligatur. Trots idog diskussion i över ett århundrade råder det fortfarande ingen enighet kring vilken ligaturnivå som är den bästa. En teoretisk fördel med hög ligatur är att den skulle kunna möjliggöra avlägsnandet av aortanära lymfkörtlar, eventuellt innehållande spridda cancerceller som annars lämnas orörda, och därigenom förbättra det cancerrelaterade (onkologiska) utfallet. Å andra sidan skulle hög ligatur kunna leda till försämrat funktionellt resultat, eftersom den utförs i närheten av nervfibrer som svarar för viktiga aspekter av tarm-, urinvägs- och sexualfunktion. Det är dock ej i dagsläget klarlagt huruvida något av dessa teoretiska resonemang har betydelse i praktiken.

Den enskilt vanligaste operationsmetoden är en så kallad främre resektion, där tumören avlägsnas och tarmändarna sammanfogas i en tarmskarv (anastomos). Efter operationen drabbas ungefär tio procent av patienterna inte tjeond patient av att anastomosen havererar. Detta leder i värsta fall till döden, medan de överlevande har ökad risk för bland annat permanent stomi ("påse på magen") samt troligen även för canceråterfall. Eftersom ett sådant anastomosläckage ofta medför en påtaglig inflammation i bäckenet och inte sällan leder till nya operationer, skulle ett läckage även kunna försämra det funktionella långtidsresultatet. Detta är sparsamt undersökt tidigare, särskilt vad gäller urinvägs- och sexualfunktion.

Under det senaste årtiondet har ett flerta l publicerade observationsstudier varnat för att smärtbehandling efter främre resektion med läkemedel av typen NSAID (nonsteroidal anti-inflammatory drug), såsom ibuprofen (Ipren®), kan öka risken
Populärvetenskaplig sammanfattning på svenska (summary in Swedish)

Målsättning

Avhandlingens målsättning är att öka kunskaperna kring intra- och postoperativ behandling av ändtarmscancer, i syfte att minska risken för canceråterfall och komplikationer efter kirurgin, samt att förbättra det funktionella utfallet.

Delarbeten

Det Svenska Kolorektalcancerregistret har använts i avhandlingens alla fyra bakåtblickande observationsstudier för att hitta och samla information kring patienter. Ett flertal olika statistiska metoder ingår i delarbetena för att kontrollera för bland annat störfaktorer och därigenom öka resultatens tillförlitlighet.


Background

Rectal cancer epidemiology

Colorectal cancer has the third highest incidence and second highest death toll of all cancers, being accountable for over 1.8 million new cases and nearly 900,000 deaths each year\(^1\). While the majority of colorectal cancers are situated in the colon, almost 40% are located in the rectum\(^1\).

Rectal cancer incidence rates vary greatly between regions, where the highest rates, found in Europe, Australia/New Zealand, Northern America and Eastern Asia, can be up to six times as high as those found in regions in southern Asia and Africa\(^1\). With a clear positive association between the incidence rate and the degree of social and economic development in each country, rectal cancer can be considered a marker of developmental transition\(^1\). A westernized lifestyle might be an attributable cause, with corresponding changes in risk factors related to diet, physical activity and metabolic status, though these factors seem to be more strongly connected to cancer in the colon than in the rectum\(^3\). Increasing incidence and mortality rates are seen in many low and middle-income countries, while reversing trends can be seen in many highly developed countries, along with decreasing rates of mortality\(^3\). The 5-year survival rate today is around 60% in Sweden and is steadily increasing\(^8\).

Diagnosis, staging and non-surgical treatment

Most rectal cancer patients initially present with rectal bleeding, while symptoms such as rectal pain and obstruction generally occur in later stages of the disease\(^9\). Initial investigations include endoscopy and imaging studies, such as pelvic magnetic resonance imaging and computerized tomography of the chest and abdomen. A colonoscopy is required to exclude synchronous tumors in the colon and a rigid sigmoidoscopy to measure the height of the rectal cancer – in this dissertation defined as having a distance of not more than 15 cm from the anal verge to the lowest border of the tumor. After diagnosis, the tumor is staged, generally by using the system provided by the American Joint Committee on Cancer: the TNM staging system\(^10\). This acronym is derived from the following parameters:
Tumor: the histological extent of tumor growth, based on what layer of the rectal wall the tumor has penetrated – graded T1-T4
• Node: the presence of lymph node metastases – graded N0-N2
• Metastasis: the occurrence of distant metastasis – graded M0-M1

After these factors have been determined, the TNM stage is established. The general principles for this are seen in Table 1. Clinical TNM refers to the preoperative staging, while the pathological TNM is determined by the histopathological examination of the surgical specimen.

Table 1. General principles of tumor staging based on TNM classification. T: Tumor; N: Node; M: Metastasis.

<table>
<thead>
<tr>
<th>Stage</th>
<th>T</th>
<th>N</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>T1-T2</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>II</td>
<td>T3-T4</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>III</td>
<td>Any T</td>
<td>N1-N2</td>
<td>M0</td>
</tr>
<tr>
<td>IV</td>
<td>Any T</td>
<td>Any N</td>
<td>M1</td>
</tr>
</tbody>
</table>

After tumor staging, the ensuing treatment is individually determined for each patient. Neoadjuvant therapy is often administered to reduce the tumor extent before surgery and to decrease the risk of distant and locoregional recurrence. This is commonly given either as short-course radiotherapy (5x5 Gy), or as long-course radiotherapy (1.8-2 x 25 Gy) for the most advanced tumors, combined with chemotherapy9. In some selected cases, where no remaining tumor can be detected after neoadjuvant therapy (i.e. complete clinical response), a conservative non-surgical treatment strategy called Watch-and-Wait is sometimes used, though the scientific support for this approach, outside of clinical trials, is today generally considered insufficient11. For patients with histopathological risk factors for recurrence such as locoregional lymph node metastasis, adjuvant therapy is sometimes given after surgery, usually in the form of chemotherapy9.

Surgical methods

Despite the importance of non-surgical therapies, the centerpiece in rectal cancer treatment remains surgery. Although for the last decade there has been a persistent trend in Sweden of an increased portion of conservatively treated patients,
Background

especially among those with metastasized disease, the majority still undergo surgery with curative intention\textsuperscript{8}. The choice of surgical procedure is based on a number of factors, including patient comorbidity, tumor stage and tumor height. Local excision is sometimes offered to selected cases with low risk tumors\textsuperscript{12,13}, while the large bulk of patients are operated abdominally using one of three methods: anterior resection, abdominoperineal excision or Hartmann’s procedure.

Anterior resection is the most common procedure in Sweden, used for tumors in the upper and mid rectum\textsuperscript{8}, and in highly selected cases also for tumors in the lower rectum. The procedure involves the construction of an anastomosis in order to achieve intestinal continuity and obviate the need of a permanent stoma, thereby aiming to improve quality of life\textsuperscript{14-16}. The anastomosis is generally either constructed by having the bowel ends joined end-to-end, side-to-end, or as a so-called J-pouch. In Sweden, an end-to-end anastomosis is mainly used for high tumors, while the two last variants are used for tumors closer to the sphincter complex, to mimic the storage capacity of the healthy rectum by constructing a defecatory reservoir.

Abdominoperineal excision was described by the British surgeon Miles as early as 1908\textsuperscript{17} and is today reserved for low tumors close to or infiltrating the sphincter complex. It is the most extensive method, involving complete removal of the distal colon, rectum and anal sphincter complex, resulting in a permanent colostomy\textsuperscript{17}. The mortality was initially substantial at 42%, though it improved after consecutive advancements in technique and perioperative care\textsuperscript{17,18}.

As a response to the high mortality after abdominoperineal excision, in 1922 the French surgeon Hartmann presented his less invasive method, which had a considerably lower mortality of 8%\textsuperscript{19}. In Hartmann’s procedure, the sphincter is preserved, instead leaving the rectum as a stump and fashioning a permanent colostomy. In Sweden today, this procedure is the least common one\textsuperscript{8}, with the main indications being: severe comorbidity, unsatisfactory preoperative sphincter function, or incurable disease.

Though these methods differ in important aspects, they share some similarities. This includes mesorectal excision – a concept that was introduced by the British surgeon R.J. Heald in 1982. Since then it has become the gold standard in all abdominal rectal cancer surgery, as it was found to substantially reduce local recurrence rates\textsuperscript{20,21}. In this technique, the embryological planes are followed and respected during dissection, and the tumor-bearing segment is removed \textit{en bloc} along with its mesorectum. The degree of removed mesorectum depends largely on the tumor height; for low tumors, a total mesorectal excision is performed, while a
Background

Partial mesorectal excision is generally considered sufficient for high tumors, for which a 5 cm margin has been shown to be oncologically safe. An open approach with laparotomy has long been the modus operandi for all three methods, but minimally invasive surgical techniques, i.e. conventional or robot-assisted laparoscopy (Figure 1), are gaining increasing popularity. The reason is that these have been shown to give similar oncologic results, but with improved short-term outcomes such as reduced blood loss, postoperative pain and length of hospital stay.

Level of vascular tie

Another factor in common for these procedures is the decision regarding the level at which the main feeding vessel is divided. A schematic illustration of the different ligation sites from the Swedish Colorectal Cancer Registry is seen in Figure 2. A high tie is defined as central ligation of the inferior mesenteric artery at level 10, close to its origin at the aorta. Conversely, ligation of one or more vessels distal to the departure of the left colic artery constitutes a low tie, corresponding to levels 12–14. Sometimes, a low tie is combined with ligation of the left colic artery itself (level 11), as this, like a high tie, allows for improved mobilization of the left colon and thereby reduces tension in the anastomosis. This also enables removal of the sigmoid colon, sometimes containing diverticular disease, instead allowing anastomosis with the descending colon. To aid in differentiation between these variants, a complementary terminology could be used; perfusive or oncologic tie level. A perfusive high tie is either a regular high tie or a combined tie, as the resulting impact on blood flow is equal. But in terms of oncologic tie, the combination tie would instead be considered low, along with a regular low tie, as the lymphatic harvest close to the aorta would be equal. Throughout this dissertation, the combination tie is referred to as a low tie, unless otherwise stated. An additional description of the tie level can be used; a distal low tie. This refers to a low tie where ligation has not been made directly distally to the left colic artery at level 12 (i.e. central tie of the superior rectal artery), instead only ligating at level 13 or 14.
Despite intense discussion for more than a century regarding the benefits of a high or a low tie, there is still no consensus regarding the preferred vascular tie level. A theoretical advantage of a high tie is that it could increase the lymph node yield by enabling removal of central nodes close to the aorta. This would benefit the patient by mainly two mechanisms: one, metastatic (positive) lymph nodes could be removed that would otherwise have been left untouched, thus improving the oncologic outcome in patients in whom no further tumor spread has occurred; two, an increased number of retrieved positive nodes would improve the tumor staging, increasing the probability that appropriate adjuvant therapy is given when needed.

However, there is no consensus regarding whether a high tie actually increases the number of harvested lymph nodes. More importantly, a clear oncologic benefit of high tie is yet to be proven, with many prior studies being inadequately powered, conducted before the introduction of total mesorectal excision, or not directly comparing patients operated with high or low tie. Additionally, the available randomized studies have not been able to demonstrate an oncologic advantage for high tie, which, aside from the potential lack of benefit, might be due to deficient statistical power.

Figure 2. Schematic illustration from the Swedish Colorectal Cancer Registry of the levels of tie in colorectal surgery. Ligation at level 10 corresponds to a high tie, whereas ligation of one or more vessels at levels 11-14 constitutes a low tie.

Background

Anastomotic leakage

A dreaded complication following anterior resection is *anastomotic leakage* (AL). Though incidence rates vary between studies, largely due to differences in definition, about one in ten patients are affected\(^43-46\). Diagnosis is made around two weeks after surgery for most patients, but AL does not occur until after more than 30 days after surgery in about a third of patients\(^47, 48\). Such late leaks seem to behave differently to those occurring early and are not seldomly asymptomatic, being radiologically detected *en passant* or in connection with stoma reversal – raising the question whether they compose a distinct disease entity\(^47-49\). Besides from potentially leading to death\(^44, 46\), the survivors of an AL face an increased risk of permanent stoma formation\(^50, 51\) and possibly also an inferior oncologic outcome, with an increased risk of local recurrence and reduced cancer-specific survival\(^52, 53\).

There are a number of identified risk factors for AL, such as low tumor height, male sex and intraoperative bleeding, but the exact etiology and underlying mechanisms still need to be fully explained\(^43, 44, 54\). Due to its allegedly negative effect on anastomotic vascular perfusion, a high vascular tie has been proposed as a possible culprit, though this is not corroborated\(^36, 45, 55\). In order to decrease the risk of at least symptomatic AL and the need for reoperation, a diverting stoma is often constructed at the time of the anterior resection, especially for low tumors, but a safe postoperative recovery can still not be guaranteed\(^51, 56\).

The definition of AL varies greatly between prior studies. In the dissertation studies (Studies III and IV), the definition is: leakage from any staple or suture line, a rectovaginal fistula, or a pelvic abscess close to the anastomosis (even without a proven anastomotic communication). This is based on the consensus definition proposed in 2010 by the International Study Group of Rectal Cancer (ISREC), where AL is defined as a communication between the intra- and extraluminal compartments due to a defect of the intestinal wall integrity at the anastomotic site, including leaks in suture and staple lines of constructed reservoirs, as well as pelvic abscesses\(^57\).
Grading of AL in Study IV was done according to the ISREC definition, where leaks graded A require no active treatment (typically corresponding to asymptomatic leaks), leaks graded B require treatment other than re-laparotomy, while grade C entails re-laparotomy. In Study III, leaks were instead graded according to the Clavien-Dindo system, in which the grade also relates to the required treatment: grade I – no treatment; grade II – pharmacological treatment; grade IIIa – intervention not under general anesthesia; Grade IIIb – intervention under general anesthesia; Grade IV – management at the intensive care unit due to a life-threatening complication; Grade V – death.

**Nonsteroidal anti-inflammatory drugs (NSAIDs)**

During the last decade, a number of observational and experimental studies have indicated a relation between AL and postoperative use of nonsteroidal anti-inflammatory drugs (NSAIDs). These drugs inhibit the enzymes cyclooxygenase (COX) 1 and 2, which are involved in synthesis of prostaglandins – a group of active lipid compounds that have a variety of functions, including inducing fever and promoting inflammation and pain. COX-1 is considered a housekeeping gene, meaning that it is constitutively expressed in most tissues and has many physiological actions, including preserving the integrity of the gastric mucosa. COX-2 is, unlike COX-1, upregulated in sites of inflammation and disease. After NSAIDs were introduced, it became known that they increased the risk of a gastric ulcer, which is why it was believed that the optimal therapeutic agent would inhibit only COX-2, not affecting COX-1. After the COX-2 selective agents were introduced to the market, concerns were instead raised regarding increased risks of cardiovascular adverse events, though this is not yet fully established. Examples of non-selective agents include ibuprofen, nabumetone, ketorolac and naproxen, while examples in the COX-2 group are diclofenac, celecoxib, etoricoxib and parecoxib.

NSAIDs have been widely used in surgery as part of accelerated postoperative care programs such as Enhanced Recovery After Surgery, in order to reduce the need of opioids, known for their side effects including respiratory depression, addiction and impaired gastrointestinal motility. By reducing opioid use, the aim is to hasten return of bowel function and reduce the length of hospital stay. However, following the reports of a possible association to AL, many surgical centers ceased...
Background

using NSAIDs as postoperative analgesics after surgery where an anastomosis had been fashioned, although the NSAID-leakage link has not been confirmed to be causal in nature. The underlying mechanisms behind this potential effect remain unclear, though proposed hypotheses include reduced collagen production and impaired angiogenesis after NSAID treatment. Questions have been raised whether the association differs between non-selective and COX-2 selective agents, but a decisive answer still lies ahead. It is also not known whether any effect of NSAIDs would be the same on colonic and colo-rectal anastomoses, though previous studies have mainly examined the association in colorectal surgery in general.

Functional outcome

As the long-term survival after anterior resection is gradually improving, another aspect after surgery is becoming increasingly important – the functional outcome. The degree of defecatory symptoms such as urgency, fecal incontinency and high defecation frequency is closely related to the quality of life after anterior resection, and these symptoms are so common that they have collectively been given their own name: Low Anterior Resection Syndrome (LARS). There is no consensus regarding the definition, how it should be measured, or at what time point after surgery a patient can be considered to have developed the syndrome. Incidence estimations are therefore difficult, but at least 50–70% of patients are affected to some degree. Risk factors include low anastomotic height, radiotherapy, and a prior diverting stoma. Moreover, urogenital disturbances are also common after surgery, with symptoms such as urinary incontinence, voiding difficulties, erectile dysfunction and vaginal dryness.

A proposed additional risk factor for a worse functional outcome is the level of vascular tie. The main rationale behind this is that a high tie is performed close to the inferior mesenteric plexus (Figure 5), which contains sympathetic nerve fibers responsible for a wide range of functions. From this plexus, the nerves travel down to the superior hypogastric plexus (also known as the preaortic plexus), and from there continue as the paired hypogastric nerves further down in the pelvis to the inferior hypogastric plexus (also known as the pelvic plexus). From here, the fibers rise to organs and structures such as the bladder, rectum, internal anal sphincter and genital structures. The exact location of the inferior mesenteric plexus in relation to the ligation site is however disputed in cadaver studies, which disagree regarding whether a high tie should be able to cause damage to these nerves.
Nerve damage at the plexus could nonetheless potentially lead to symptoms such as stress and urge urinary incontinence, fecal incontinence, increased defecatory frequency, defecation at night, as well as anejaculation or retrograde ejaculation.

The parasympathetic nerves are derived from the second, third and fourth sacral nerves and merge with the sympathetic fibers in the inferior hypogastric plexus. Consequently, they are located deep in the pelvis, and can be assumed to be undamaged by a high tie. Damage to this plexus, however, could occur when a total mesorectal excision is performed, as this involves dissection all the way to the pelvic floor. Though the long-lived notion that these nerves are parasympathetic recently has been challenged, and have been proposed to in fact be sympathetic\(^9\), damage to these nerves is nonetheless believed to cause voiding difficulties and overflow incontinence due to urinary retention, as well as erectile dysfunction in both women and men, and vaginal dryness in women.

Another potential mechanism behind the alleged negative affect of high tie on defecatory function is that it would result in a longer denervated colon segment (Figure 6). The basis for this is that sympathetic autonomous nerves innervating the colon descend along with the arteries and are thus cut along with any ligated vessel. Consequently, as both high and low tie directly or indirectly denote ligation of the superior rectal artery, supporting the sigmoid colon, this segment would be denervated irrespective of the ligation level. However, a substantial part of the descending colon, supported by the left colic artery, would theoretically be denervated only after high tie. The same would apply to low tie surgery where the left colic artery itself has been ligated (high perfusive tie). The parasympathetic fibers, on the other hand, are considered to emerge from the inferior hypogastric
plexus and ascend in the mesentery contiguous with the bowel, innervating the descending colon and the distal portion of the transverse colon. These fibers are therefore expected to be cut when the tumor-bearing bowel segment is excised, resulting in denervation of the colon proximal to the transection, regardless of the level of ligation. Apart from nerve damage, additional mechanisms related to a high tie might also play a role, such as reduced vascular perfusion to the remaining colonic segment due to a more central tie, although this is not fully established.

The effect of tie level on functional outcome has been investigated only sparsely. Results regarding defecatory function are conflicting, while the effect on urogenital function, to the author’s knowledge, had never been previously evaluated when Study II was published. Moreover, although radiotherapy is a known risk factor for inferior functional outcome, no prior study has investigated whether this effect could be modified by the level of tie.

Another possible risk factor of impaired functional outcome is AL, as it often leads to a marked inflammatory response with peritonitis, as well as increasing the risk of reoperation. One concrete manifestation of affected defecatory function after AL can be considered the increased risk of having a newly fashioned stoma as a consequence of AL, or that an existing stoma, originally intended to be temporary, becomes permanent. The defecatory function could, however, also be affected by an AL in patients without a permanent stoma, though data regarding this are conflicting. Little is known of the effect of AL on urogenital function, especially regarding sexual function in women.

Figure 6. Illustration of the denervation (yellow) that occurs during vascular ligation due to damage of descending sympathetic nerve fibers. To the left: high tie with a long denervated segment, involving the sigmoid colon and a part of the descending colon. To the right: low tie with a short denervated segment, involving the sigmoid colon only.
Aims

The general aims of this dissertation were to increase the knowledge of intra- and postoperative treatment for rectal cancer, with the goal of improving the oncologic and functional outcomes, as well as reducing postoperative complications.

The specific aims were:

Study I. To perform a population-based study on whether a high vascular tie affects the oncological results after abdominal surgery for rectal cancer.

Study II. Primarily to evaluate whether high tie during anterior resection for rectal cancer affects the long-term defecatory, urinary or sexual functional outcome. A secondary aim was to explore whether the tie level modifies the effect of preoperative radiation on functional outcome.

Study III. To study whether anastomotic leakage after anterior resection for rectal cancer affects long-term defecatory, urinary or sexual function in a population-based setting.

Study IV. To investigate whether postoperative pain treatment with NSAIDs increases the risk of anastomotic leakage after anterior resection for rectal cancer, and to evaluate whether this association differs between non-selective and COX-2 selective agents.
Materials and Methods

Data collection: registers, questionnaire and chart review

We used the *Swedish Colorectal Cancer Registry* (SCRCR) in all of the dissertation studies to identify patients for inclusion. It has been found to have more than 97% coverage of rectal cancer patients in Sweden, thus providing a reliable way of identification\textsuperscript{113, 114}. The almost complete coverage is achieved by regular cross-checks against the national cancer register, which receives access to new cancer cases by treating clinicians as well as pathologists. Additionally, the register is intermittently validated and contains an extensive amount of prospectively registered information, including details regarding patient characteristics, the surgical procedure, postoperative course, oncological treatment, histopathological assessment and follow-up data, and has been found to have generally good validity\textsuperscript{113-115}. We have therefore used this register widely in all studies to acquire information on pertinent variables, often including exposure and/or outcome. Technical surgical details, such as the tie level, are reported to the register either directly by the surgeon, or indirectly through a nurse or assistant surgeon.

We used two additional registries in Study I to assess the oncologic outcome. First, the *Swedish Cause of Death Register* was used to evaluate the cancer-specific mortality\textsuperscript{116}. Since 1952 this register has recorded the cause of death for all persons permanently residing in Sweden, regardless of whether the person deceased in Sweden or abroad, and from 2012 has also included all deaths occurring in Sweden for individuals without a Swedish civil registration\textsuperscript{116}. It is administered by the National Board of Health and Welfare and is based on The *International Statistical Classification of Diseases, Injuries, and Causes of Death* (ICD-10). Unregistered deaths are virtually non-existing, and the degree of completeness regarding cause of death exceeds 99\%\textsuperscript{116, 117}. A validation on the registered causes of deaths for patients deceased in 1995 found a general agreement of 77\% with case summaries, though the percentage varied greatly between different diagnostic groups, being highest in malignant neoplasms at 90\%\textsuperscript{118}. The definition of the underlying cause of death in ICD-10 is\textsuperscript{119}:

\begin{quote}
\textit{(a) the disease or injury which initiated the train of morbid events leading directly to death, or (b) the circumstances of the accident or violence which produced the fatal injury.}
\end{quote}
Based on this, death caused by, for example, pneumonia in a cancer patient who is bedridden due to cancer, would be considered as caused by the cancer and thus categorized as cancer-specific mortality. The same goes for any postoperative complication after cancer surgery leading to death.

The second additional register in **Study I** was the Human Mortality Database\(^{120,121}\), which was used to obtain mortality rates for the Swedish general population in order to calculate the relative survival. This database was first published in 2002 and is a collaboration between the Department of Demography at the University of California, Berkeley (USA) and the Max Planck Institute for Demographic Research (Germany). It presently contains detailed population and mortality data for 41 countries and areas, where the data regarding Sweden are collected mostly from Statistics Sweden (Statistiska centralbyrån)\(^{120}\).

![Figure 7. An English version of the questionnaire used in Studies II and III. The questionnaire can be seen in full in the appendix.](image)

To assess the functional outcome, a questionnaire was used in **Studies II and III** (Figure 7). This questionnaire has its roots in the Rotterdam Symptoms Checklist, a well-validated Dutch questionnaire developed to measure psychological and physical symptoms of cancer patients participating in clinical research\(^{122}\). After being supplemented with additional questions regarding defecatory, urinary and sexual function, it was first used in its present form in a Dutch trial carried out in the second half of the 1990s, and investigated the efficacy of preoperative radiotherapy with total mesorectal excision for rectal cancer\(^{79,98}\). This modified version of the questionnaire has, to the author’s knowledge, never been the subject of formal validation, and there is no standardized way of analyzing and presenting the data. After being professionally translated to Swedish, it was used in the dissertation studies. It includes 20 questions related to current and preoperative
defecatory, urinary and sexual function, as well as stoma status at follow-up. The current function pertains to the last three months. The majority of questions regarding functional symptoms offer 4–6 alternatives on an increasing scale, ranging from asymptomatic to severely symptomatic, while a minority of questions were dichotomous (yes/no) or, in the case of defecation frequency, numerical (times per day or week). An English version can be seen in full the appendix (Supplementary Figure 1).

In Study IV, information on the study exposure and outcome, as well as some of the covariates, was retrieved during a chart review. This was done by individual examiners in each of the three healthcare regions. A mutual survey form (Figure 8), as well as a set of declared decision rules for variables where uncertainties could arise were therefore used to strengthen the inter-rater reliability.
Materials and Methods

Study design

All of the dissertation’s studies are retrospective cohort studies, where Studies I–III are nationwide population-based and Study IV is a multi-center study. All studies were approved by the regional ethical review boards at Umeå University.

Study I: tie level and oncologic outcome

All patients in Sweden who underwent rectal cancer surgery in 2007–2014, with either anterior resection, abdominoperineal excision or Hartmann’s procedure, were eligible for inclusion. We excluded all patients with distant metastasis (TNM IV) and in whom the resection margin had not been evaluated as completely tumor-free, either histologically by the pathologist or macroscopically by the surgeon. We also excluded patients with intraoperative intestinal perforation, related to their potential risk increase of cancer recurrence. Included patients were followed until 15 August 2017 to detect the occurrence of any outcome, i.e. for a minimum of about 3.5 years.

Exposure was high tie. The primary outcome was cancer-specific survival, while secondary outcomes were overall and relative survival, locoregional and distant recurrence, as well as number of retrieved lymph nodes. In addition to the main analyses, we analyzed the results for a potential interaction between high tie and clinical T-stage (T1-T2 vs. T3-T4) as well as between high tie and tumor height (≤12 vs. >12cm). Additionally, results were stratified by clinical tumor stage and operation type.

We performed three different sensitivity analyses. Firstly, we excluded patients deceased within the first 90 postoperative days, aiming to remove deaths unrelated to the oncologic outcome. Secondly, when assessing the risk of distant metastasis, we excluded those occurring during the first 90 postoperative days, as they presumably might have already been present at the time of surgery. Thirdly, we excluded patients operated with a distal low tie, since this could be an oncologically unsatisfactory method.

Studies II and III: tie level / anastomotic leakage and functional outcome

All patients who underwent anterior resection for rectal cancer in Sweden were eligible for inclusion; in Study II from April 2011 until September 2012, while the time period in Study III was extended to June 2013. We sent the above described questionnaire to all patients by postal mail at 2 years +/- 6 weeks after surgery to assess the outcome, defined as any current defecatory, urinary or sexual dysfunction. We dichotomized the outcomes (e.g. no vs. any degree of voiding
difficulties) to increase the statistical robustness and to facilitate comparison of results with other studies. Non-responders were sent one reminder questionnaire. Respondents were contacted by telephone once if an answer was missing or considered contradictory. To reduce the risk of multiple testing, we analyzed only a predefined subset of questions. As the portion of patients with preoperative dysfunction was similar between the exposure groups (except for a somewhat reduced urinary incontinence prevalence among AL patients in Study III), we did not account for preoperative function in subsequent analyses. In the main analysis of defecatory function, we excluded patients with a permanent stoma, while patients who were sexually inactive before surgery were excluded from the analysis of sexual symptoms.

Exposure in Study II was high tie. In Study III, exposure was AL. The latter was retrieved from the SCRCR by combining its AL variable with complementary free text information in the register regarding any complications or reoperations, as we have described previously. We assessed the free text information in accordance with the ISREC-based definition. In the register, AL is not explicitly defined and is instead registered according to the judgement of the surgical staff. However, registration should only be made of AL graded higher than Clavien-Dindo I diagnosed in the same hospital stay as the index surgery or, if appearing after discharge, within 30 days after surgery.

As a secondary analysis, Study II investigated any potential interaction between high tie and preoperative radiotherapy on different functional symptoms. Studies II and III both included a sensitivity analysis, where the included patients where compared with the non-responders to the questionnaire (in Study III divided into whether being alive at follow-up or not). Also, Study III included two additional sensitivity analyses: firstly, to investigate any dose-response relationship, we divided the exposure variable, AL, into three groups where no AL was reference, and the ALs were divided based on whether or not they required surgery with general anesthesia (no AL vs. Clavien-Dindo grade II–IIIa vs. grade >IIIb); secondly, with the aim of accounting for the fact that a permanent stoma is sometimes provided for patients with unacceptable defecatory function, we included all patients with a permanent stoma in the analysis of defecatory function, instead of omitting them, as if they were symptomatic in relation to each of the questions.

**Study IV: NSAID and anastomotic leakage**

Inclusion criteria were anterior resection for rectal cancer at 15 different hospitals in the Northern, Southern and Western healthcare regions in Sweden during 2007–2013. The exposure was defined as treatment with any NSAID for at least two days within the first postoperative week. The outcome was symptomatic AL before the
91st postoperative day. We excluded all patients with an AL diagnosed during the first two postoperative days, as these leaks probably were caused by a technical error, and not due to NSAID treatment. To support this decision, anastomotic strength has been found to be minimal during the first two postoperative days, and during that time relies on sutures/staples for integrity, before new collagen can be produced. We retrieved some of the clinical and demographic variables from the SCRCR, while the remainder, including data on exposure and outcome, was collected from examination of medical records.

As a secondary analysis, we categorized NSAID exposure into whether only non-selective or COX-2 selective agents had been used (excluding those who received both types), after which we estimated the effect on AL for each subtype. We performed two sensitivity analyses. Firstly, we investigated a potential dose-response relationship by examining whether the potential effect of NSAIDs on AL was affected in relation to the total dosage, duration (<4 vs. ≥4 days), or onset of treatment (postoperative day 1–3 vs. 4–6). Secondly, to evaluate any confounding by contraindication, where patients with certain risk factors are less likely to receive the treatment, but also have a different risk of developing the outcome, we excluded patients if they had any predefined characteristics generally contravening NSAID treatment: kidney disease (requiring treatment), ischemic cardiac disease, systemic cortisone treatment, ASA class III–IV or age >80 years.

Statistical methods and analyses

All studies can be afflicted by various types of bias, leading to incorrect and misleading results. Of the different types, arguably the most important is confounding. In the dissertation studies, the patients were not randomly allocated to a certain exposure. Therefore, any variable, such as age, sex and tumor height, that is associated with the investigated exposure will be unevenly distributed between the exposed and unexposed patients. If this variable is also related to the outcome, without being a mediator of effect, it is considered a confounder. This is exemplified in Figure 9, where tumor height has the properties of a confounder, while reoperation is a mediator. As confounders can cause substantial distortion of the estimates, they are necessary to adjust for when considering a causal association. Fortunately, the area of epidemiology and statistics is gradually improving, and there is an array of statistical and methodological ways by which confounding can be prevented or at least alleviated, thus theoretically approaching the results of a properly conducted randomized study.
To adjust for confounding, all of this dissertation’s studies include some form of multivariable regression. The choice of regression model is logistic and linear regression for dichotomous and continuous variables, respectively, while Cox proportional-hazards regression is included in Study I to analyze survival data. Additionally, Study I also includes propensity score matching. The purpose of this method is to adjust for confounding by emulating the properties of a randomized control trial, where all patients have the same probability of receiving the investigated exposure or treatment. Each patient’s probability of receiving the treatment is measured with a so-called propensity score, which is calculated by accounting for predictive covariates. It is notable that only observed covariates can be used to construct the propensity score, leaving this method as vulnerable as conventional regression modelling to unmeasured confounding. However, in Study III, sexual symptoms could not be analyzed with regression analyses (except anejaculation) due to small patient numbers and were instead analyzed univariably with Fisher’s exact test.

The choice of confounders in the statistical models is a delicate process, where including too few leads to residual bias, while a surplus of variables can result in over-adjustment, possibly leading to incorrect and imprecise results. In addition, it is also important not to include variables that mediate the effect of the exposure on the outcome, as this gives biased results. In order to alleviate these problems, we have constructed one or several directed acyclic graphs (DAGs) in most of the dissertation studies (Studies I, III and IV). These graphs are used to aid in determining the smallest possible set of confounders needed to estimate the total effect of the exposure on the outcome. A simplified example of this (from www.dagitty.net) is shown in Figure 9, where each arrow indicates the author’s

Figure 9. A simplified example of a directed acyclic graph. Tumor height, being associated with both the exposure anastomotic leakage and the outcome fecal incontinence, constitutes a confounder. As reoperation is a mediator of effect on the outcome, it is not a confounder.
perceived direction of each variable’s causal pathway(s). In this example, only tumor height should be adjusted for when estimating the effect of AL on fecal incontinence.

The choice of included covariates in Study II was instead based on biological rationale and previous research. The exact choice of adjusted covariates can be seen in each individual study (appendix).

To further improve the reliability of the results, we have used additional statistical methods. These include multiple imputation, which we have used in all dissertation studies to reduce the potential impact of missing data on bias and loss of precision. This is done by predicting what value the missing observation would have, were it not missing\textsuperscript{129}. Another frequently used method (Studies I, II and IV) is adjustment for clustering within hospitals. This is conducted to address the issue that patients operated at the same hospital share more similarities with each other than with patients operated at other hospitals (e.g. being treated by the same surgical and anesthesiologic team), thus violating the assumption that all patients constitute independent observations – a necessity for standard statistical methods. Performing this adjustment corrects the standard errors, generally widening the confidence intervals\textsuperscript{130}.

Univariable analyses were conducted on continuous variables with Mann-Whitney U test or Student’s t-test, on categorical variables with Fisher’s exact test, while ordinal variables were analyzed with Mann-Whitney U test, Goodman and Kruskal’s gamma or Fisher’s exact test. In Study I, survival and recurrence results were visualized using Kaplan-Meier curves, and differences were assessed with log-rank tests. Results for continuous variables were presented as medians or means with ranges or interquartile ranges (IQRs), while categorical variables were presented as proportions. Regression results were presented as odds ratios (ORs) for categorical variables, as coefficients for continuous variables and as hazard ratios (HRs) for Cox regression analyses, along with 95% confidence intervals (CIs) for all regression estimates. Test results with a P-value below 0.05 were considered statistically significant.

Analyses were performed with Stata version 13.1 or 15.1 (StataCorp. 2013 or 2017. Stata Statistical Software: Release 13 or 15. College Station, TX, USA: StataCorp LLC), or with R Core Team (2017) (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria).
Results

Study I: tie level and oncologic outcome

A total of 9653 patients matched the inclusion criteria. After exclusion of 1366 patients, 8287 remained for analysis. Among these, 60.0% (n=4968) had surgery with a low tie, 36.8% (n=3049) with a high tie, while no data were available for 3.3% (n=270). Follow-up was in median 5 years and 37 days. For all patients, after propensity score matching, the cancer-specific 5-year survival was 86%. The corresponding relative survival was marginally better, and the overall-survival 76%, while the rate of locoregional and distant recurrence after 5 years was 2.6% and 18%, respectively.

Cox regression on the propensity score matched data demonstrated no clear effect of high tie on cancer-specific (HR 0.92; 95% CI 0.79–1.07), overall (HR 0.98; 95% CI 0.89–1.08), or relative survival (HR 1.05; 95% CI 0.85–1.28), nor on locoregional (HR 0.85; 95% CI 0.59–1.23) or distant (HR 1.01; 95% CI 0.88–1.15) recurrence. This is visualized in Kaplan-Meier curves in Figure 10A-E. The mean total lymph node harvest was, after propensity score matching, slightly higher after high tie (17.7 vs. 16.7; p<0.01), while the mean number of positive nodes was similar between the tie groups (1.29 vs. 1.38; p=0.72).

After stratification for operation type, a trend of reduced overall survival after high tie was seen after abdominoperineal excision (HR 1.17; 95% CI 0.98–1.39). However, all other stratified analyses, including tumor stage, as well as the interaction analyses, which included T-stage and tumor height, showed no differences. Also, no differences were seen in the sensitivity analyses, where exclusion was made of patients who deceased or had a distant recurrence within 90 days, or who had distal low tie surgery.
Results

Figure 10A-E. Kaplan-Meier curves and log-rank tests on oncologic outcomes after propensity score matching, by level of tie. A: Cancer-specific survival; B: Overall survival; C: Locoregional recurrence; D: Distant recurrence; E: Relative survival
Study II: tie level and functional outcome

We identified 1024 patients who fulfilled the inclusion criteria. As 87 (13%) were dead at follow-up, we sent out 937 questionnaires, of which 86% (n=807) were answered. We excluded two patients due to no information on tie level, leaving 805 included patients. A high tie was performed in 46% (n=373) of patients and a low tie in 54% (n=432). Non-responders to the questionnaire (alive or dead), compared with responders, were older, had higher American Society of Anesthesiologists (ASA) class and AL frequency, as well as a more advanced tumor stage and a lower prevalence of diverting stoma, while the use of high tie was comparable (46% vs. 51%; p=0.32).
Regarding defecatory function, 12% (n=99) of all responding patients had a permanent stoma at follow-up, with no significant differences between the tie groups, and these patients were thus excluded from analysis. All multivariable results are seen in Table 2. This shows an increased need of defecation at night after high tie (OR 1.44; 95% CI 1.02–2.03), while there was no clear effect on the risk of fecal incontinence, use of incontinence products, or defecation frequency.

Table 2. The risk of defecatory, urinary and sexual symptoms two years after anterior resection for rectal cancer for patients treated with high tie, compared with low tie. Multivariable logistic and linear regression in 805 patients, using both complete case and multiple imputation.

<table>
<thead>
<tr>
<th>High tie vs. low tie</th>
<th>Multivariable with complete case</th>
<th>Multivariable with imputation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Defecatory function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Defecatory frequency (added movements/day)</td>
<td>-0.16 (-0.60–0.28)</td>
<td>-0.22 (-0.63–0.19)</td>
</tr>
<tr>
<td>Fecal incontinence</td>
<td>0.96 (0.58–1.57)</td>
<td>0.85 (0.59–1.22)</td>
</tr>
<tr>
<td>Aid use for fecal incontinence</td>
<td>0.84 (0.51–1.37)</td>
<td>0.75 (0.51–1.12)</td>
</tr>
<tr>
<td>Defecation at night</td>
<td>1.64 (1.11–2.44)</td>
<td>1.44 (1.02–2.03)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Urinary function</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary incontinence (any)</td>
<td>0.93 (0.61–1.43)</td>
<td>0.94 (0.63–1.41)</td>
</tr>
<tr>
<td>Stress incontinence</td>
<td>0.98 (0.66–1.44)</td>
<td>0.94 (0.67–1.32)</td>
</tr>
<tr>
<td>Urge incontinence</td>
<td>1.07 (0.75–1.51)</td>
<td>1.10 (0.78–1.55)</td>
</tr>
<tr>
<td>Combined</td>
<td>0.93 (0.62–1.38)</td>
<td>0.90 (0.61–1.31)</td>
</tr>
<tr>
<td>Aid use for urinary incontinence</td>
<td>1.07 (0.63–1.83)</td>
<td>1.00 (0.61–1.66)</td>
</tr>
<tr>
<td>Voiding difficulty</td>
<td>0.82 (0.56–1.21)</td>
<td>0.84 (0.57–1.23)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sexual function</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dyspareunia</td>
<td>0.67 (0.27–1.66)</td>
<td>0.78 (0.33–1.84)</td>
</tr>
<tr>
<td>Vaginal dryness during coitus</td>
<td>0.49 (0.19–1.25)</td>
<td>0.73 (0.27–1.95)</td>
</tr>
<tr>
<td>Decreased sexual activity</td>
<td>1.12 (0.42–3.01)</td>
<td>1.33 (0.53–3.34)</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erectile dysfunction</td>
<td>0.89 (0.44–1.80)</td>
<td>0.86 (0.45–1.64)</td>
</tr>
<tr>
<td>Anejaculation</td>
<td>1.60 (0.96–2.67)</td>
<td>1.33 (0.83–2.13)</td>
</tr>
<tr>
<td>Decreased sexual activity</td>
<td>0.86 (0.33–2.21)</td>
<td>0.87 (0.36–2.08)</td>
</tr>
</tbody>
</table>

We analyzed urinary function with no notable effect of high tie on the risk of urinary incontinence (OR 0.94; 95% CI 0.63–1.41) or any of its subtypes (stress, urge and
Results

combined incontinence), and with comparable results regarding the risk of voiding difficulties and aid use for urinary incontinence.

We saw no noteworthy differences between the tie groups regarding the risk of dyspareunia, vaginal dryness during coitus or reduced sexual activity in women, nor the risk of erectile dysfunction, anejaculation or reduced sexual activity in men.

No manifest interaction could be found between fecal incontinence and preoperative radiotherapy on the risk of the different symptoms, with generally wide confidence intervals.

Study III: anastomotic leakage and functional outcome

Eligible for inclusion were 1563 patients. At follow-up, 128 (8%) were deceased and one had emigrated, resulting in 1434 mailed questionnaires. After 82% had responded, 1180 patients remained for analysis. Due to external reasons, those who were operated between October 2012 and June 2013, corresponding to 31% of included patients, received their questionnaires later (median 2 years and 5 months after surgery; range 24–37 months). The frequency of AL was 7.5% (n=89). The Clavien-Dindo grading of the leaks was II for 18%, IIIa for 44% and IIIb for 30%, while the grade was missing for 8%.

A permanent stoma was reported in 44% of AL patients and 9% of non-AL patients (p<0.01), leaving 49 and 981 stoma-free patients with and without AL, respectively, in the main analysis of defecatory function. All regression analyses (including for urogenital function) are listed in Table 3. The use of aid products for fecal incontinence (e.g. panty liners, sanitary pads or nappies/diapers) was increased after AL (OR 2.27; 95% CI 1.20-4.30), while the defecation frequency trended at being increased (added movements/day: 1.06; 95% CI -0.04–2.17). There was no clear effect on the risk of fecal incontinence itself, nor the need of defecation at night.

The risk of urinary incontinence (0.53; 95% CI 0.31–0.90) and aid use for urinary incontinence (0.41; 95% 0.18–0.92) was reduced after AL, while the risk of each incontinence subtype (stress/urge/combined) only trended at being decreased. The risk of voiding difficulties was however not associated with AL.

The sexual function in men was not clearly affected by AL, with similar risks of anejaculation and frequencies of erectile dysfunction in leak and non-leak patients (95% vs. 85%; p=0.21). Among women, there was a trend of increased risk of dyspareunia after AL (83% vs. 50%; p=0.06), while the frequency of vaginal dryness
Results
during coitus was virtually identical between AL (67%) and non-AL (66%) patients. For all patients, AL was associated with an increased frequency of reduced sexual activity after surgery (90% vs. 82%; p<0.01).

Table 3. Risk of defecatory, urinary and sexual symptoms two years after anterior resection for rectal cancer for patients with anastomotic leakage, compared with those without. Univariable and multivariable logistic and linear regression of 1180 patients, with both complete case and multiple imputation analysis.

<table>
<thead>
<tr>
<th>Anastomotic leakage vs. no leakage</th>
<th>Univariable</th>
<th>Multivariable with complete case</th>
<th>Multivariable with imputation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient (95% CI)</td>
<td>Coefficient (95% CI)</td>
<td>Coefficient (95% CI)</td>
</tr>
<tr>
<td><strong>Defecatory function</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Defecation frequency (added movements/day)</td>
<td>1.29 (0.05–2.53)</td>
<td>1.17 (0.01–2.33)</td>
<td>1.06 (-0.04–2.17)</td>
</tr>
<tr>
<td>Fecal incontinence</td>
<td>1.38 (0.74–2.57)</td>
<td>1.26 (0.63–2.51)</td>
<td>1.23 (0.62–2.41)</td>
</tr>
<tr>
<td>Aid use for fecal incontinence</td>
<td>1.84 (1.03–3.29)</td>
<td>2.28 (1.19–4.36)</td>
<td>2.27 (1.20–4.30)</td>
</tr>
<tr>
<td>Defecation at night</td>
<td>1.54 (0.79–2.99)</td>
<td>1.15 (0.57–2.33)</td>
<td>1.28 (0.63–2.57)</td>
</tr>
<tr>
<td><strong>Urinary function</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary incontinence (any)</td>
<td>0.50 (0.31–0.81)</td>
<td>0.54 (0.31–0.92)</td>
<td>0.53 (0.31–0.90)</td>
</tr>
<tr>
<td>Stress incontinence</td>
<td>0.42 (0.22–0.83)</td>
<td>0.52 (0.25–1.11)</td>
<td>0.53 (0.25–1.11)</td>
</tr>
<tr>
<td>Urge incontinence</td>
<td>0.67 (0.39–1.14)</td>
<td>0.68 (0.38–1.20)</td>
<td>0.69 (0.40–1.20)</td>
</tr>
<tr>
<td>Combined</td>
<td>0.32 (0.14–0.76)</td>
<td>0.37 (0.15–0.92)</td>
<td>0.50 (0.21–1.17)</td>
</tr>
<tr>
<td>Aid use for urinary incontinence</td>
<td>0.35 (0.17–0.74)</td>
<td>0.40 (0.18–0.91)</td>
<td>0.41 (0.18–0.92)</td>
</tr>
<tr>
<td>Voiding difficulty</td>
<td>1.27 (0.80–2.03)</td>
<td>1.11 (0.68–1.80)</td>
<td>1.11 (0.69–1.79)</td>
</tr>
<tr>
<td><strong>Sexual function</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anejaculation (men only)</td>
<td>1.58 (0.75–3.36)</td>
<td>1.21 (0.54–2.74)</td>
<td>1.32 (0.60–2.95)</td>
</tr>
</tbody>
</table>

In the sensitivity analysis of a potential dose-response relationship, we found no such pattern. Moreover, when we included all patients with a permanent stoma in the defecatory analysis as if they were symptomatic, the relationship to AL was strengthened even further regarding aid use for fecal incontinence (OR 4.02; 95% CI 2.33–6.93), and there was a statistically significantly increased risk of defecation at night (OR 2.27; 95% CI 1.19–4.34) and fecal incontinence (OR 2.24; 95% CI 1.22–4.13). When comparing responders and non-responders to the questionnaire, the frequency of AL was higher in both alive (9.4%) and deceased (12.5%) non-responders. Compared to patients alive at follow-up, deceased patients had higher
Results

...age and ASA class, more advanced tumor stage, and more often received neoadjuvant chemoradiotherapy. Among those who were alive, responders had slightly lower BMI and higher tumor height.

Study IV: NSAID and anastomotic leakage

As seen in the flowchart in Figure 11, 1568 patients matched the inclusion criteria. After exclusion of 73 (5%) patients at chart review, 1495 remained for analysis. NSAID treatment was given according to the study definition (i.e. for at least two days within the first postoperative week) to 28% \((n=411)\) of patients. Table 4 shows the characteristics of NSAID treatment. For those treated, non-selective agents were used in 84\% \((n=345)\) and COX-2 selective agents in 16\% \((n=67)\), while one patient received treatment with both types. The non-selective NSAID ibuprofen was the most used drug, administered to 70\% \((n=286)\) of NSAID patients, while diclofenac was the most common COX-2 selective agent, given to 13\% \((n=54)\). The 90-day mortality after surgery was lower in NSAID patients than in non-NSAID patients \((0.2\% \text{ vs. 2.2}\%; \ p<0.01)\).

![Study flowchart](image)

Symptomatic AL occurred in 14\% \((n=203)\) of all patients, in median being diagnosed on postoperative day 14 (IQR: 8–24). The grade, according to ISREC’s system, was for most leaks B \((73\%; n=149)\), while 27\% \((n=54)\) were graded C. No leaks were graded A. In the multivariable regression after multiple imputation, NSAID...
Results

treatment was not associated with an increased risk of anastomotic leakage (OR 0.88; 95% CI 0.65–1.20). Similar results were seen after treatment with only non-selective (OR 0.91; 95% CI 0.62–1.35) or COX-2 selective agents (OR 0.82; 95% CI 0.63–1.06). The portion of leaks being graded C was similar between those who received NSAID (26%; 12/47) and those who did not (27%; 42/156). The risk of having a grade B leak trended at being decreased in NSAID patients (OR 0.76; 95% CI 0.56–1.04), while the risk of grade C leaks pointed at being increased, although with a wide confidence interval (OR 1.42; 95% CI 0.63–3.18).

The risk of AL after NSAID treatment was not affected by the total dosage, duration or onset of treatment, thus not supporting a dose-response relationship. Similarly, the point estimate was barely affected after exclusion of 23% (n=340) of patients due to high age and/or comorbidity (OR 0.90; 95% CI 0.58–1.38) in a sensitivity analysis.

Table 4. Properties of NSAID treatment

<table>
<thead>
<tr>
<th>NSAID type</th>
<th>Exposed patients</th>
<th>Postoperative day of onset</th>
<th>Duration of use (days)</th>
<th>Total dosage (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>Median (range)</td>
<td>Median (range)</td>
<td>Median (range)</td>
</tr>
<tr>
<td><strong>Non-selective</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>286 (19.1)</td>
<td>3 (0–6)</td>
<td>4 (2–8)</td>
<td>4600 (800–9600)</td>
</tr>
<tr>
<td>Nabumetone</td>
<td>55 (3.7)</td>
<td>2 (0–3)</td>
<td>5 (2–8)</td>
<td>10 000 (4000–16000)</td>
</tr>
<tr>
<td>Ketorolac</td>
<td>3 (0.2)</td>
<td>3 (1–3)</td>
<td>3 (3–3)</td>
<td>105 (40–165)</td>
</tr>
<tr>
<td>Naproxen</td>
<td>1 (0.1)</td>
<td>1 (1–1)</td>
<td>7 (7–7)</td>
<td>2250 (2250–2250)</td>
</tr>
<tr>
<td><strong>COX-2 selective</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diclofenac</td>
<td>54 (3.6)</td>
<td>4 (1–6)</td>
<td>3 (2–7)</td>
<td>300 (100–850)</td>
</tr>
<tr>
<td>Etoricoxib</td>
<td>11 (0.7)</td>
<td>0 (0–2)</td>
<td>5 (4–8)</td>
<td>600 (300–960)</td>
</tr>
<tr>
<td>Celecoxib</td>
<td>1 (0.1)</td>
<td>6 (6–6)</td>
<td>2 (2–2)</td>
<td>800 (800–800)</td>
</tr>
<tr>
<td>Parecoxib</td>
<td>1 (0.1)</td>
<td>1 (1–1)</td>
<td>2 (2–2)</td>
<td>80 (80–80)</td>
</tr>
</tbody>
</table>
Discussion

General methodological considerations

All studies in this dissertation are cohort studies and thus observational – a study design generally considered to be below randomized controlled trials in the medical evidence hierarchy\textsuperscript{131}, albeit having numerous important advantages. To start with, this design facilitates accrual of large study populations – a great advantage when studying rare outcomes in order to reach sufficient statistical power. This can be exemplified with locoregional recurrence in Study I, occurring in barely 3% of patients within five years after surgery. To assess this outcome by conducting a trial in which the tie level is randomized would require unfathomable resources. Matters would also be complicated by the fact that an intended low tie procedure is sometimes converted to a high tie to enable better mobilization of the colon. Such occurrences are usually handled in randomized trials with an intention to treat analysis, where the exposure is registered as it was originally intended regardless of any possible conversion. This, however, inevitably causes dilution of any potential impact of the exposure due to mixing of patients, thus increasing the risk of missing an actual effect (i.e. type II error).

The observational nature also allows evaluation of exposures that cannot, for practical and ethical reasons, be assessed by randomized studies. Such is definitely the case for Study III, where it would not have been ethically imaginable to randomize patients into having an AL or not. Such may also be the case for Study IV, where the indications of an increased risk of AL after NSAID treatment in previous studies could be argued to render such a randomization unethical.

The main objective of every scientific study is to acquire results with high validity. Validity can be divided into internal and external validity, where the former refers to the capability of the study to accurately answer its research question and is threatened by, for example, confounding. The external validity, on the other hand, relates to the study’s generalizability, meaning how well the results translate to the actual setting and patient category in which they are meant to be applied.

For all clinical studies, a potential threat to the validity is selection bias. This is introduced if the tendency of a participant to be included in the study is linked to the exposure or outcome. If this occurs, results can be altered as the selected study sample differs from the target population. As this is difficult to control for when participants already have been included, it is better to prevent in advance. An
example of when this can appear is when performing single-center studies, as the character of the hospital (e.g. university vs. rural) can influence what types of patients are recruited. If Study I, for example, had been conducted in a rural hospital, operating mainly low-risk tumors in relatively healthy individuals, any difference between the ligation levels would have been attenuated and the internal validity thus impaired, as the statistical power decreases with diminishing incidence rates of the outcome. Also, the external validity would have come into question, as the results from such a study do not necessarily translate into patients with more advanced disease. To address this, Studies I–III are population-based, involving patients from all of Sweden. In Study IV, it was not possible to retrieve patient records from every hospital in each healthcare region, resulting in a multi-center design instead.

Apart from being related to the study design, selection bias can arise due to characteristics in the patient, for example influencing the patient’s probability of accepting participation or being lost to follow-up. This is prevented almost completely in many register-based studies, such as Studies I and IV, as inclusion and follow-up are conducted without the conscious knowledge of the patient. In Studies II and III, however, the final decision of study inclusion was made by the patient, based on whether the questionnaire was answered or not. This can introduce selection bias, as patients with worse dysfunction, for instance, may choose to decline participation to a greater extent. To prevent this, non-responding patients were reminded with a new mailed questionnaire. The resulting response rate was high in the studies at 82% and 86%, respectively, thus reducing any consequence of such bias. We have also described the attributes of the non-participating patients in both these studies, to discern how the results could be affected.

Another important part of the internal validity is the reliability of the used variables, where an essential factor is the degree of misclassification. Misclassification is categorized as either differential or non-differential, where the latter occurs at random and thus has the same probability of afflicting all patients. This usually causes dilution of any studied effect and increases the risk of a type II error. On the other hand, differential bias is systematic, meaning that its likelihood of arising is connected to some characteristic in the patient. For example, due to the concerns regarding a potential relationship between NSAID treatment and AL, clinicians may be more prone to investigate postoperative disturbances with, for example, a computerized tomography in NSAID patients than in non-NSAID patients, potentially causing undiagnosed ALs to be more common in non-NSAID patients. This could introduce bias and alter the results in any direction, potentially demonstrating an effect that is not true (i.e. a type I error).
Another mutual characteristic of the dissertation’s studies, besides being cohort studies, is their retrospective design. This, in general, opens up to an increased risk of misclassification in comparison with prospective studies, as the used information is interpreted in hindsight, often by an external individual, meaning that the exposure is registered after the outcome is known. This possibly increases the risk of non-differential misclassification but, most importantly, also of differential misclassification. However, all variables in Studies I–III, and the majority in Study IV, are prospectively registered as part of the continuous registration of data in the SCRCR. In addition, the questionnaires in Studies II and III measured the outcome in a prospective manner, except for the degree of preoperative function which was assessed retrospectively. This would imply that any misclassification occurring in these variables probably should be non-differential only. The only other retrospectively collected variables are found in Study IV, where both the exposure with NSAIDs and the outcome AL, as well as some covariates, were registered à posteriori during chart review. The reason for this is that these variables are either absent in the register or are included, but with known poor quality.

Regarding the questionnaire in Studies II and III, a compromising factor is that it has never previously been validated and that there is no established uniform way to interpret, analyze or present the data. Currently, a large number of validated questionnaires exist for both defecatory urogenital function, including the LARS score, which was developed to detect and quantify the degree of LARS. However, when these studies were designed and data collection was initiated, no other questionnaires than the one used were found that were considered capable of successfully addressing the study aims.

Tie level and oncologic outcome (Study I)

In this study, we did not find that high tie affected any oncologic outcomes in relation to low tie, except for a slightly increased total lymph node harvest, while the number of positive nodes was similar. No differences were seen in the sensitivity, subgroup and interaction analyses.

Any oncologic advantage of a high tie can in theory be assumed to be infrequent. In a previous study, a positive lymph node at the most central part of the inferior mesenteric artery, generally unreachable with a low tie, was found in 1.7% of patients undergoing high tie surgery. But the proportion of patients actually having a meaningful survival benefit of a high tie can be assumed to be even lower, considering that an unknown share of these node-positive patients has probably already developed microscopic distant or additional locoregional metastases which
Discussion

are undetected and not removed at the time of surgery. In addition, about a quarter of these patients irrespectively die within five years after surgery, regardless of ligation level, from factors related or unrelated to the cancer. Therefore, a major strength of this study is the large sample size – to the author's knowledge, unprecedented. This assures high statistical power and decreases the risk of a type II error, which is important when confronted with negative results.

However, a source of a type II error could be misclassification of some of the variables. The ligation level variable has previously been found to have acceptable validity, with a general agreement of 86% with case records. But as surgeons have been found to have limited perception of the actual tie level performed, predominantly believing it to be more central than it is, even case records are potentially flawed. However, we conducted a post hoc sensitivity analysis, where we randomly selected patients, in whom the tie level was re-classified according to the demonstrated misclassification. The estimates continued to indicate statistical insignificance in 99 of 100 simulations, corroborating our negative results. Also, data on cancer-specific survival and recurrence may also be misclassified for some of the patients. We therefore included relative survival as an outcome to circumvent such issues, comparing the overall survival with that of the general population. Moreover, it could also be argued that the inclusion of patients with less advanced tumors might mask a true effect of high tie, as these patients are less likely to benefit from a high tie. However, no such indication was seen in the interaction analysis of T-stage, nor after stratification by tumor stage.

The risk of a type II error could in theory be further increased due to a proportion of patients undergoing low tie surgery where the inferior mesenteric artery is skeletonized, i.e. fully dissected of its mesenteric surroundings. This method is mainly used in Asia, with the purpose of achieving equal lymph harvest as in high tie surgery without the risk of compromising vascular anastomotic perfusion. While it has been shown to be a safe technique, no gains in oncologic outcome in relation to an ordinary low tie have been proven. Its use in Sweden is also, to the author's knowledge, negligible or even non-existent. This should therefore not present an issue in this study.

Our main results are supported by previous systematic reviews and meta-analyses, which have also found no differences in survival or recurrence between ligation levels. Corresponding findings are seen in the available randomized controlled trials, though insufficient statistical power could likely be an important alternative explanation, considering that the largest study only included 331 patients. If there actually is an oncologic benefit with high tie, it seems to be neutralized by another factor with opposing effect. An example of this could in theory be increased
postoperative mortality after high tie due to more extensive surgery. But, in our sensitivity analysis where patients with early mortality were excluded, no support for such a hypothesis could be seen.

Regarding our finding of increased total lymph node harvest after high tie, this has previously been both supported\textsuperscript{37} and contradicted\textsuperscript{35, 36, 45, 137}. Of note is that the latter studies also showed a difference between tie levels equal to, or even greater than in the present study, but failed to reach statistical significance, probably owing to their smaller study sizes. However, as positive nodes in our study were equally frequent in both tie groups, it is questionable whether an increase in the total number of nodes truly extends to any patient-oriented benefit. It is reasonable to assume that at least a part of this difference is due to the fact that a high tie is often associated with a more extensive colonic resection, as the entire sigmoid colon is commonly removed. Since the SCRCR does not offer information regarding the location from which the nodes are harvested, the additional node, on average, could originate from the mesocolon in the longer colon segment, rather than apically from the base of the inferior mesenteric artery. This would therefore hardly confer a survival benefit.

However, though the most apparent oncologic benefit would be in patients in whom positive nodes are detected during the pathologist’s postoperative assessment, also, patients in whom nodal involvement remains unverified could potentially be helped. Reasons for this include the histopathological assessment possibly turning out to be falsely negative, and hidden cancer cells in the lymphatic tissue that have not yet reached the lymph nodes being able to be removed. This notion is supported by the fact that distant metastases occur also in node-negative patients with early stages of cancer\textsuperscript{138}.

**Tie level and functional outcome (Study II)**

We found an increased need of defection at night after high tie, while other aspects of defecatory, urinary and sexual function were not clearly affected. No interaction could be demonstrated between preoperative radiation and the tie level.

A weakness of this study is that the analyses of sexual function suffered from many excluded patients due to sexual inactivity, thus reducing the statistical power and increasing the risk of a type II error. This can also negatively affect the statistical robustness by causing overfitting in the multivariable regression model (i.e. too few events in relation to the number of included variables)\textsuperscript{139}. The risk of a type II error could be further increased due to misclassification of the tie level variable, as
Discussion

discussed above. An additional source of a type II error could in theory be dilution of results due to patients undergoing low tie with skeletonizing of the inferior mesenteric artery, which could cause similar nerve damage to a proper high tie. But as argued above, this should not be an important consideration in Swedish data.

However, this study has several important strengths. This is, to the author’s knowledge, the largest study to this day, including 805 patients. It was also the first study investigating urogenital function in relation to this research question. Regarding non-responders, though they differed from the responders in various clinical aspects (e.g. older and more comorbid), results should not be substantially biased in any given direction, as their frequency of high tie was similar between groups. If the non-responders had been numerous, the statistical power could have been reduced, as that would entail loss of a patient group with, presumably, higher frequencies of dysfunction. But the response rate in this study was strong at 86%, thus consolidating the study’s validity and limiting any potential effect of non-response.

In a previous study evaluating the importance of the denervated colonic segment, patients who were considered to have a long denervation (high tie, or low tie with extended lymphatic dissection around the inferior mesenteric artery) had impaired colonic motility compared with regular low tie patients, with reduced propagated contraction down to the rectum and increased spastic minor contractions. These motility disorders were in turn associated with defecatory dysfunction, such as multiple evacuations and urgency, but a direct association between the length of denervated colon and defecatory dysfunction could not be shown with statistical significance, probably owing to lacking statistical power. However, the consequence of an increased length of denervated colon after high tie remains to be fully demonstrated, as it in reality may be counteracted by the fact that high tie is often accompanied by a more proximal colonic transection. This would reduce the length of the denervated segment – conceivably even to a lesser extent than after low tie, as the denervated and potentially also underperfused sigmoid colon might be removed more often in high tie surgery. Unfortunately, data on the level of transection are not available in the register. But, as reference, the use of splenic flexure mobilization, a technique used to improve colonic mobility and ensure a tension-free Anastomosis, was recorded in a Swedish study assessing patient records. This maneuver was used in 73% of high tie and 29% of low tie operations, respectively – a difference likely mirroring the discrepancy of extended colonic resection.

A direct comparison of defecatory function between high and low tie has, to the author’s knowledge, only been done twice previously; both were randomized
controlled trials from 2015. In the first, including 100 patients, no differences were found between high and low tie\textsuperscript{55}, while the other, with 128 patients, found that high tie increased defecatory frequency three months following surgery, and led to a more prevalent use of laxatives one year after surgery, while other outcomes were similar (only abstract assessed due to full article in Chinese)\textsuperscript{95}.

Our finding of increased nightly defecation after high tie could be explained by sympathetic nerve damage, but could also be a consequence of multiple testing, considering the numerous analyses and the risk of the other defecatory symptoms associated with sympathetic damage (fecal incontinence and increased defecatory frequency) seeming to point in the opposite direction.

Regarding urogenital function, results can only be compared with a randomized controlled trial from 2018, published after our study, which examined 214 patients with questionnaires and uroflowmetric examination\textsuperscript{36}. Genitourinary function was found to be worse after high tie than after low tie 9 months after surgery, though female sexual dysfunction was not analyzed due to a high prevalence of sexual inactivity.

**Anastomotic leakage and functional outcome (Study III)**

We found evidence supporting that AL increased the risk of reduced sexual activity and increased aid use for fecal incontinence, while the risk of urinary incontinence and aid use for urinary incontinence was unexpectedly lower for those with AL. These patients also had a trend of increased defecation frequency, as well as a trend of increased dyspareunia in women, while the remainder of symptoms were similar between patients with and without AL.

A weakness of this study is, similar to Study I, that it had limited response rates regarding sexual symptoms. This is especially true among women, where only 5 patients with an AL were included. A notable risk of a type II error must therefore be taken into account. The low participation rate also hindered multivariable analysis for the majority of these symptoms, increasing the influence of confounding. Regarding defecatory function, a type II error may have been introduced due to underreporting of AL in the register, which we have found to occur at a rate of 29\%\textsuperscript{124}. Moreover, a portion of the patients received their questionnaires later, after it was decided that this study would be executed as an extension of Study II. The prevalence of some symptoms may therefore have been affected. But as this occurred in a non-differential manner, results should not be biased.
The main strengths of this study are connected to its novelty. It is, to the author's knowledge, the largest study, as well as the first performed in a population-based setting, investigating this research question. It also belongs to a rare breed of studies investigating the association to urogenital function. As non-responders to the questionnaire had higher rates of AL and differed slightly in a few variables (e.g. lower tumor height), selection bias could arise. However, the high response rate of 82% reduces the consequences of such a bias.

In previous studies, some have found AL to negatively affect the defecatory function\textsuperscript{103-105, 107-109, 111} – in accordance with our findings – while others have found no such effect\textsuperscript{77, 102, 106, 110, 112}. The risk of a type II error due to lack of statistical power must however be acknowledged for the latter studies, considering their generally very small study sizes. Urinary function has previously been found to be both unaffected\textsuperscript{105} and negatively affected after AL\textsuperscript{103, 106}, while our finding of improved function appears to be unique. As regards the association to sexual function, no clear impact has been demonstrated in previous studies; apart from the true absence of an effect, inadequate statistical power is likely an important explanation to this finding\textsuperscript{103, 105, 106}.

In this study, we found an increased use of aid products for fecal incontinence after high tie, but not an increased risk of fecal incontinence itself. This may indicate that AL impairs defecatory function by other means than incontinence, thereby reducing the patients’ trust in their bowels and causing them to use incontinence pads as a safety measure. An example of this could be urgency, which is not captured by the questionnaire. However, the risk of all defecatory symptoms could be underestimated, as patients with a permanent stoma were excluded from analysis. The basis for this is that defecatory symptoms have been found to be the reason for stoma permanence in 8–53\% of such patients\textsuperscript{51, 100}. The sensitivity analysis, where these patients were included in evaluation of defecatory symptoms, was performed to reflect this. This analysis rendered increased risk for all three outcomes with both statistical and clinical significance. These values are meant to be interpreted as extremes, and the true value should be somewhere in between.

It was surprising to us that the risk of urinary incontinence and associated aid use was reduced among leak patients, and the biological basis for such an association is unclear. An explanation is possibly found in the preoperative frequencies (table is found in the appendix to the original study), where leak patients had lower rates of urinary incontinence (5.6\%) than non-leak patients (12.6\%). The differences at follow-up (28% vs. 44\%) could be an extension of those differences.
NSAID and anastomotic leakage (Study IV)

We found that the risk of anastomotic leakage was not affected by postoperative treatment with NSAIDs. This was also not related to the type of NSAID used.

A limitation of this study is its observational design, which opens up to various forms of bias and confounding. An example of this is confounding by contraindication, by which NSAID treatment is less likely to be given to elderly and comorbid patients, thus possibly leading to an underestimated leak risk in this group. This is indicated in our study by the reduced comorbidity and postoperative mortality among NSAID patients. But in a sensitivity analysis, where patients with known contraindications for NSAID treatment were excluded, the results were unaffected, indicating that this is not an important factor to consider. Another potential mechanism, affecting the results in the opposite direction, is protopathic bias. This means that NSAID treatment may be more likely to be initiated in patients with abdominal pain caused by a yet undiagnosed AL. This form of reverse causality can for this group lead to an exaggerated risk estimate. Regardless of these limitations, this study has several strengths. This includes the reliable and generally complete patient records from which many of the variables were collected, as well as the substantial study size.

Many of the early studies on this research topic indicated a detrimental effect of NSAID treatment on the risk of AL. This is supported by a recent experimental study, where both mice and humans with genetically little or no COX-2 expression had increased risk of AL. This was partly reversed in the mice after administration of prostaglandin, a product of the COX pathway, and the authors concluded that COX-2 is essential in anastomotic healing. In a meta-analysis from 2016, the risk of AL after NSAID treatment was increased without statistical significance in the included 6 randomized controlled trials (OR 1.96; 95% CI 0.74–5.16), while the 11 observational studies displayed a more distinct effect (OR 1.46; 95% CI 1.14–1.86). However, in several recent clinical studies, including one of our own, no such connection was found, raising the question whether publication bias may be an issue.

An important factor to consider is that any effect of NSAID treatment on anastomotic healing may differ depending on the location of the anastomosis. This is suggested by animal studies that found a more unfavorable effect on ileal than colonic anastomoses, as well as a stronger impact on anastomoses in the proximal than in the distal colon. This may explain why no negative effect of NSAID treatment on AL was found in this study. Another explanation for this could be that any potential detrimental effect of NSAIDs is balanced by an advantageous effect. An
example of this could be through an indirect effect on the intestinal microbiota – an area that is gaining increasing attention as a potential culprit in the development of anastomotic leakage\textsuperscript{150}. In an experimental study in rats, morphine treatment was found to increase the risk of AL\textsuperscript{151}. It was also found to increase the adhesiveness and collagenase production of \textit{Enterococcus faecalis} at the anastomosis, causing breakdown of the anastomotic integrity\textsuperscript{151}. The reduced need of opioids during NSAID treatment might therefore indirectly lead to beneficial effects.
Conclusion

In Study I, the level of tie did not affect the oncologic outcome. This would allow the surgeon to set aside oncological considerations when deciding on the tie level, instead considering factors such as personal technical preferences and the patient’s anatomical attributes.

In Study II, while we could not see any difference between the tie levels for the vast majority of symptoms, high tie was associated with an increased need of defecation at night – a finding of uncertain significance that warrants further investigation. We also found no interaction between preoperative radiation and ligation level. Based on this study, a high tie does not seem to increase the risk of major dysfunction, again reducing the complexity of the surgeon’s choice on tie level.

In Study III, AL increased the risk of aid use for fecal incontinence, while the finding of increased defecation frequency did not reach statistical significance. As the impact on defecatory function was not obviously detrimental, the incentive of maintaining gastrointestinal continuity after AL may be greater than has been believed. However, as indicated by a sensitivity analysis, defecatory function might be worse in leak patients than is indicated by the main analysis. Urinary function, in terms of urinary incontinence and aid use for urinary incontinence, was surprisingly improved in leak patients, while voiding difficulties were unaffected. An explanation might be differences in preoperative frequencies, unaccounted for in the analysis. Regarding sexual function, sexual activity was reduced after AL, and dyspareunia in women trended at being increased, while other symptoms were not affected. However, as patient groups were small due to high frequencies of sexual inactivity, the reliability of these analyses may be reduced; further investigation is required.

In Study IV, NSAID treatment was not found to increase the risk of symptomatic anastomotic leakage, irrespective of the type of NSAID used. This would increase the treatment arsenal when managing postoperative pain.
Future aspects

The connection between a high vascular tie and the oncologic outcome is absent in our study. This finding suggests that there is no clinically meaningful effect, but needs to be replicated. As any potential advantage of a high tie appears to be minute, future researchers could consider including only patients with an advanced tumor stage (e.g. only TNM III) to increase the probability of detecting an effect. An alternative to a cohort study could be a case-control design using as cases, for instance, patients with locoregional recurrence. But as confounding factors can influence the choice of tie level in an observational setting, a randomized study would be preferable, though achieving an adequately sized trial seems all but impossible. However, a new study design has emerged during recent years that could be a feasible alternative, namely a register-based randomized clinical trial. This would provide the advantages in causal inference that come with randomization, while facilitating many aspects by using a register, such as the SCRCR, as a way of including patients and collecting data relating to them, including long-term outcomes.

If a register-based clinical trial on the level of tie were to be conducted, additional outcomes could be investigated concurrently, such as the functional outcome. Data could be complemented at the time of surgery with information on splenic flexure mobilization and the level of colonic transection, which could be used to assess whether any autonomic nerve damage at the inferior mesenteric plexus is balanced by the level of colonic transection. Also, the impact of the level of colonic transection in itself could be assessed (e.g. proximally vs. distally to the junction between the sigmoid and descending colon), as this has not, to the author’s knowledge, been evaluated. Information on functional symptoms could be assessed by mailing validated questionnaires to the patients before and after surgery. This would enable paired difference tests, where each patient is used as its own control, further decreasing the influence of confounding. It would also be interesting to evaluate the function at different points of time, for instance after 6, 12, 24 and 36 months, to assess whether any effect is temporally dynamic.

Regarding the effect of anastomotic leakage on the functional outcome, further research is needed. The fact that AL occurs only in a minority of patients presents difficulties in achieving sufficient statistical power, especially regarding sexual function in women, where sexual inactivity pertains to the vast majority. Accounting for this when future studies are planned is therefore paramount. To aid in achieving statistical power, a matched cohort study could be a legitimate alternative, matching patients who have had AL with patients who have not. If only defecatory function is
to be assessed, registries can be used to exclude patients with a permanent stoma even before the questionnaires are mailed\textsuperscript{153}.

According to our study, NSAID treatment seems to be safe in relation to AL. This finding along with similar results in other recent observational studies not only illustrates the necessity of a well-powered randomized controlled trial, but also provides ethical legitimacy to such a study. Preferably, such a trial would be large enough to enable stratified subgroup analysis depending of the type of anastomosis (ileo-colic vs. colo-colic vs. colo-rectal), as these may constitute different entities in respect to NSAID-induced AL. Moreover, investigations regarding the effect of opioid treatment on the risk of AL would also be of great interest. Further research is also required concerning the role of intestinal microbiota in the development of AL and how its potential effect can be regulated. Also, as morphine might cause bacteria with tissue-degrading properties to adhere to the anastomosis, it is important to discern whether NSAIDs have similar effects.
Acknowledgements

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Appendix

Supplementary Figure 1. An English version of the questionnaire used in Studies II and III to assess the functional outcome

Department of Surgical and Perioperative Sciences

Personal ID number:…………………………………

Name:……………………………………………

Questionnaire: Symptoms after surgery for rectal cancer

1. Date completed: ……/……/ …… (yyyymmdd)

2. Do you have or have you had a stoma (bag on your stomach)?
   □ Yes, the stoma is still there
   □ Yes, but it was reversed (closed) a while after the surgery
   □ No, I was not given a stoma in connection with the operation

   If you still have the stoma, go to question 9 and continue to the end of the questionnaire from there. If you never got a stoma or do not still have the stoma, go to question 3 and continue to the end of the questionnaire from there. Note that all questions pertain to the symptoms you have had in the past three months.

3. How often do you have bowel movements?

   If you generally have a bowel movement more than once a day: ... times a day.
   Or
   If you generally have a bowel movement less than once a day: Once every ... days.
4. Do you sometimes have to go to the bathroom at night for a bowel movement?
   - No, never
   - Yes, sometimes
   - Yes, often
   - Yes, always

5. The next question concerns how often bowel incontinence occurs, meaning involuntary bowel movements due to leakage. Do you ever have bowel incontinence (involuntary bowel movements)?
   - Never
   - Sometimes, more specifically once a week or less
   - Often, more specifically more than once a week
   - Always, more specifically every day

6. Do you ever have bowel incontinence (involuntary bowel movements) at night?
   - Never
   - Sometimes, in other words once a week or less
   - Often, in other words more than once a week
   - Always, in other words every night

7. Do you use absorbent products, such as panty liners, sanitary pads and nappies/diapers due to bowel incontinence (involuntary bowel movements)?
   - Not at all
   - Only at night
   - Only if I am outdoors
   - At night and if I am outdoors
   - Throughout the day
   - Day and night
8. Do you feel that you are restricted in one or more of the following areas due to the symptoms (involuntary bowel movements, diarrhoea, etc.) that your bowel function gives rise to:

<table>
<thead>
<tr>
<th>Activity</th>
<th>Not at all</th>
<th>A little</th>
<th>Somewhat often</th>
<th>Very often</th>
</tr>
</thead>
<tbody>
<tr>
<td>Work and/or housekeeping</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outdoor activities (such as shopping, visiting somebody)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Going to the cinema or theatre, etc.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

9. Do you ever have involuntary urination (urine leakage)?
   - No
   - Yes, less than once a week
   - Yes, once a week
   - Yes, a few times a week
   - Yes, every day

10. Do you use absorbent products (nappies/diapers or the like) due to involuntary urination (urine leakage)?
    - No
    - Yes

11. Is there any connection between the occurrence of involuntary urination (urine leakage) and:
    - Coughing, sneezing, squeezing, lifting, standing up, exercise and/or laughing
    - A sudden strong need to urinate (urgency) that leads to involuntary urination
    - A combination of both (urgency and simultaneous coughing, sneezing, squeezing, etc. as per above)
12. Do you have problems urinating (do you have to, for example, exert yourself, push, to get the urinary stream going)?
   - Not at all
   - A little
   - Quite a lot
   - Very much

13. Have you noticed any decrease in your sexual activity?
   - Not at all less active
   - Slightly less active
   - Somewhat less active
   - Much less active
   - It was like that before my surgery, but afterwards I am no longer sexually active
   - I was not sexually active before my illness and surgery and am not currently sexually active

The following two questions pertain to women. If you are a man, continue to question 16.

14. Does it hurt during intercourse?
   - No, never
   - Yes, sometimes
   - Yes, mostly
   - Yes, always
   - Not applicable (do not have intercourse)

15. Do you experience vaginal dryness during intercourse?
   - No, never
   - Yes, sometimes
   - Yes, mostly
   - Yes, always
   - Not applicable (do not have intercourse)
The following two questions pertain to men. If you are a woman, continue to question 18.

16. Do you have difficulty getting or keeping an erection?
   - No, never
   - Yes, sometimes
   - Yes, mostly
   - Yes, always
   - Not applicable (not sexually active)

17. Do you ever fail to ejaculate, meaning that no sperm comes out upon orgasm?
   - No, never
   - Yes, sometimes
   - Yes, mostly
   - Yes, always
   - Not applicable (not sexually active)
Appendix

The final questions are about how you felt before the treatment for rectal cancer.

18. Did you suffer from involuntary bowel movements (bowel incontinence) before you were treated for rectal cancer?
   - No
   - Yes, but it got worse after the treatment for rectal cancer
   - Yes, I had similar symptoms before the treatment for rectal cancer
   - I do not remember

19. Did you suffer from involuntary urination (urine leakage) before you were treated for colorectal cancer?
   - No
   - Yes, but it got worse after the treatment for rectal cancer
   - Yes, I had similar symptoms before the treatment for rectal cancer
   - I do not remember

20. Did you have difficulty emptying your bladder before you were treated for rectal cancer?
   - No
   - Yes, but it got worse after the treatment for rectal cancer
   - Yes, I had similar symptoms before the treatment for rectal cancer
   - I do not remember

21. Did you have problems with your sex life before the treatment for rectal cancer?
   - No
   - Yes, but it got worse after the treatment for rectal cancer
   - Yes, I had similar symptoms before the treatment for rectal cancer
   - I do not remember

Thank you for your participation!