Permanent stoma after anterior resection for rectal cancer

Prevalence and mechanisms

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Till Martina
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Abstract

While sphincter-saving surgery constitutes standard treatment for rectal cancer, anterior resection still harbours a significant risk of a permanent stoma in the long run. Although anastomotic leakage plays a major role in this surgical dilemma, the exact mechanisms are not known, while surveys indicate a stoma-free outcome is essential for a majority of patients. To address this issue, the overall aim of the present thesis was to investigate the permanent stoma prevalence in patients undergoing anterior resection for rectal cancer in Sweden, and to identify plausible mechanisms that impede prospects of a stoma-free outcome.

In a population-based cohort, chart review of patients who had anterior resection for rectal cancer in the Northern healthcare region in Sweden between 2007 and 2013 showed that 75 out of 316 (24%) patients ended up with a permanent stoma. Of 274 patients (87%) primarily defunctioned with a stoma, 229 underwent stoma closure, 21 (9%) of whom suffered major complications that required return to theatre or worse. A permanent stoma was shown to be more common among patients with anastomotic leakage and an advanced tumour stage.

A registry-based method to estimate nationwide stoma outcome after anterior resection for rectal cancer was developed, using data from the Swedish Colorectal Cancer Registry and the National Patient Registry. With a chart-reviewed cohort as reference, stoma outcome was assessed with a positive predictive value of 85.1%, and a negative predictive value of 100.0%. In patients operated in Sweden between 2007 and 2013, the registry-based method determined that 942 out of 4768 (19.8%) had a permanent stoma, while stoma rates varied substantially between different healthcare regions.

In a 1:1 matched case-control study of 82 patients who had curative resection for non-disseminated colorectal cancer, a subgroup analysis of 34 patients with rectal cancer displayed biomarker aberrations in serum measured preoperatively in those with anastomotic leakage. Compared to complication-free controls, 15 proteins related to inflammation were elevated, of which two (C-X-C motif chemokine 6, and C-C motif chemokine 11) remained significant after adjustment for multiple testing.

Based on a cohort of 4529 patients who had anterior resection, tumour height served as a proxy to determine the extent of mesorectal excision, while long-term stoma outcome was classified using a previously validated registry-based method. Defunctioning stomas significantly decreased chances of a stoma-free outcome, especially in patients undergoing partial mesorectal excision; for these patients,
faecal diversion was also least beneficial in terms of reducing anastomotic leakage.

In conclusion, every fifth patient undergoing anterior resection for rectal cancer in Sweden eventually ends up with a permanent stoma. Although construction of a defunctioning stoma decreases the risk of symptomatic anastomotic leakage, subsequent takedown surgery carries a substantial risk of major complications, while chances of a long-term stoma-free outcome become significantly reduced. To facilitate selective use of faecal diversion, novel markers to identify high-risk anastomoses prior to surgery have been identified, but require validation in larger prospective settings. Anterior resection without a defunctioning stoma should be considered in appropriately informed patients for whom a stoma-free outcome is of importance. In particular, this holds true for patients eligible for partial mesorectal excision, where anastomatic dehiscence is less frequent and the advantageous effects of a defunctioning stoma are limited.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>AJCC</td>
<td>American Joint Committee on Cancer</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>BR</td>
<td>Baseline Risk</td>
</tr>
<tr>
<td>CCL11</td>
<td>C-C motif chemokine 11</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence Interval</td>
</tr>
<tr>
<td>cTNM</td>
<td>clinical TNM stage</td>
</tr>
<tr>
<td>CXCL6</td>
<td>C-X-C motif chemokine 6</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>HR</td>
<td>Hazard Ratio</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>high-sensitivity C-reactive Protein</td>
</tr>
<tr>
<td>I-FABP</td>
<td>Intestinal fatty-acid binding Protein</td>
</tr>
<tr>
<td>LARS</td>
<td>Low Anterior Resection Syndrome</td>
</tr>
<tr>
<td>NPV</td>
<td>Negative Predictive Value</td>
</tr>
<tr>
<td>OR</td>
<td>Odds Ratio</td>
</tr>
<tr>
<td>PME</td>
<td>Partial Mesorectal Excision</td>
</tr>
<tr>
<td>PPV</td>
<td>Positive Predictive Value</td>
</tr>
<tr>
<td>pTNM</td>
<td>Final pathological assessment of TNM stage</td>
</tr>
<tr>
<td>RD</td>
<td>Risk Difference</td>
</tr>
<tr>
<td>RR</td>
<td>Relative Risk</td>
</tr>
<tr>
<td>TME</td>
<td>Total Mesorectal Excision</td>
</tr>
<tr>
<td>TNM</td>
<td>Tumour, Node, Metastasis</td>
</tr>
<tr>
<td>U-CAN</td>
<td>The Uppsala-Umeå Comprehensive Cancer Consortium</td>
</tr>
<tr>
<td>UICC</td>
<td>Union for International Cancer Control</td>
</tr>
</tbody>
</table>
Original Papers

The present thesis is based on the following papers, which are referred to in the text by their Roman numerals (I–IV).


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Populärvetenskaplig sammanfattning (Summary in Swedish)

Bakgrund
Rektalcancer är en vanlig cancerform med drygt 2100 nyinsjuknanden i Sverige varje år. Kirurgisk standardbehandling utgörs av så kallad främre resektion och inbegriper utöver borttagande av det sjuka tarmsegmentet förfärdigande av en anastomos (tarmkarv), med målsättning att patienten ska slippa stomi (på magen) – inte minst eftersom långtidsöverlevnaden för rektalcancer är förhållandevis god. Dessvärre utfaller likväl omkring 20 % med permanent stomi på sikt, där en starkt bidragande orsak är tarmkarvsläckage (anastomosläckage). Anastomosläckage utgör i sig en allvarlig komplikation som gjort att tillfällig stomi under senaste decenniet rutinmässigt anläggs i slutet av de flesta operationer för att minska konsekvenserna om ett läckage skulle inträffa; detta innebär dock att ytterligare ett kirurgiskt ingrepp behövs för att patienten ska bli stomifri, och utan att på förhand veta vilka patienter som faktiskt drar nytta av den tillfälliga stomin som sådan.

Syfte
Det övergripande syftet med den här avhandlingen var dels att utvärdera aspekter kopplat till användning av tillfälliga stomier vid främre resektion för rektalcancer i Sverige, dels att fastställa långtidsförekomst av permanent stomi. Målsättningen var att försöka identifiera bakomliggande mekanismer till varför vissa patienter på sikt inte lyckas bli stomifria, för att i sin tur öka vår förståelse kring hur kirurgiska behandlingsstrategier kan matchas mot den enskilda patientens önskemål och förutsättningar.

Metoder
I delarbete II användes kompletterande data från Patientregistret för att med en registerbaserad metod på nationell nivå försöka fastställa långtidsförekomst av permanent stomi efter främre resektion. Med hjälp av den journalgranskade patientkohorten från delarbete I kunde metodens träffsäkerhet valideras och sedan användas för att undersöka eventuella regionala skillnader mellan olika sjukvårdsregioner i Sverige.


Resultat

I delarbete I, II och IV framkom att 20–24 % av alla patienter som idag opereras med främre resektion på sikt har permanent stomi. Delarbete II visade att den registerbaserade metoden med hög träffsäkerhet kan fastställa stomiuutfall, samtidigt som förekomsten av stomi varierade i betydande utsträckning mellan olika sjukvårdsregioner i Sverige.

I delarbete I, som inkluderade 312 patienter, var anastomosläckage och avancerad tumörsjukdom faktorer kopplade till att patienter i högre utsträckning hade permanent stomi. Tillfällig stomi var också vanligare hos patienter som på sikt hade permanent stomi men inte till en statistiskt säkerställt nivå. Delarbete IV som inkluderade betydligt fler patienter (n = 4529) kunde dock visa att tillfällig stomi i sig ökar risken för permanent stomi markant, och särskilt hos patienter som har tumörer belägna högre upp i lilla bäckenet, samtidigt som
permanent stomi i negligerbar utsträckning berodde på färre anastomosläckage efter avlastande stomi. I delarbete I framkom också att var tionde patient som fick sin tillfälliga stomi nedopererad drabbades av en allvarlig komplikation (n = 21/229). De flesta av dessa behövde intensivvård eller reoperation, medan en patient avled till följd av sin stominedläggning.

I delarbete III inkluderade vi 82 patienter fördelat på hälften fall och hälften kontroller. Analys visade att 15 proteiner kopplade till inflammation var stegrade redan före operationen hos de som senare drabbades av anastomosläckage. Efter justering för upprepade analyser var fortfarande C-X-C motif chemokine 6, och C-C motif chemokine 11 klart förhöjda hos läckagedrabbade patienter med rektalcancer. Däremot visade den kliniskt mer väletablerade markören hs-CRP endast en skillnad hos patienter med koloncancer.

**Slutsatser**

Introduction

Background
Colorectal cancer remains the third most common type of cancer, with 1.8 million new cases diagnosed worldwide in 2018. Historically, it has mainly been a disease in Western countries; however, colorectal cancer incidence rates are rapidly increasing in developing countries. Diet, physical inactivity and smoking are some of several modifiable risk factors that contribute to the development of colorectal cancer. Additional known risk factors include genetic predispositions, such as a family history of colorectal cancer or inflammatory bowel disease, as well as a few genetic disorders. Although colorectal cancer is the second most common cause of cancer deaths globally, the prevalence is high as the prognosis is nonetheless relatively good, with an overall 5-year disease-specific survival of 65% in Sweden. Internationally, survival rates in developed countries are similar. Consequently, there are millions of colorectal cancer survivors globally, and long-term function and quality of life following surgery have become increasingly important.

Diagnosis, staging and preoperative management
Virtually all colorectal cancers are thought to arise from adenomatous polyps of uncontrolled proliferation in the cells lining the large intestine. While the detailed mechanisms of colorectal carcinogenesis are beyond the scope of the present thesis, it is nonetheless important to understand how the adenoma-carcinoma sequence requires accumulation of one or a combination of several mutations and takes years to decades to complete. Benign adenomatous polyps are therefore often possible to detect before transformation to invasive cancer, and the majority of these lesions de facto never become malignant. To differentiate benign lesions from invasive cancer, however, biopsy from a suspicious area or lesion using endoscopy is required, and biopsy constitutes the gold standard to diagnose colorectal cancer.

In Sweden, the annual incidence of colorectal cancer amounts to more than 6700 newly diagnosed cases each year. While cancer of the colon and rectum share most features, increased understanding of some important differences have resulted in the two conditions being viewed as two separate entities. One of the papers in the current thesis (Paper III) includes both diagnoses due to their putative similarities in terms of anastomotic healing after surgery; however, the main focus hereafter will be on rectal cancer.
Roughly one third of colorectal malignancies originate in the rectum, and rectal cancer shows a slight male predominance. Despite possible anatomical differences between women and men, the rectum is most often defined as the large intestine within 15 cm from the anal verge. Herein, rectal cancer is considered as any malignant tumour with its lower margin within 15 cm of the anal verge, measured using rigid sigmoidoscopy.

Apart from endoscopy and biopsy to confirm the diagnosis and to rule out metachronous colon cancer, preoperative examination for rectal cancer includes computerized tomography of the abdomen and the thorax to evaluate distant metastasis, as well as pelvic magnetic resonance imaging to determine degree of invasion of the rectal wall and the mesorectal fascia, infiltration of lymph nodes, and extramural vascular involvement. In patients with suspected liver metastases, extent of disease is usually determined by magnetic resonance imaging of the liver, while positron emission tomography with concomitant computerized tomography is sometimes employed to rule out occult extrahepatic spread, which in turn can result in a change in treatment strategy.

Preoperative assessment is mainly done to enable correct tumour staging and was historically performed according to the Dukes’ classification. Presently, the Dukes’ classification has largely been replaced by the TNM-classification (Tumour, Node, Metastasis), which is the taxonomy most widely used internationally, and constitutes current standard practice in Sweden (Table 1).
**Table 1.** The TNM-classification in relation to the AJCC/UICC staging system.

<table>
<thead>
<tr>
<th>TNM classification</th>
<th>AJCC/UICC staging system*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T1-2 No Mo</strong></td>
<td>Stage I</td>
</tr>
<tr>
<td>T1 = invasion into the submucosa</td>
<td></td>
</tr>
<tr>
<td>T2 = invasion into the muscularis propria</td>
<td></td>
</tr>
<tr>
<td>No = no involvement of lymph nodes</td>
<td></td>
</tr>
<tr>
<td>Mo = no distant metastases</td>
<td></td>
</tr>
<tr>
<td><strong>T3-4 No Mo</strong></td>
<td>Stage II</td>
</tr>
<tr>
<td>T3 = invasion into the serosa or perirectal fat</td>
<td></td>
</tr>
<tr>
<td>T4 = invasion of adjacent organs and/or breaching of the visceral peritoneum</td>
<td></td>
</tr>
<tr>
<td>No = no involvement of lymph nodes</td>
<td></td>
</tr>
<tr>
<td>Mo = no distant metastases</td>
<td></td>
</tr>
<tr>
<td><strong>T1-4 N1-2 Mo</strong></td>
<td>Stage III</td>
</tr>
<tr>
<td>N1 = 1-3 perirectal lymph nodes involved</td>
<td></td>
</tr>
<tr>
<td>N2 ≥ 4 perirectal lymph nodes involved</td>
<td></td>
</tr>
<tr>
<td>Mo = no distant metastases</td>
<td></td>
</tr>
<tr>
<td><strong>T1-4 No-2 M1</strong></td>
<td>Stage IV</td>
</tr>
<tr>
<td>M1 = distant metastases</td>
<td></td>
</tr>
</tbody>
</table>

*The 8th ed. of the American Joint Committee on Cancer and the Union for International Cancer Control TNM staging system.

While staging was mainly developed to reflect the patient’s prognosis and survival, TNM classification is a fundamental step to aid decisions regarding overall treatment strategy, and oncological management in particular. Although final pathological assessment of TNM stage (hereafter referred to as “pTNM”) is available only after surgery, preoperative staging refers to the clinical TNM stage (cTNM) as derived from the assessment made prior to tumour resection (if resection is performed at all).
All patients diagnosed with colorectal cancer are discussed at a multidisciplinary team meeting to provide the best up-to-date treatment tailored to the individual patient, including neoadjuvant therapy and operative approach\(^4\). As we will review later on, local recurrence has historically represented a formidable problem in rectal cancer treatment, partly resolved by ground-breaking advances in surgical treatment; however, introduction of radiotherapy has also helped to reduce local recurrence rates\(^12\). While the effect is also partly achievable with postoperative irradiation, preoperative radiotherapy has better oncological results and is associated with less morbidity\(^13\). Interestingly, ongoing research suggests an increasing number of patients may qualify for non-operative treatment in the future\(^14\); however, surgical resection remains gold standard and is offered to the lion’s share of patients with rectal cancer in Sweden\(^15\).

**Surgical treatment options**

To understand the principles of current surgical options for rectal cancer, a historical perspective is necessary. The first operation with restored bowel continuity dates back to 1885, performed and described by German surgeon Paul Kraske\(^16\). In his *Sacrale Methode* coined “posterior resection” the rectum was accessed through a posterior incision that entailed coccygectomy, often with significant postoperative morbidity and poor oncological outcome as a result. Along with increased understanding of tumour spread, the refined version of the abdominoperineal excision with permanent colostomy, presented in 1923 by Miles\(^17\), instead became the gold standard of rectal cancer surgery that prevailed throughout the majority of the twentieth century. Although sphincter-saving “anterior resection” (hereon via an anterior, transabdominal approach) was introduced already in the nineteen forties\(^18\), it was not until the advent of mechanical staplers some thirty years later that rectal resections with a primary anastomosis became more frequent\(^19\); eventually, anterior resection was established as standard treatment for rectal cancer. Furthermore, the third transabdominal operation considered in some patients with rectal cancer is Hartmann’s procedure. Also introduced to the surgical armamentarium back in the nineteen twenties, Hartmann’s procedure evolved from a two-step operation into a one séance technique with sigmoidostomy construction and closure of the rectal stump followed by tumour removal\(^20\). It is, however, generally reserved for patients where there is doubt about ability to tolerate major surgery, as well as in palliative settings.
Figure 1. Displaying a schematic illustration of an anterior resection. The blue shaded area in the left picture denotes the bowel segment (rectum and part of the sigmoid colon) that is removed together with its surrounding lymphovascular mesorectum (not displayed in the picture). The right image depicts the primary anastomosis fashioned after the bowel ends have been joined together by means of a stapling device.

During the pioneering era summarised in the previous paragraph, local recurrence rates for rectal cancer were reported to be as high as 30–40%\cite{17, 21, 22}. A paradigm shift came with the introduction of total mesorectal excision (TME) by Heald in 1982\textsuperscript{23}. Principles of TME involve dissection in embryological planes and en bloc resection of the tumour together with all of the mesorectum. In his series, Heald reported a 5-year local recurrence rate lower than 5\%\textsuperscript{24}. While these results were initially subject to some debate\textsuperscript{25}, there are several reports on recurrence rates around 10\%\textsuperscript{21, 26}, and the local recurrence rate for rectal cancer in Sweden is currently at 5\%\textsuperscript{27}. Thus, the TME technique is believed to be an important factor behind the vast improvement in rectal cancer survival rates\textsuperscript{26}. However, these advancements probably also derive from better oncological treatment (radiotherapy for rectal cancer in particular), aided by more accurate staging, as well as improved management of distant metastasis.
In summary, anterior resection constitutes current standard treatment for rectal cancer in Sweden whenever oncologically possible. Approximately half of all patients diagnosed with a rectal malignancy are considered for such surgery, and it is the only transabdominal surgical option with a sphincter-saving intent. While abdominoperineal excision and Hartmann’s procedure also adhere to TME principles, both methods involve immediate permanent end colostomy construction, for which implications will be reviewed in detail further on.

**Anterior resection and its associated morbidity**

**Anastomotic leakage**

The Achilles’ heel of sphincter-saving resections is the subsequent risk of anastomotic leakage. While a plethora of different definitions permeates the surgical literature, anastomotic leakage is generally considered a defect of the integrity of the intestinal wall leading to communication between the intra- and extraluminal spaces. It afflicts all colorectal surgery and is associated with profound morbidity, and also mortality; however, leakage occurs at a

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*. Local procedures by means of e.g. transanal endoscopic microsurgery can be employed in patients with locally growing T1 cancer. Pathological staging indicating deeply invasive cancer will, however, require subsequent transabdominal resection in patients where a curative intent is the aim.
significantly higher rate in rectal surgery compared to other parts of the gastrointestinal tract\textsuperscript{31}. The reported leakage incidence following anterior resection amounts to 9–19\% in population-based series\textsuperscript{30, 32–33}, and apart from the immediate consequences of faecal peritonitis, often ensues in permanent stoma construction\textsuperscript{34}. Recent reports also suggest that anastomotic leakage increases the risk of local relapse\textsuperscript{35}, and it is evident why leakage constitutes a surgical conundrum that surgeons attempt to avoid at almost any cost.

Although there is moderate evidence indicating that a low anastomosis confers an increased risk of leakage (Table 2)\textsuperscript{36}, results of an extensive research effort to identify other predictors remain somewhat inconclusive\textsuperscript{37}. While the understanding of the multifactorial pathogenesis behind an anastomotic breakdown is not complete, leakage is generally considered a failed wound healing process\textsuperscript{38}. Previous research has, as a consequence, focused on postoperative markers associated with wound healing, in order to detect anastomotic leaks early after surgery, before such a leak is clinically obvious\textsuperscript{39–41}. However, the inflammatory milieu in patients with colorectal cancer has been reported to differ from healthy controls already before surgery\textsuperscript{42, 43}, and, even after complete tumour removal, factors associated with invasion and inflammation remain elevated for several weeks\textsuperscript{44}. Interestingly, preoperative systemic inflammation has also been shown to correlate with a higher rate of postoperative infections, out of which 20\% were anastomotic leaks\textsuperscript{45}. Ideally, biomarkers denoting an unfavourable intestinal environment could be used to identify patients at a higher risk of anastomotic leakage prior to surgery\textsuperscript{46}. This could in turn facilitate more selective surgical strategies, e.g. identify situations where surgery without a primary anastomosis could be the judicious option to consider, as well as motivate construction of a temporary stoma.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Number of patients/number of studies</th>
<th>Regarded a risk factor (based on available evidence)</th>
<th>Pooled odds ratio (95% CI, meta-analysis)</th>
<th>Quality of evidence (GRADE)</th>
<th>Newcastle-Ottawa score (median for included studies)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (high)</td>
<td>17 493/7</td>
<td>No</td>
<td>0.99 (0.89–1.10)</td>
<td>Low</td>
<td>8</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>93 016/11</td>
<td>Yes</td>
<td>1.48 (1.37–1.68)</td>
<td>Low</td>
<td>8</td>
</tr>
<tr>
<td>Low anastomosis</td>
<td>7175/6</td>
<td>Yes</td>
<td>3.26 (2.31–4.62)</td>
<td>Moderate</td>
<td>8</td>
</tr>
<tr>
<td>ASA class</td>
<td>17 254/7</td>
<td>?</td>
<td>1.71 (1.09–2.67)</td>
<td>Very low</td>
<td>8</td>
</tr>
<tr>
<td>BMI</td>
<td>72 436/3</td>
<td>?</td>
<td>1.00 (0.93–1.07)</td>
<td>Very low</td>
<td>8</td>
</tr>
<tr>
<td>Preoperative radiotherapy</td>
<td>3820/2</td>
<td>Yes</td>
<td>1.65 (1.06–2.56)</td>
<td>Low</td>
<td>8</td>
</tr>
<tr>
<td>Tumour stage</td>
<td>18 084/8</td>
<td>No</td>
<td>0.86 (0.67–1.10)</td>
<td>Low</td>
<td>8</td>
</tr>
</tbody>
</table>

ASA, American Society of Anaesthesiology; BMI, body mass index; GRADE, Grading of Recommendations Assessment, Development and Evaluation.

Table 2. Displaying results from a meta-analysis on preoperative risk factors for anastomotic leakage and their quality of evidence according to the GRADE approach\textsuperscript{36}. Reprint was made with publisher’s permission.
**Defunctioning stoma**

While knowledge on leakage prediction is generally lacking, there is robust evidence that “temporary faecal diversion” reduces symptomatic leaks and mitigates consequences if it occurs\(^{47,48}\). More specifically, faecal diversion means that a loop-stoma is constructed, usually in immediate conjunction with the index operation (occasionally preoperatively) in order to divert the faecal stream away from the primary anastomosis during the postoperative period. The defunctioning stoma is then reversed at a later time point, and routine use of such stomas have during the past decades become widespread in colorectal surgical practice\(^{49,50}\). Paradoxically, leakage rates after anterior resection for cancer, despite intensive use of defunctioning stomas, have not decreased in population-based studies\(^{49}\), nor has a mortality reduction been shown\(^{51}\). Consequently, faecal diversion remains controversial and subject to debate\(^{49,52}\). Additionally, of loop stomas fashioned with a temporary intent, some 18–25% are reported to remain in place or become converted to end colostomies\(^{53,54}\), while the subsequent takedown surgery entails a risk of major complications in itself\(^{55}\). Given that defunctioning stomas also result in significantly more short-term complications\(^{56-58}\), several reports conclude that a more selective use of defunctioning stomas is needed\(^{52,59}\); however, identifying the patients for whom there will be an actual benefit from a defunctioning stoma remains a Gordian knot yet to be cut.

**Stoma outcome**

Although anterior resection is a sphincter-saving treatment for rectal cancer, the long-term permanent stoma prevalence is reported at \(19–25\%\)\(^{34,60}\). Anastomotic leakage is one of the leading causes of stoma permanence\(^{34}\), while defunctioning stomas left in place, and severe bowel dysfunction, also seem to contribute to this phenomenon\(^{34,53,60}\). As long-standing surgical stomas are associated with both increased morbidity\(^{58,61,62}\) and decreased health-related quality of life\(^{63,64}\), further research concerning prevalence and mechanism behind different stoma outcomes after anterior resection is warranted.

**Permanent stoma type**

With focus on the high permanent stoma prevalence after anterior resection, it is important also to understand key features and functionality of different stoma types. To begin with, the loop ileostomy has, when performing anterior resection in Sweden, generally triumphed over the loop transverse colostomy due to the slightly lower risk of overall complications\(^{48}\); specifically, lower prevalence of sepsis, prolapse and parastomal hernia\(^{65}\). Nevertheless, this topic has historically been subject to much debate, and continues to recur in updated reviews\(^{66}\). While drawbacks of loop ileostomies include a higher incidence of intestinal obstruction and ileus, the main criticism is aimed at the risk of a high stoma output, which
basically only afflicts patients with stomas on the small intestine\textsuperscript{66, 67}. Putatively, loop ileostomies are for these reasons not well suited for permanence, with several studies reporting frequent hospital readmissions and chronic kidney failure attributable to ileostomy-related dehydration and electrolyte imbalance\textsuperscript{58, 62, 68, 69}. Furthermore, there is also increasing awareness that presence of an ileostomy during adjuvant treatment is a predictor of severe chemotherapy-induced diarrhoea, which in turn may interfere with the treatment regime and impact long-term survival\textsuperscript{70}.

As many loop stomas are reported to remain in place after anterior resection, such stomas merit comparison with end colostomy, which would have been the outcome had the patient been managed with abdominoperineal excision or Hartmann’s procedure instead. However, to the best of our knowledge, there have hitherto been no such investigations comparing loop stomas left in place, with end colostomy after non-sphincter-saving procedures. While surveys indicate that a stoma-free outcome ranks highest for most patients (Figure 3)\textsuperscript{71}, it is somewhat puzzling that a Cochrane review only managed to demonstrate a minor negative influence of a permanent end colostomy on health-related quality of life, when comparing patients treated with abdominoperineal excision or Hartmann’s procedure to those managed with anterior resection\textsuperscript{72}. A plausible explanation is that several of the studies in the report did not include data on tumour height or level of anastomosis, factors known to correlate with anorectal dysfunction, which may impede patient satisfaction after anterior resection\textsuperscript{73}. Also of note, the apparent lack of differences may be attributable to response shift\textsuperscript{74}, meaning that patients may adapt to their new condition with time, appreciating the notion that stoma permanence was considered inevitable for the patient and, as such, a small price for survival. Moreover, a more recent Swedish report showed that overall health-related quality of life was inferior in patients with a permanent stoma compared to those who were stoma-free\textsuperscript{64}. 
In conclusion, while a stoma-free outcome may be the preferred situation for most patients, an end colostomy is most likely the second-best option and is important to remember when reviewing aspects of surgical strategies in patients undergoing treatment for rectal cancer.

**Extent of mesorectal excision and functional results**

Due to an initial increase in the frequency of anastomotic leakage, TME at first seemed yellow to the jaundiced eye of some surgeons\(^7^5\); however, leakage rates ultimately improved with experience\(^7^6\). To further decrease leakage rates and reduce bowel dysfunction, partial mesorectal excision (PME: Figure 4) has developed into a widely accepted option for tumours in the upper rectum that allows construction of higher anastomoses with a subsequent lower risk of leakage\(^7^7\). Namely, 32 out of 396 (8.1%) patients treated with TME had anastomotic leakage, compared to 3 out of 226 (1.3%) of those managed with PME, according to the benchmark study presented by Law et al in 2004. In the same report, PME also rendered less intraoperative blood loss and shorter hospital stay, while oncological results were similar between the two groups, and morbidity also showed a tendency favouring PME.
Figure 4. Demonstrating the difference between PME (left), and TME (right), for rectal cancer. The tumour itself is indicated with filled black areas in the images, together with adjacent mesorectal infiltration. The dashed lines illustrate how an adequate resection margin below the lower tumour border will be sufficient in PME without dissection down to the pelvic floor, as required in TME.

To delve even further into the rationale behind why routine TME for upper rectal cancer became challenged, results of detailed histological examination of the distal mesorectum requires mentioning. Several reports found evidence that rectal cancers do not spread beyond 5 cm distal of the tumour border\cite{23, 79, 80}, and Lopez-Kostner et al managed to corroborate this clinically by demonstrating similar local recurrence rates and survival between surgery for sigmoid cancer and those who had PME for upper rectal cancer\cite{81}. Further supported by the treatment outcomes presented by Law et al, and conforming results from more recent reports\cite{82}, PME is generally considered sufficient and advisable whenever an oncologically safe transection of the bowel and mesorectum can be made at a minimum of 5 cm below the tumour\cite{83}. Consequently, most proximal tumours that originate above the peritoneal reflection are currently managed with such surgery, and high rectal cancers originating at 13–15 cm from the anal verge in particular\cite{50, 84-86}.

In addition to increased leakage occurrence, anterior resection with TME sometimes results in a poor functional outcome\cite{87}, under what has commonly become referred to as low anterior resection syndrome (LARS)\cite{88, 89}. The term LARS includes faecal and gas incontinence, urgency, frequent bowel movements, clustering of stools and difficulty emptying. Although it is generally acknowledged that up to 90% of patients undergoing anterior resection will experience a change in bowel habits, the prevalence of LARS varies greatly between different studies\cite{89}; presumably, non-responders to surveys and subsequent selection bias might explain these discrepancies. Notwithstanding, in
a large international multicentre study with a 75% response rate, Juul and colleagues managed to demonstrate that the severity of LARS was closely associated with the health-related quality of life of the queried patients99. Importantly, the authors found that quality of life did not differ significantly between asymptomatic patients and those with minor LARS, whereas roughly half of all patients in the cohort suffered from major LARS and fared considerably worse in basically all aspects of health-related quality of life. To conclude, it is important to bear in mind the functional impact of anterior resection, as some patients who end up stoma-free will sustain major LARS and may ultimately opt for construction of a permanent stoma34.

PME not only features a lower risk of anastomotic dehiscence, but also a decreased likelihood of a poor anorectal function. The high anastomosis and less common use of neoadjuvant radiotherapy are some of the plausible mechanisms behind the improved functional outcomes reported after PME75; moreover, lower leakage incidence after PME probably also reduces the risk of dysfunction, as dehiscence leads to higher risks of LARS in itself94. With these important aspects in mind, the need for a defunctioning stoma in patients treated with PME becomes less obvious. Also, all randomised controlled trials supporting the use of a defunctioning stoma were conducted exclusively with patients undergoing TME for tumours predominantly located in the mid and lower rectum. Hence, there are as yet no clinical trials nor observational reports, to support the use of defunctioning stomas in patients undergoing rectal resections with PME; however, such stomas are in use for anterior resection with PME, with roughly every third patient defunctioned according to available reports50, 82.
Aims of the Thesis

The overall objective of the current thesis was to evaluate aspects of the use of defunctioning stomas for anterior resection for rectal cancer in Sweden, and to establish benchmark data on the permanent stoma prevalence after such surgery. The aim was also to identify mechanisms behind different stoma outcomes to provide insights on how treatment strategies can be tailored to the individual patient’s preferences.

Specific aims

**Paper I:** To investigate the permanent stoma prevalence after anterior resection for rectal cancer in the Northern healthcare region in Sweden, as well as factors influencing stoma outcome, and complication rates following stoma reversal.

**Paper II:** To validate a registry-based method to estimate long-term stoma outcome after anterior resection for rectal cancer in Sweden, and to explore regional differences regarding permanent stoma prevalence after such surgery.

**Paper III:** To identify novel biomarkers to help predict high-risk anastomoses prior to surgery for colorectal cancer.

**Paper IV:** To quantify the effect of defunctioning stomas on the long-term stoma outcome and how the extent of mesorectal excision affects this potential relationship.
Patients and Methods

Ethics and approvals

The research featured in the present thesis was conducted according to the regulatory norms and standards of the 1975 Helsinki Declaration. All four studies (Papers I–IV) were approved by the regional ethical review board in Umeå. The approval registration numbers are provided below:

**Paper I:** Dnr 2011-234-31M, Dnr 2015-6-32M and Dnr 2015-122-32M

**Paper II:** Dnr 2011-234-31M, Dnr 2015-6-32M and Dnr 2015-122-32M

**Paper III:** Dnr 2015-425-31M


Data from the Swedish Quality Registries enlisted below were requested in written form and authorised by each registry holder before data extraction. Access to biobank samples in **Paper III** was approved by the Regional Biobank Centre in Northern Sweden and the Diagnosis Specific Expert Group for colorectal cancer at the Uppsala-Umeå Comprehensive Cancer Consortium (U-CAN). Patients in the U-CAN programme had signed written consent prior to blood sampling and biobank storage, and in any case where an informed agreement was missing, patients were excluded from further study.

Data sources

The present thesis is based on observational research, in which different Swedish quality registries were used to identify patients eligible for study inclusion as well as to collect clinically related data. The Swedish Colorectal Cancer Registry was used in all four studies (Papers I–IV) to identify the study cohorts, whereas the National Patient Registry and the U-CAN database constituted supplementary sources from which information was added. A detailed introduction to these registries will follow, while the implementation of each registry’s data will be outlined separately for each study.

**The Swedish Colorectal Cancer Registry**

The Swedish Colorectal Cancer Registry covers all patients diagnosed with rectal cancer in Sweden from 1995 onwards and, since 2007, also encompasses colonic cancer. It is continually cross-checked against the National Cancer Registry for
completeness and has been validated twice, both times with figures denoting a near complete degree of coverage. Data reported to the registry include patient characteristics, surgical details, postoperative course, final pathological assessment and a 5-year follow-up. In accordance with this thesis, rectal cancer is defined in the registry as any malignancy originating with its lower margin within 15 cm of the anal verge, measured using rigid sigmoidoscopy. Tumour height as assessed before surgery is comprised in the patient characteristics, while surgical details on the extent of mesorectal excision have hitherto not been introduced to the national registry form and were therefore ascertained through chart review in Paper I and classified using tumour height in Paper IV. Data on stoma reversal surgery evaluated in Paper II have been recorded from the year 2011 onwards, while a few other variables have also been added to the registry or undergone minor updates through the years, but without any influence on the studies covered herein, and are therefore left without further comment. Importantly, however, implications of the definition of anastomotic leakage and how it is reported to the registry require particular attention and will be reviewed in a separate section below.

The National Patient Registry
Starting in 1964, data on inpatient care in Sweden have been recorded in the National Patient Registry. The registry is run by the National Board of Health and Welfare and, since 1987, has featured nationwide coverage. It includes all inpatient surgery performed in Sweden, and procedure codes are registered according to the Swedish version of the NOMESCO Classification of Surgical Procedures version 1.9. The degree of completeness has been demonstrated to exceed 99%, while previous validation reports on specific diagnoses also indicate a high correlation between the registry data and review of medical records as reference.

The Uppsala-Umeå Comprehensive Cancer Consortium
U-CAN started as a strategic research effort funded by the Swedish government back in 2010. Since its inception, the U-CAN programme features longitudinal collection of blood and tissue samples from patients treated for various malignant conditions, including colorectal cancer. While additional centres have been introduced during recent years, the two university hospitals of Uppsala and Umeå constitute the foundation of the collaboration, which encompasses a joint total population of more than 600,000 citizens from which patients are recruited. Like the aforementioned nationwide quality registries, U-CAN has near complete coverage, while its performance has been rewarded with high ratings, and nearly all patients registered (98%) have had blood samples drawn at time of inclusion. Samples are typically drawn the day before surgery, and generally before any therapeutic intervention at all. Inter alia, blood sampling includes EDTA plasma...
and serum, and frozen aliquots stored in 0.5 ml microvials (Micronic, Lelystad, the Netherlands) are usually attained within four hours of the sampling occasion, preserved at -80°C.

**Study cohorts**

**Paper I** and **Paper II** included patients who had had an anterior resection for rectal cancer in Sweden between 1 January 2007 and 31 December 2013, with follow-up until 31 December 2014 regarding stoma outcome. While **Paper I** comprised a chart-reviewed cohort of those operated in the Northern healthcare region in Sweden, **Paper II** constituted a registry-based cohort of all patients who received treatment in Sweden during the study period. In both studies (**Paper I & Paper II**), any patient erroneously registered with rectal cancer with a tumour height above 15 cm from the anal verge, as well as any case of emergency surgery, were excluded, whereas the following ineligibility criteria were applied to the chart reviewed cohort (**Paper I**) exclusively:

- Any mortality within 30 days of index surgery, as this was considered unrepresentative of the long-term stoma outcome
- Operative charts indicating the patient did not undergo surgery with a primary anastomosis
- The patient was lost to follow-up
- Medical records and data were unattainable

**Paper III** was based on a cohort of patients who had elective surgery for non-disseminated colorectal cancer, with a primary anastomosis, between 1 January 2010 and 31 December 2015 at either of the two university hospitals of Uppsala and Umeå in Sweden. Patients who had anastomotic leakage were identified using complications reported to the Swedish Colorectal Cancer Registry and matched 1:1 to complication-free controls. Any patient with non-registered anastomotic leakage with respect to the registry, determined during chart review to have such a complication, was considered eligible for study inclusion and subsequently added to the study cohort whenever matching to a complication-free control was possible. Patients who could not be matched due to there being no eligible controls were excluded, as well as any case where an informed consent turned out to be missing, or where a preoperative blood sample had not been obtained.

In the fourth study (**Paper IV**), patients who had had an elective anterior resection for rectal cancer between 1 January 2007 and 31 December 2016 were considered and followed until the end of 2017 regarding stoma outcome. Patients erroneously registered with rectal cancer with a tumour height above 15 cm from the anal verge, as well as any emergently operated patients, were, as with the first
two reports (Paper I & Paper II), excluded as a consequence. Also, any patient with missing data for tumour height was excluded as a consequence.

**Study designs**

**Papers I–II**

**Paper I study design**
In the first study (Paper I), presence of a defunctioning stoma prior to surgery, or fashioned during the index operation, was extracted from medical records and recorded to type. Patient characteristics available from the Colorectal Cancer Registry were retrieved and complemented with chart-reviewed data on anastomotic leakage and the extent of mesorectal excision, as specified for each study.

**Stomas: long-term outcomes**
For descriptive purposes, stoma outcome was divided into three different groups: ‘never stoma’, indicating that neither did the patient receive a defunctioning stoma, nor had any stoma fashioned at a later time point; ‘reversed stoma’, meaning that the patient at some time point had a stoma present (either defunctioned primarily or received a stoma at a later time point) but ultimately underwent reversal and ended up stoma-free; ‘permanent stoma’, denoting that the patient had a stoma present at end of follow-up, regardless of whether this was a primary stoma left in place or a secondary stoma that had been constructed after the index operation. To provide a dichotomous stoma outcome in the subsequent analyses, ‘never stoma’ and ‘reversed stoma’ were merged into a ‘stoma-free’ group. Permanent stomas were categorized into ileostomy, colostomy (including transverse colostomies remaining in place, and end colostomy after salvage surgery without excision of the anal canal) or abdominoperineal excision, to illustrate the stoma permanence origin. Any stoma left in place at end of follow-up was considered to be permanent irrespective of whether or not the patient was scheduled for takedown surgery at a date outside the study period.

**Stoma reversal complications**
Postoperative complications within 90 days of stoma reversal surgery were recorded and graded according to the Clavien-Dindo taxonomy. Events where the patient required return to theatre, intensive care or went *ad mortem*, corresponding to Clavien Dindo IIIB–V, were defined as major complications.

**Paper II study design**
In Paper II, inpatient procedure codes registered during the study period were
obtained from the National Patient Registry for all patients in the study cohort. Data on construction of a defunctioning stoma recorded in the Swedish Colorectal Cancer Registry was used to classify whether patients had been managed with primary faecal diversion or not, whereas any stoma fashioned at a later time point than the index operation was defined as a secondary stoma.

**Registry-based stoma outcome**

An algorithm-based classification system, hereafter coined “registry-based method”, was developed and applied, using procedure codes to identify surgical events corresponding to creation or reversal of a stoma. Stoma reversal was registered using the following procedure codes: (JFG)00, 10, 20, 23, 26, 29, 30, 33, 36. Construction of a secondary stoma was defined as the presence of any of the procedure codes: (JFF)10, 11, 13, 20, 21, 23, 24, 26, 27, 30, 31, 96, 97. Events indicating reversal of a secondary stoma, as well as subsequent further stoma creation were taken into account; the classification system was looped until no more events were recorded and final stoma outcome was established. Hartmann’s procedure ([JGB]10, 11; [JFB]60, 61, 63, 64), and abdominoperineal excision ([JGB]30, 31, 33, 34, 36) were also registered, and any such surgery denoted a permanent stoma outcome immediately. Stoma type was derived from the procedure codes at the time of the index operation or from secondary stoma surgery: ileostomy (JFF10, 11), colostomy (JFF20, 21, 23, 24, 26, 27, 30, 31), or unspecified (JFF13, 96, 97). Any stoma left in place at the end of follow-up was considered a permanent stoma.

**Reference data**

The chart-reviewed cohort of patients who had had surgery for rectal cancer in the Northern healthcare region in Paper I was used as reference cohort to internally validate the accuracy of the registry-based method and reporting on stoma reversal surgery to the Swedish Colorectal Cancer Registry. The geographical district covered in the reference cohort is one out of a total of six healthcare regions in Sweden, and encompasses nearly one million inhabitants.

**Paper III**

**Paper III study design**

**Paper III** featured a case-control study design, in which matching criteria were sex (male or female), age (±5 years, at time of surgery), tumour location (colon, or rectum), pTNM (I, II or III) and operating hospital (Umeå University Hospital, or Uppsala University Hospital). The tumour location was scrutinized so that all patients recorded with rectal cancer had a tumour height reported to the registry within 15 cm of the anal verge. To ensure true case or control status, all patients
underwent chart review, while the pTNM stage reported to the registry was crossed-checked by examination of reports on histopathological assessment of tumour stage. Dates when blood samples had been drawn and stored in the biobank were retrieved. Additionally, to account for potential immune aberrations due to transfusion-related immunomodulation, any allogenic blood transfusion within 90 days prior to surgery was ascertained during review of medical records.

After collection of data, matching was completed. For all potential controls matchable to more than one case, prioritisation was performed to ensure all cases had a minimum of one control. Additionally, for all cases that had more than one eligible control, relaxation rules were applied using the following criteria: blood sampling corresponding to sampling completeness of the case (while a preoperative sample was an absolute criterion for study inclusion, postoperative samples were also considered in this context, as the study cohort derives from a larger project including different research questions), nearest date of surgery relative to the case, surgical approach (open, or laparoscopic) and neoadjuvant treatment (no treatment, radiotherapy, chemoradiotherapy).

**Blood sample analyses**

**Olink Proteomics**

Serum samples from all study participants were requested from the biobank and sent for analysis to Olink Proteomics, Uppsala, Sweden. The predefined biomarker panel “Olink Inflammation” (available through the company website), was selected for analysis. In brief, the Olink panel is run through adding 92 protein-specific pairs of antibodies labelled with unique complementary oligonucleotides (PEA probes) to 1 µl of each serum sample. The antibody pairs target a specific protein, and once attached to their target, the unique PEA probes nearby allow for hybridization, which in turn generates a DNA template that can act as a protein marker. Templates are thereafter extended by means of DNA polymerase and pre-amplified by quantitative polymerase chain reaction using universal primers. Excess primers are cleared before quantification by a microfluidic chip (96.96 Dynamic array IFC, Fluidigm Biomark), which is run in a quantitative polymerase chain reaction platform (BioMark HD System). For more information, detailed descriptions are readily available through the Olink website. All analyses were performed blinded to the study endpoint.

The biomarker panel analysis generates output in the form of Normalized Protein eXpression, which is an arbitrary unit on the log2 scale. Consequently, measurements are only applicable for relative quantification for each protein.
individually between samples, and noncomparable in terms of absolute numbers or between analyses performed at different time points. With results derived on a log2 scale, each one-unit increase in Normalized Protein eXpression corresponds to a doubling in protein concentration.

**Enzyme-linked immunosorbent assay**

Based on the findings of previous research on early leakage prediction\(^{46}\), high-sensitivity C-reactive protein (hs-CRP) and intestinal fatty-acid binding protein (I-FABP) were also evaluated. As these proteins were not included in the Olink inflammation panel, analyses were performed in-house by means of enzyme-linked immunosorbent assay (ELISA)\(^{102}\) and took place at a later time point.

In brief, ELISA is a “wet lab” biochemistry assay technique, in which quantification of an analyte is performed by adding liquid sample (i.e. patient serum herein) to a reaction chamber (usually a microtiter plate), after which measurements are derived by means of spectrophotometry\(^{102}\)(Figure 5). In terms of the present analyses specifically, hs-CRP and I-FABP protein standards were first diluted with ultrapure water according to the manufacturers’ protocol. Prepared dilution gradients across 96 well plates were then transferred onto the premanufactured ELISA plates coated with target antibodies against hs-CRP and I-FABP, respectively, and left to incubate (i.e. to allow protein standards to adhere to capture antibodies). Before thawed patient serum samples were added for incubation, serum samples were also diluted to reduce the risk of concentrations exceeding the spectrophotometer’s upper limit of detection. Subsequently, plates were washed either once or repeatedly, depending on the specific test kit, in order to remove uncaptured antigens. Next, a specific antibody aimed at the antigen was added; hence, giving rise to the label “sandwich” for this particular type of ELISA, as the antigen figuratively gets stuck between the two antibodies in this step. Enzyme-linked secondary antibodies were then added as detection antibodies that bind specifically to the Fc regions of the antibodies already in place. Finally, a chemical which becomes converted into a fluorescent signal by the enzymes-linked antibodies was added to the solutions. Spectrophotometry was then used to quantify antigen concentrations.
(1) Plate is coated with a capture antibody; (2) sample is added, and any antigen present binds to capture antibody; (3) detecting antibody is added, and binds to antigen; (4) enzyme-linked secondary antibody is added, and binds to detecting antibody; (5) substrate is added, and is converted by enzyme to detectable form. © Jeffrey M. Vinocur, Wikipedia.

While what was described in the preceding paragraph refers to “sandwich” ELISA, there are also other types (e.g. direct ELISA, competitive ELISA, etc.). Sandwich ELISA is limited to proteins with at least two binding sites as it uses both a capture and detection antibody between which the protein is “sandwiched”. Compared to standard ELISA, sandwich ELISA has a higher sensitivity and does not require the samples to be purified before analysis. To clarify, very low concentrations can be detected as the capture antibody only grabs the protein of interest while remaining proteins are washed away. However, the foremost disadvantage of sandwich ELISA is that antibody optimization can be difficult, as it is important to reduce cross-reactivity between capture and detection antibodies, which otherwise can result in false positives.

In Paper III specifically, blood samples had been stored frozen at -80 °C and were thawed on ice, vortexed and spun prior to analyses. Circulating protein levels were then measured using ELISA kit for hs-CRP (Cloud-Clone Corp, TX, USA) and I-FABP, Human, ELISA kit (Hyocult Biotech, Uden, The Netherlands).

Paper IV
In Paper IV, the registry-based method developed in Paper II was used to estimate stoma outcome at the time points 1, 2 and 3 years after anterior resection. Data on surgical details from the Swedish Colorectal Cancer Registry were used to classify whether or not the patient received a defunctioning stoma in connection to the index operation.

To account for stoma non-reversals owing to major postoperative complications, patients deceased within 90 days of index surgery were deemed ineligible and
subsequently excluded in any analysis with the long-term stoma outcome as the
dependent variable. Also, as delayed stoma reversals are not uncommon,
sometimes taking place more than a year after an anterior resection, the stoma
outcome was established at the time point 2 years after surgery in the main
analyses. Consequently, patients operated in the year 2016 were omitted.
Likewise, patients operated in 2015 were also excluded in sensitivity analyses
where a 3-year time point was used for determination of permanent stoma
presence.

Definitions

Anastomotic leakage
As outlined in the introduction, the lack of a uniform definition of anastomotic
leakage long posed a major obstacle in colorectal research, reflected, for instance,
by incidence rates that varied considerably between different studies. Hence,
leakage-related outcomes have historically been difficult to compare between
reports. To remedy the situation a consensus definition was eventually proposed
by the International Study Group of Rectal Cancer in 2010, including a grading
of severity:

A defect of the intestinal wall integrity at the colorectal or colo-anal anastomotic
site (including suture and staple lines of neorectal reservoirs) leading to a
communication between the intra- and extraluminal compartments. A pelvic
abscess close to the anastomosis is also considered as anastomotic leakage.

Grade

A Anastomotic leakage requiring no
active therapeutic intervention

B Anastomotic leakage
requiring active therapeutic
intervention but manageable
without re-laparotomy

C Anastomotic leakage requiring
re-laparotomy

Figure 6. Demonstrating an anastomotic leakage. Illustration: © Aimee Rowe, TeachMeSurgery.
In the present thesis, the consensus definition was employed whenever anastomotic leakage was ascertained as per chart review (Paper I & Paper III), while the remaining studies (Paper II & Paper IV) used data on complications extracted from the Swedish Colorectal Cancer Registry. More specifically, all postoperative complications within 30 days are currently reported to the registry, regardless of whether they occur in-hospital or appear after discharge, and potential anastomotic complications were derived using the following variables: anastomotic insufficiency (checkbox only), reoperation for anastomotic insufficiency (checkbox only), other complications (checkbox, explained in free text), and other reoperations (checkbox, explained in free text). Importantly, although the consensus definition has started to gain wide acceptance, it has not yet been included in the instructions to registrars reporting to the registry; thus, a formal definition is lacking, while there is no further explanation of the registry variables mentioned above. Nevertheless, the two variables with complications entered in free text were reviewed manually for all patients in search of potential leaks that were not registered as such in the first two variables, most often corresponding to events where a pelvic abscess or a rectovaginal fistula was reported.

Additionally, surgical complications historically used to be reported within 30 days; however, more recent research suggests a longer follow-up time is required to adequately capture all postoperative events related to surgery\textsuperscript{104}. More importantly, up to one-third of anastomotic leaks are reported to occur beyond 30 days of surgery\textsuperscript{105,106}, while the frequent use of defunctioning stomas may also delay their diagnosis in some cases\textsuperscript{104}. Consequently, any anastomotic leakage within 90 days of surgery was recorded in studies where patients were reviewed by medical records (Paper I & Paper III), while leaks reported to the registry were confined to a 30-day follow-up time (Paper II & Paper IV).

To sum up, the definition of anastomotic insufficiency and how it is determined have important implications for any study investigating leakage-related outcomes. The validity of registry data and how leakage ascertainment might influence study results will be reviewed in detail in relation to each study in the discussion.

**Extent of mesorectal excision**
As explained earlier, extent of mesorectal excision may influence both the observed anastomotic leakage incidence as well as functional outcomes. In Paper I, the extent of mesorectal excision was derived through scrutiny of medical records and operative charts, while Paper IV used tumour height reported to the registry as a proxy for such, which will be clarified next.
As outlined in the introductory chapter, PME is sufficient and advisable in patients with high rectal cancers, where a 5 cm margin below the tumour is oncologically and technically achievable\textsuperscript{77, 81, 107, 108}; the majority of most proximal tumours (i.e. originating above the peritoneal reflection, and at 13–15 cm from the anal verge in particular) are generally managed with such surgery\textsuperscript{85, 86, 109-111}. In accordance, data on tumour height reported to the registry were used in Paper IV to classify whether the patient had undergone TME or PME. Specifically, any tumour registered $\leq$ 10 cm from the anal verge was considered to have undergone TME, whereas patients with a tumour height of $\geq$ 13 cm were deemed highly likely to have been treated with PME. Patients with tumours originating in between these levels were considered a stand-alone segment in which extent of mesorectal excision could not reliably be determined.

**Epidemiological considerations**

As the current thesis is based on observational research, important epidemiological aspects deserve attention. All four studies (Papers I–IV) used registry data, and except for the validation done in Paper II, the overall aim of any statistical analysis was to quantify unbiased effect estimates between predefined exposures and outcomes; i.e. to derive causal inferences based on specific scientific questions outlined \textit{à priori}\textsuperscript{112}. To do so, the issue of confounding had to be addressed, and domain knowledge was essential to outline the causal structure of each research question under study. Thus, the collective empirical knowledge of the co-authors, including senior clinicians and researchers, was employed to identify causal pathways between the exposure and the outcome, and to differentiate actual confounders from covariates mediating the effect of interest. To overcome the potential complexity of these multivariable relationships and to better understand implications of a certain analysis, \textit{directed acyclic graphs} were drawn and also used to chart the direct and indirect pathways conveying the effect of interest\textsuperscript{113, 114}. Furthermore, although there are other ways to cope with bias in observational studies, the above approach was considered pertinent for the studies in this thesis, and has also been suggested by statistical editors in the surgical literature\textsuperscript{115}. Finally, while several journals may advise authors to refrain from the use of causal language, suggested euphemisms such as “correlation” or “association” risk confusing the reader about the research aims\textsuperscript{116}. As a consequence, causal language developed successively through the papers featured in this thesis; e.g. from “risk factors” in Paper I to “causal effects” in Paper IV.

To visualize the causal relations assumed in Paper I & Paper IV, directed acyclic graphs for both studies are provided in the Appendix (Supplementary Figures 1 & 2).
Statistical analyses

**Paper I**

In *Paper I*, age, tumour height above the anal verge, intraoperative bleeding, cTNM, pTNM, preoperative radiotherapy and type of mesorectal excision were categorised for analysis purposes. Age was divided into three similarly sized groups (≤62 years, 62–70 years or ≥70 years) and tumour height was split into three levels, to reflect groups of low, mid and high rectal cancer (≤6 cm, 7–12 cm or ≥13 cm), while intraoperative bleeding was dichotomised using the median (<350 ml or ≥350 ml). cTNM and pTNM were categorised with stages I and II merged into a reference level, while retaining separation of stages III and IV. Pre-operative radiotherapy (treatment, or no treatment) and type of mesorectal excision (TME, or PME) were treated as binary variables.

Risk factors for a permanent stoma were evaluated using multiple logistic regression, and results were reported with odds ratios (ORs) and 95% confidence intervals (CIs). Factors limiting stoma reversal were evaluated using survival analyses: Kaplan-Meier curves, log-rank tests and Cox regression models, with hazard ratios (HRs) and 95% CIs.

Symptomatic anastomotic leakage, defunctioning stoma at the index operation, pTNM, and type of mesorectal excision were selected *à priori* as the exposures of interest. With stoma permanence as the dependent variable, multiple logistic regression was used to calculate adjusted effect estimates for each exposure separately. Leakage, pTNM and type of mesorectal excision were also analysed as risk factors in the survival analysis for non-reversal of a defunctioning stoma. In the causal models for all regression analyses, sex, radiotherapy, type of mesorectal excision, cTNM, intraoperative bleeding and tumour height were treated as potential covariates causally related to the exposure and the outcome. Each selected risk factor was adjusted for a minimal subset of actual confounders identified with the help of the causal diagrams. Only primary defunctioning stomas fashioned in connection to the anterior resection were considered in the survival analysis for stoma non-reversal.

Variables with missing values were managed with multiple imputation in the statistical analyses, using chained equations. Thus, the reported ORs and HRs stem from analyses performed on imputed data sets, although complete case analyses were also made for comparison. Furthermore, logistic regression models were assessed for goodness of fit by means of the Hosmer-Lemeshow test, while survival analyses were evaluated with regard to the proportional-hazards assumption, using Schoenfeld’s residuals. Plausible violations of the
proportional-hazards assumptions were investigated using Aalen’s linear hazards model, which was also applied to pinpoint time-varying effect shifts\textsuperscript{118}.

**Paper II**

In the principal analysis in Paper II, the accuracy of the registry-based method to identify stoma outcome for all patients was assessed. We calculated positive and negative predictive values, nominal agreement and the level of agreement with Kappa values\textsuperscript{119}. Although interpretation of the Kappa value is a somewhat arbitrary measure, a value of 0–0.6 is generally considered to be in the range of poor to moderate agreement, whereas 0.6–0.8 indicates substantial conformity and a value greater than, or equal to, 0.8 denotes almost perfect agreement\textsuperscript{120}. We also performed a subgroup analysis in patients with a defunctioning stoma at the index operation, evaluating accuracy to identify primary stoma reversals. Furthermore, agreement between primary stoma reversals reported to the Swedish Colorectal Cancer Registry, and reference data, were also investigated.

After validation, the registry-based method was used to estimate stoma outcome in all patients treated with an elective anterior resection for rectal cancer in Sweden during the study period. We also evaluated the effect of mortality on the national permanent stoma prevalence, excluding all patients who deceased during the follow-up period. Possible regional differences in stoma prevalence were also presented by estimating stoma outcomes separately for all six healthcare regions in Sweden. Geographical discrepancies in the presence of a stoma were thereafter examined using the \(\chi^2\) test. Additionally, distributions of types of treatment given for all patients diagnosed with rectal cancer in Sweden during the study period, according to Colorectal Cancer Registry data, were demonstrated with figures broken down for each of the six regions.

Finally, primary stoma reversals after anterior resection over time were presented with interquartile ranges and illustrated by a Kaplan-Meier diagram. Any stoma left in place at end of follow-up was reported and, when possible, classified to type using procedure codes registered at the time of stoma creation.

**Paper III**

In the Paper III statistics, BMI was classified using the conventional taxonomy (<25 kg/m\(^2\): normal weight; 25–30 kg/m\(^2\): overweight; >30 kg/m\(^2\): obesity), while neoadjuvant treatment was divided into three different categories (no neoadjuvant treatment, radiotherapy or chemoradiotherapy). pTNM was also treated as a categorical variable, retaining all three classes (I, II and III) separated. Sex (male or female), tumour location (colon or rectum), preoperative blood transfusion (no or yes) and defunctioning stoma (no or yes) were managed as binary variables. Due to small numbers and similar relations to the main
outcome, ASA classes I and II were merged into a reference category, while ASA III was treated as a separate category owing to a possible association with anastomotic leakage\textsuperscript{121, 122}. The variables age and intraoperative bleeding were kept as continuous and presented with interquartile ranges.

As detailed previously, the main confounding factors were identified à priori and controlled through matching at time of inclusion, while any remaining baseline differences between cases and controls were evaluated using Fisher’s exact test for categorical variables, and the Student’s t-test for continuous variables.

In the main analysis, a further developed version of orthogonal projections to latent structures (OPLS)\textsuperscript{123}, OPLS-effect projections (OPLS-EP)\textsuperscript{124}, designed for dependent 1:1 matched samples, was used. This method employs latent variables, and the probability level of each OPLS-EP model was formally assessed by means of the CV-ANOVA diagnostic\textsuperscript{125}. Models with a p value below 0.05 were considered significant and used to identify potential biomarkers displaying differences between cases and controls. The interpretation of univariate associations of OPLS-EP models corresponds to that of t-tests of individual variables\textsuperscript{126}, while p values from two tailed t-tests were considered statistically significant at a level below 0.05. Also, to control for a plausible false discovery rate, the Benjamini-Hochberg procedure was used\textsuperscript{127}, with a threshold level set at 0.10.

Results of ELISA assays were evaluated using the dependent t-test and the Wilcoxon signed rank sum test. P values were reported and supplemented with mean as well as median fold-changes, to display numerical differences between cases and controls. Since only two predefined proteins were selected for analysis, adjustment for multiple testing was omitted in this case.

Both the biomarker panel analysis, and the ELISA measurements, were followed by subgroup analyses for colon and rectal cancer patients performed separately. Also, in order to control for any inflammatory response to neoadjuvant radiotherapy, all outcomes were reevaluated with a sensitivity analysis of patients with concordant oncological treatment.

To illustrate significant serum protein level differences noted between the two groups in the biomarker panel analysis, graphs with distributions divided into tertiles were presented and displayed together with receiver operating characteristic curves, along with a grouped discriminant analysis.
**Paper IV**

In the main analysis in **Paper IV**, defunctioning stoma was hypothesised as an exposure for a permanent stoma outcome. Aided by the directed acyclic graph illustrated in Supplementary Figure 2, anastomotic leakage was acknowledged as an indirect effect mediator, while confounding pathways were identified and used to calculate unbiased effect estimates (confounders are denoted as red circles in the acyclic graph). A separate analysis was also performed to assess the protective effect of a defunctioning stoma on anastomotic leakage, in which the same variables were taken into account. Except for cTNM and age, the same subset of variables as above was considered to confound the effect of interest with regard to defunctioning stoma as the exposure for anastomotic leakage.

Continuous variables (age, intraoperative bleeding and hospital volume) were managed as linear variables after ruling out presence of major threshold effects. The remaining variables were handled as categorical. ASA classes III and IV were merged due to few observations in the latter group, while retaining separation of classes I and II. Neoadjuvant treatment was divided into three groups (no treatment, preoperative radiotherapy only or preoperative chemoradiotherapy). Healthcare region was divided into six groups (Northern, Stockholm-Gotland, Southern, South-Eastern, Uppsala-Örebro and Western).

To derive both total, direct and indirect effects, the probit regression method proposed by Lindmark et al in 2018 was used\(^{128}\). Namely, the total effect of a defunctioning stoma on a permanent stoma outcome was estimated, together with the indirect effect mediated by anastomotic leakage, and the direct effect attributable to defunctioning stoma alone. In accordance with the study hypothesis, there was a significant interaction between defunctioning stoma and extent of mesorectal excision, while a similar relationship between defunctioning stoma and healthcare region was also noted. Thus, a probit regression model for the mediator anastomotic leakage was first fitted, in which leakage was conditioned on defunctioning stoma and the predefined confounder subset, including interactions between defunctioning stoma and the extent of mesorectal excision, as well as healthcare region. Subsequently, a corresponding probit regression model for the outcome permanent stoma was fitted, including the exposure variable (defunctioning stoma), the mediator (anastomotic leakage) and the confounding variables, also with interactions between defunctioning stoma and 1) extent of mesorectal excision; 2) healthcare region; 3) anastomotic leakage, and between anastomotic leakage and 1) extent of mesorectal excision; 2) healthcare region. Finally, the causal effects of interest were estimated through integration of the coefficients obtained from the two regression models\(^{128}\). Also, sensitivity analyses were done with stoma outcome determined at the time points 1 and 3 years, after the index surgery.
Furthermore, to estimate the protective effect of a defunctioning stoma on anastomotic leakage, confounder-adjusted probit regression was performed as in the first step outlined previously, although without any exclusions made regarding early mortality. An interaction between defunctioning stoma and the extent of mesorectal excision, as well as defunctioning stoma and healthcare region were also noted in the secondary analysis and were consequently incorporated in the regression model. There was no mediator variable of interest identified between the exposure and outcome, and calculated figures therefore correspond to total effect estimates.

To reduce potential bias due to variables with missing data, multiple imputation by chained equations was performed\textsuperscript{117, 129}. Results from 10 imputed datasets were pooled in a t-based procedure\textsuperscript{130}. Thus, all displayed figures stem from imputed datasets; however, estimates were compared throughout to the output of complete case analyses. Finally, we attempted to estimate susceptibility to unmeasured confounding, in which the relationships exposure-mediator, mediator-outcome and exposure-outcome were evaluated using the method proposed by Lindmark et al\textsuperscript{128}. As unobserved confounding is prone to induce correlation between the error terms in the models, strong residual confounding can be expected to present with associations of a greater magnitude. Thus, results from sensitivity analyses on unmeasured confounding were reported, with figures of correlation required to bias calculated outcomes towards a null or reversed effect.

**Significance levels and statistical software**

In all four studies (Papers I–IV), two-tailed p values were calculated and considered statistically significant at a level below 0.05. In Paper III, a false discovery rate adjusted p-value (q-value) of 0.10 was considered pertinent to fit the explorative study design.

In Papers I–III, the statistical software STATA (StataCorp, Houston, TX, USA) was used. Specifically, all calculations were performed using the STATA version 13.1 in Paper I, while Paper II & Paper III used STATA version 15.1. However, in Paper III, only baseline characteristics and univariate associations were computed with STATA, while OPLS-EP statistics were computed using MATLAB release 2017a (The MathWorks, Inc, Natick, MA, USA). Finally, all statistical analyses in Paper IV were performed using the computer software R version 3.5.2 (R Core Team, Vienna, Austria).
Results

Paper I: High stoma prevalence and stoma reversal complications following anterior resection for rectal cancer: a population-based multicentre study

Of 323 patients enrolled in Paper I, a total of 316 remained eligible following exclusions for treatment with Hartmann’s procedure (n = 2), early mortality (n = 2), lost to follow-up (n = 2) and data unattainable (n = 1). Some 274 (87%) patients received a defunctioning stoma in connection with the anterior resection, of which one patient had a stoma fashioned preoperatively that was reversed during the index operation. In total, 273 (86%) had a primary defunctioning stoma in place after the index procedure and patient characteristics stratified for stoma outcome are presented in Table 3.

Stoma outcome

Of the 316 patients included in Paper I, 206 (65%) at some time point had a stoma present but ultimately became stoma-free after a stoma takedown. Of the remaining patients, 75 (24%) had a permanent stoma at the end of follow-up, while 35 (11%) never had a stoma. Stoma outcome was determined with a median follow-up time of 1331 days (range: 34–2906 days), while the permanent stoma indications are listed in Table 4.

Risk factors for a permanent stoma

In the multiple logistic regression analyses, anastomotic leakage had a strong effect on the permanent stoma rate (OR 7.44; 95% CI 3.58–15.44). Tumour stage IV also proved to be a risk factor for stoma permanence, with an OR of 5.97 (95% CI 2.23–15.99). Additionally, patients who underwent PME had a reduced risk of a permanent stoma outcome, demonstrated by an OR of 0.35 (95% CI 0.13–0.97). The use of a defunctioning stoma increased the risk of a permanent stoma with an adjusted OR of 1.71 (95% CI 0.56–5.26), although this estimate was not formally statistically significant. Complete case and multiple imputation analyses were similar (results not shown).
Table 3. Patient demographic displaying 316 patients undergoing an anterior resection who either had a permanent stoma or were stoma-free at the end of follow-up.

<table>
<thead>
<tr>
<th>Categorical variables</th>
<th>Permanent stoma (n = 75)</th>
<th>Stoma-free (n = 241)</th>
<th>P-value *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
<td></td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>42 (55%)</td>
<td>137 (57%)</td>
<td>0.895</td>
</tr>
<tr>
<td>Female</td>
<td>33 (45%)</td>
<td>104 (43%)</td>
<td></td>
</tr>
<tr>
<td><strong>pTNM</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-II</td>
<td>34 (45%)</td>
<td>143 (59%)</td>
<td>0.011</td>
</tr>
<tr>
<td>III</td>
<td>27 (36%)</td>
<td>77 (32%)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>12 (16%)</td>
<td>14 (6%)</td>
<td></td>
</tr>
<tr>
<td><strong>ASA class</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASA I</td>
<td>12 (16%)</td>
<td>47 (20%)</td>
<td>0.215</td>
</tr>
<tr>
<td>ASA II</td>
<td>43 (57%)</td>
<td>149 (62%)</td>
<td></td>
</tr>
<tr>
<td>ASA III</td>
<td>17 (23%)</td>
<td>34 (14%)</td>
<td></td>
</tr>
<tr>
<td><strong>Radiotherapy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>56 (75%)</td>
<td>142 (59%)</td>
<td>0.014</td>
</tr>
<tr>
<td>No</td>
<td>19 (25%)</td>
<td>99 (41%)</td>
<td></td>
</tr>
<tr>
<td><strong>Defunctioning stoma</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>70 (93%)</td>
<td>203 (84%)</td>
<td>0.053</td>
</tr>
<tr>
<td>No</td>
<td>5 (7%)</td>
<td>38 (16%)</td>
<td></td>
</tr>
<tr>
<td><strong>Symptomatic leakage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>28 (36%)</td>
<td>28 (12%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>No</td>
<td>47 (64%)</td>
<td>213 (88%)</td>
<td></td>
</tr>
<tr>
<td><strong>Mesorectal excision</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TME</td>
<td>66 (88%)</td>
<td>191 (79%)</td>
<td>0.073</td>
</tr>
<tr>
<td>PME</td>
<td>7 (9%)</td>
<td>45 (19%)</td>
<td></td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 62</td>
<td>20 (27%)</td>
<td>85 (35%)</td>
<td></td>
</tr>
<tr>
<td>62 - 70</td>
<td>24 (32%)</td>
<td>71 (29%)</td>
<td></td>
</tr>
<tr>
<td>≥ 70</td>
<td>31 (41%)</td>
<td>85 (35%)</td>
<td>0.369</td>
</tr>
<tr>
<td><strong>Tumour height</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 6 cm</td>
<td>9 (12%)</td>
<td>17 (7%)</td>
<td></td>
</tr>
<tr>
<td>6 - 12 cm</td>
<td>50 (67%)</td>
<td>178 (74%)</td>
<td></td>
</tr>
<tr>
<td>≥ 13 cm</td>
<td>16 (21%)</td>
<td>45 (19%)</td>
<td>0.284</td>
</tr>
<tr>
<td><strong>Intraoperative bleeding</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 350 ml</td>
<td>28 (37%)</td>
<td>118 (49%)</td>
<td>0.059</td>
</tr>
<tr>
<td>≥ 350 ml</td>
<td>45 (60%)</td>
<td>110 (46%)</td>
<td></td>
</tr>
</tbody>
</table>

pTNM = pathological tumour-node-metastasis; ASA = American Society of Anesthesiologists; TME = total mesorectal excision; PME = partial mesorectal excision. *Fisher’s exact test used for categorical variables.
Table 4. Indications for receiving a permanent stoma or having a temporary stoma reconsidered as permanent in 75 patients of 316 who had undergone an anterior resection for rectal cancer.

<table>
<thead>
<tr>
<th>Permanent stoma indications</th>
<th>N</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anastomotic leakage</td>
<td>28</td>
<td>37%</td>
</tr>
<tr>
<td>Stage IV colorectal cancer</td>
<td>18</td>
<td>24%</td>
</tr>
<tr>
<td>Anterior resection syndrome</td>
<td>6</td>
<td>8%</td>
</tr>
<tr>
<td>Other*</td>
<td>6</td>
<td>8%</td>
</tr>
<tr>
<td>Delayed anastomotic leakage†</td>
<td>5</td>
<td>7%</td>
</tr>
<tr>
<td>Local recurrence</td>
<td>3</td>
<td>4%</td>
</tr>
<tr>
<td>Stoma reversal operation complications</td>
<td>3</td>
<td>4%</td>
</tr>
<tr>
<td>Comorbidity‡</td>
<td>2</td>
<td>3%</td>
</tr>
<tr>
<td>Advanced urologic malignancy</td>
<td>2</td>
<td>3%</td>
</tr>
<tr>
<td>Patient unwilling to undergo more surgery</td>
<td>2</td>
<td>3%</td>
</tr>
</tbody>
</table>

*Index operation complications, high age, anastomotic stricture, poor nutritional status, extensive index operation and lacking compliance. †Anastomotic leakage more than 90 days after index surgery. ‡Advanced cardiovascular disease and stroke.

Stoma reversal complications
Median time to stoma closure was 272 days (range: 55–1142 days), and of the 273 patients with a defunctioning stoma, 229 underwent stoma reversal. Major complications (Clavien-Dindo IIIB–V) occurred in five (14.7%) loop transverse colostomy reversals, and 16 (8.2%) loop ileostomy reversals. Consequently, 21 (9.2%) primary stoma reversals sustained major complications in total, the most frequent being a bowel obstruction (n = 10). The remaining complications were stoma reversal anastomotic failure (n = 3), intraoperative complications (n = 2), incisional hernia (n = 1), cardiac arrest (n = 1), respiratory insufficiency (n = 1), stroke (n = 1), sepsis (n = 1), and abscess formation (n = 1). Most patients who suffered a major complication needed return to theatre (n = 18), and of the remaining three, complications leading to cardiac arrest resulted in mortality in one patient, whilst two required intensive care subsequent to organ failure. When accounting for all complications, the complication rate following stoma reversal amounted to 31% (71/229), comprising a postoperative mortality rate of 0.4% (1/229).
Among 229 primary stoma reversals, three patients had a previously undiagnosed leakage in the rectal anastomosis immediately following stoma closure. These leakages were not considered as complications to the reversal procedure itself.

**Risk factors for stoma non-reversal**

Some 273 patients had a defunctioning stoma fashioned primarily and were included in the survival analyses regarding factors limiting stoma reversal. A violation of the proportional-hazards assumption was noted at 12 months after the index procedure concerning anastomotic leakage and pTNM stage. Analyses were therefore done separately for stoma reversal before and after 12 months.

The relative likelihood of stoma reversal within 12 months was much lower in patients who had suffered a leakage compared to those who did not, as shown by a HR of 0.25 (95% CI 0.13–0.47) in the multivariable analysis. This effect persisted after 12 months, but was not as pronounced (HR 0.45; 95% CI 0.24–0.84). With pTNM stage I–II as reference, both stage III (HR 0.33; 95% CI 0.22–0.50) and stage IV patients (HR 0.27; 95% CI 0.12–0.62) had a reduced likelihood of undergoing stoma reversal within the first year following surgery. Conversely, stage III patients were after 12 months more likely to have their stomas reversed (HR 1.87; 95% CI 1.05–3.32). Stage IV patients, however, experienced a similar risk of stoma reversal as stage I–II patients a year after surgery (HR 1.27; 95% CI 0.49–3.17). Complete case analyses did not differ significantly from analyses performed on imputed data sets (results not shown).

With the chart reviewed cohort in Paper I as reference, the registry-based method developed in Paper II had a positive predictive value of 85.1%, and a negative predictive value of 100.0% in identifying a permanent stoma outcome. Nominal agreement was 95.9% and the Kappa value was 0.89.

In total, 13 (4.2%) misclassifications occurred, all incorrectly denoting presence of a permanent stoma in patients who ended up stoma-free. The main reason for misclassification was due to missing data on reversal surgery in the National Patient Registry (n = 11). Of the remaining two patients, one had mistakenly been reported as having a primary defunctioning stoma in the Swedish Colorectal Cancer Registry, consequently with no reversal surgery recorded; the other patient had a defunctioning stoma constructed preoperatively, which was reversed during the anterior resection but not recorded in the operative charts. Further, one patient correctly classified as stoma-free was, according to chart review, supplied with a primary stoma and later underwent reversal; yet, reporting on primary defunctioning to the Colorectal Cancer Registry was incorrect for this patient, while data on reversal surgery were coincidentally missing as well.

Results from a subgroup analysis confined to the patients provided with a defunctioning stoma primarily (n = 270) are displayed in Table 5.

<table>
<thead>
<tr>
<th>Data source</th>
<th>PPV</th>
<th>NPV</th>
<th>Agreement</th>
<th>Kappa</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPR*</td>
<td>99.1  (96.7–99.9)</td>
<td>81.1  (68.0–90.6)</td>
<td>95.6  (92.4–97.7)</td>
<td>0.85  (0.77–0.93)</td>
</tr>
<tr>
<td>SCRCR†</td>
<td>98.8  (95.6–99.8)</td>
<td>75.0  (57.8–87.9)</td>
<td>94.4  (90.2–97.2)</td>
<td>0.80  (0.68–0.91)</td>
</tr>
</tbody>
</table>

Positive predictive value (PPV) and negative predictive value (NPV) refers to detection of primary stoma reversal. Displaying 95% confidence intervals (CIs). Subgroup analysis excluding all mortality indicating analysis was performed with exclusion of patients who died during the follow-up period.

*Using data from the National Patient Registry indicating primary stoma reversal. †Using reporting on primary stoma reversal to the Swedish Colorectal Cancer Registry.
Among patients with a primary stoma, Swedish Colorectal Cancer Registry data on reversal surgery turned out to be missing to a significant degree in the reference cohort (27.4%). The missing data were not explained by a lack of reporting in patients who had their index operation prior to 2011, i.e. in the period before the stoma reversal variable was introduced into the registry (results not shown). However, a complete case analysis of accuracy of stoma reversals reported to the registry, with medical records as reference, are shown in Table 5.

**Long-term stoma outcome following anterior resection**

During the study period, 13550 patients were diagnosed with rectal cancer in Sweden, for which distribution of specific treatments is displayed in Table 6. Of 4844 patients registered with an anterior resection, 4768 patients were eligible for this study, after exclusion of 76 patients who had emergency surgery. A total of 3633 (75.7%) were reported with a defunctioning stoma at the index operation to the Swedish Colorectal Cancer Registry. At end of follow-up, regardless of receiving a primary stoma or not, 942 (19.8%) patients had a stoma present according to the registry-based method, whilst 3826 (80.2%) were classified as stoma-free. Among patients who ended up stoma-free, 2830 (59.4%) were registered as having undergone stoma reversal surgery, whereas 996 (20.9%) had never had any stoma present. Stoma outcome was determined with a median follow-up time of 1324 days (interquartile range: 764–2053 days).

A total of 950 (19.9%) patients deceased during the follow-up period, of which the 90-day mortality rate amounted to 87/4768 (1.8%). Some 382/950 (40.2%) of the deceased had a stoma present at time of death, resulting in an adjusted permanent stoma prevalence of 14.6% after exclusion of patients who deceased before the end of the study period.

Regional data on estimated stoma prevalence, together with distributions of types of treatment given in the different healthcare regions, are displayed below (Table 6). Stoma outcomes varied to a statistically different degree between the six healthcare regions (p <0.001).
Table 6. Displaying distributions of specific types of treatment given to 13406 patients diagnosed with rectal cancer in Sweden between 1 January 2007 and 31 December 2013. Demonstrating regional data, together with ratios of anterior resections performed, alongside estimated stoma outcomes.

<table>
<thead>
<tr>
<th>Healthcare region</th>
<th>Surgical treatment*</th>
<th>Anterior resections</th>
<th>Stoma rate†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern</td>
<td>973/1253 (77.7%)</td>
<td>348/973 (35.8%)</td>
<td>29.2%</td>
</tr>
<tr>
<td>Stockholm-Gotland</td>
<td>1996/2369 (84.3%)</td>
<td>982/1996 (49.2%)</td>
<td>19.8%</td>
</tr>
<tr>
<td>Southern</td>
<td>2058/2506 (79.3%)</td>
<td>877/2058 (42.6%)</td>
<td>17.8%</td>
</tr>
<tr>
<td>South-Eastern</td>
<td>1181/1471 (80.3%)</td>
<td>528/1181 (44.7%)</td>
<td>19.5%</td>
</tr>
<tr>
<td>Uppsala-Örebro</td>
<td>2578/3201 (80.5%)</td>
<td>1148/2578 (44.5%)</td>
<td>19.1%</td>
</tr>
<tr>
<td>Western</td>
<td>2019/2516 (80.3%)</td>
<td>961/2019 (47.6%)</td>
<td>19.1%</td>
</tr>
<tr>
<td>Sweden</td>
<td>10805/13406 (80.6%)</td>
<td>4844/10805 (44.8%)</td>
<td>19.8%</td>
</tr>
</tbody>
</table>

NB: The number of anterior resections above constitute non- scrutinised registry data and therefore differ from the rest of the manuscript displaying a chart reviewed cohort where ineligible patients have been excluded. Additionally, some 144 patients were registered to have been diagnosed with cancer of the rectum during the study period but had unspecified procedures reported to the registry and are not shown in the above table. *Using data from the Swedish Colorectal Cancer Registry, with surgical treatment denoting anterior resections, Hartmann’s procedures, abdominoperineal excisions, local excisions (including transanal endoscopic microsurgery) and laparotomy without tumour resection. †Using the registry-based method validated herein to identify a permanent stoma outcome in patients managed with anterior resection; p value of 0.001 for difference using the χ² test.

Stoma prevalence after anterior resection and permanent stoma type
Out of 942 permanent stomas present at end of follow-up, 445 (47.2%) were estimated to be loop ileostomies. Of those remaining, 304 (32.3%) were colostomies, while 193 (20.5%) stomas could not be specified to type. The 304 colostomies comprised 10 primary loop transverse colostomies left in place, whereas the remaining 294 were end colostomies. Out of these 294 patients, 232 (78.9%) had been defunctioned primarily. Furthermore, 88 of the 294 end colostomies were constructed as a consequence of an abdominoperineal excision.

Time to stoma closure
Among patients who underwent closure of a primary stoma, median time to stoma reversal surgery was 196 days (interquartile range: 125–301 days). Figure 7 demonstrates the cumulative primary stoma reversal over time.
Figure 7. Stoma reversals in 3622 patients operated with an anterior resection for rectal cancer who received a primary defunctioning stoma in Sweden between 1 January 2007 and 31 December 2013, as determined by a registry-based method.
Paper III: Preoperative biomarkers related to inflammation identify high-risk anastomoses in colorectal cancer surgery: an explorative study

During the study period between 1 January 2010 and 31 December 2015, a total of 726 patients had elective surgery for non-disseminated colorectal cancer with a primary anastomosis at either of the two university hospitals in Uppsala and Umeå, Sweden. Some 48 patients with anastomotic leakage were considered for inclusion, out of whom five could not be matched as no eligible controls were found, while a missing informed consent and a lost preoperative blood sample resulted in exclusion of another two patients. The remaining 41 patients were included and matched 1:1 against 41 controls, amounting to a total of 82 patients in the cohort.

Table 7 displays patient demographics stratified by occurrence of anastomotic leakage. While body mass index differed marginally, the two groups were highly comparable in terms of the entire cohort. In patients with rectal cancer, groups differed to a significant degree with regard to neoadjuvant treatment (p = 0.031).

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<td></td>
<td></td>
</tr>
<tr>
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<td></td>
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</tr>
<tr>
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<td>22 (53.7)</td>
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<td>&gt; 30</td>
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</tr>
<tr>
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<td>24 (58.5)</td>
<td></td>
</tr>
<tr>
<td>Rectum</td>
<td>17 (41.5)</td>
<td>17 (41.5)</td>
<td>1.000</td>
</tr>
</tbody>
</table>
Biomarker panel analyses

When investigating the entire cohort, no differences in measurable serum protein levels were observed between cases and controls. However, a subgroup analysis of the 34 rectal cancer patients displayed significantly altered serum levels of 15 proteins associated with inflammation between cases and controls in the paired analysis (Supplementary Table 1). After adjustment for a false discovery rate set at 0.10, the two proteins C-X-C motif chemokine 6 (CXCL6), and C-C motif chemokine 11 (CCL11) were considered as true positive findings. Results did not differ in a sensitivity analysis, where only matched cases and controls who had concordant oncological treatment were considered (results not shown).
Figure 8 shows the distributions of CXCL6 and CCL11 concentrations between cases and controls, divided into tertiles. Protein levels were fairly evenly distributed between the two groups in the mid tertile, while the upper and lower tertiles show evident discrepancies, with less than 10% of controls present in the upper tertile for both proteins. Accompanying receiver operating characteristics curves illustrate an area-under-the-curve of 0.837 for CXCL6 and 0.758 for CCL11, whereas a discriminant analysis of merged data, using both proteins, demonstrated a slightly higher degree of separability between cases and controls with an area-under-the-curve of 0.896.

Figure 8. Displaying tertile distributions of proteins CXCL6 and CCL11 that were significantly elevated prior to surgery in 17 patients who had anastomotic leakage after anterior resection for rectal cancer, compared to 17 complication-free controls. A grouped discriminant analysis with combined data, using both proteins, is also presented. All three graphs are accompanied by receiver operating characteristic curves and an estimation of area-under-the-curve (AUC) denoting prediction accuracy. ELISA analyses
As demonstrated in Table 8, results from ELISA analyses displayed statistically weak but significantly increased levels in hs-CRP in patients with anastomotic leakage in the entire cohort [mean fold-change: 3.595 (p = 0.044); median fold-change 2.308 (p = 0.039)] as well as in the colon subgroup [mean fold-change: 4.336 (p = 0.048); median fold-change 2.771 (p = 0.031)]. In patients with rectal cancer, levels of hs-CRP did not differ between cases and controls. A sensitivity analysis conducted with matched pairs who had concordant neoadjuvant treatment exclusively did not display any significant differences (results not shown).

<table>
<thead>
<tr>
<th></th>
<th>hs-CRP*</th>
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<tr>
<td></td>
<td>Mean fold-change</td>
<td>P value†</td>
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<td>3.595</td>
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<td>2.308</td>
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<tr>
<td>Colon</td>
<td>4.336</td>
<td>0.048</td>
<td>2.771</td>
</tr>
<tr>
<td>Rectum</td>
<td>2.545</td>
<td>0.626</td>
<td>1.729</td>
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<table>
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<tr>
<th></th>
<th>I-FABP§</th>
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<th></th>
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<tbody>
<tr>
<td></td>
<td>Mean fold-change</td>
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<td>Median fold-change</td>
</tr>
<tr>
<td>Entire cohort</td>
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<td>Rectum</td>
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<td>0.873</td>
<td>0.823</td>
</tr>
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</table>

Table 8. Displaying results of ELISA analyses performed on serum drawn prior to surgery in a matched cohort of 41 patients who had anastomotic leakage, and 41 complication-free controls, after surgical treatment for colorectal cancer in Sweden between 1 January 2010 and 31 December 2015.

Entire cohort indicating all 82 patients were included in the analysis, with Colon and Rectum denoting subgroup analyses, divided by tumour location, with a total of 48 patients in the former group, and 34 patients in the latter group. *hs-CRP = High-sensitivity C-reactive protein. †Dependent t-test for comparison of matched cases and controls. ‡Wilcoxon signed rank sum test for comparison of matched samples. §I-FABP = Intestinal fatty-acid binding protein.
Paper IV: Defunctioning stomas decrease chances of a stoma-free outcome after anterior resection for rectal cancer

During the study period of 1 January 2007 through 31 December 2016, 6584 patients were treated with an anterior resection for rectal cancer in Sweden. Patients who had their index operation in 2016 (n = 656), who deceased within 90 days of surgery (n = 103), with a tumour height missing or with its lower margin above 15 cm in the registry (n = 48), or those who were registered with a tumour originating at 11 or 12 cm from the anal verge (n = 1462) were excluded in the main study cohort concerning stoma outcome. While some patients fulfilled more than one exclusion criterion, the exact number of ineligible patients amounted to 2055, and a total 4529 patients were considered for further analysis.

Patient characteristics divided by the estimated extent of mesorectal excision, and stratified by stoma outcome, are displayed in Table 9. A total of 3573 patients (78.9%) were registered with a defunctioning stoma, while the overall anastomotic leakage incidence amounted to 419 events (9.3%). The permanent stoma prevalence at the end of follow-up was 768/4529 (17.0%). Supplementary Figure 3 shows a flowchart of all events with effect on stoma status registered during the time between the index operation and the end of follow-up.

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<td>N (%)</td>
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<td></td>
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<td>68 (61–75)</td>
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<td>450 (250–800)</td>
<td>250 (100–500)</td>
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<td>12 (13.0)</td>
<td>43 (91.5)</td>
<td>14 (8.5)</td>
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Defunctioning stoma and stoma outcome

Regardless of the extent of mesorectal excision, construction of a defunctioning stoma increased the rate of permanent stomas to a significant degree (Table 10). While confounder adjusted total risk differences were similar between TME and PME [TME: RD 0.11 (95% CI 0.09–0.13); PME: RD 0.15 (95% CI 0.13–0.16)], patients in the former group had a higher baseline risk of a permanent stoma outcome [TME: BR 0.09 (95% CI 0.08–0.10); PME: BR 0.04 (95% CI 0.03–0.06)]. This resulted in a higher relative risk for patients undergoing PME [TME: RR 2.23 (95% CI 1.43–3.02); PME: RR 4.36 (95% CI 3.05–5.68)]. Mediation analyses indicated that the indirect effect through anastomotic leakage on stoma outcome was small, compared to the substantial direct effect via construction of a defunctioning stoma (Table 10). Sensitivity analyses regarding patients with stoma outcome determined at three years after surgery displayed similar results, whereas the same trends emerged in calculations performed with permanent stoma presence one year after surgery; however, the latter indicated a stronger effect of stoma construction on stoma outcome (results not shown). Results from imputed data sets did not differ from complete case analyses (results not shown).

<table>
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<tr>
<th>Operation time (min)</th>
<th>245 (191–320)</th>
<th>250 (203–319)</th>
<th>219 (167–296)</th>
<th>247 (197–343)</th>
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<tbody>
<tr>
<td>Hospital volume (AR/y)</td>
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<td>18 (13–25)</td>
<td>18 (13–25)</td>
<td>18 (13–27)</td>
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</tbody>
</table>

The extent of mesorectal excision as derived using registry data on tumour height above the anal verge as a proxy (≤ 10 cm: TME vs 13–15 cm: PME). Another 515 patients had anterior resection for rectal cancer located at 11 or 12 centimetres during the specified study period and are excluded in the above table due to uncertainty in determining extent of mesorectal resection. Additionally, 89 patients were deemed ineligible due to early mortality, whereas 5 were already excluded among the 515 patients outlined above, thus amounting to 593 exclusions in total. *Stage (clinical) = clinical TNM staging prior to surgery. †Stage (pathological) = final pathological assessment of TNM stage following surgery. ‡AR/y = Anterior resections performed per year.

**Defunctioning stoma and stoma outcome**

Regardless of the extent of mesorectal excision, construction of a defunctioning stoma increased the rate of permanent stomas to a significant degree (Table 10). While confounder adjusted total risk differences were similar between TME and PME [TME: RD 0.11 (95% CI 0.09–0.13); PME: RD 0.15 (95% CI 0.13–0.16)], patients in the former group had a higher baseline risk of a permanent stoma outcome [TME: BR 0.09 (95% CI 0.08–0.10); PME: BR 0.04 (95% CI 0.03–0.06)]. This resulted in a higher relative risk for patients undergoing PME [TME: RR 2.23 (95% CI 1.43–3.02); PME: RR 4.36 (95% CI 3.05–5.68)]. Mediation analyses indicated that the indirect effect through anastomotic leakage on stoma outcome was small, compared to the substantial direct effect via construction of a defunctioning stoma (Table 10). Sensitivity analyses regarding patients with stoma outcome determined at three years after surgery displayed similar results, whereas the same trends emerged in calculations performed with permanent stoma presence one year after surgery; however, the latter indicated a stronger effect of stoma construction on stoma outcome (results not shown). Results from imputed data sets did not differ from complete case analyses (results not shown).
Table 10. Displaying the effect of defunctioning stoma on a permanent stoma outcome. Using probit regression models to quantify mediation, indirect effects correspond to the estimated effect attributable to anastomotic leakage. Analyses are based on a cohort of 4529 patients who had anterior resection for rectal cancer in Sweden between 1 January 2007 and 31 December 2015, with stoma outcome determined at two years after the index operation.

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<td>Point estimate (95% CI)</td>
<td>Point estimate (95% CI)</td>
</tr>
<tr>
<td>All</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Direct effect</td>
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<td>0.14 (0.13–0.15)</td>
<td>2.87 (2.40–3.33)</td>
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<tr>
<td>Indirect effect</td>
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<td>-0.02 (-0.03–0.01)</td>
<td>0.91 (0.84–0.97)</td>
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<tr>
<td>Total effect</td>
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<td>0.12 (0.11–0.14)</td>
<td>2.59 (1.76–3.43)</td>
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<td>TME</td>
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<tr>
<td>Direct effect</td>
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<tr>
<td>Indirect effect</td>
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<td>-0.03 (-0.04–0.02)</td>
<td>0.89 (0.81–0.96)</td>
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<tr>
<td>Total effect</td>
<td>0.09 (0.08–0.10)</td>
<td>0.11 (0.09–0.13)</td>
<td>2.23 (1.43–3.02)</td>
</tr>
<tr>
<td>PME</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Direct effect</td>
<td>0.04 (0.03–0.06)</td>
<td>0.15 (0.14–0.17)</td>
<td>4.56 (4.05–5.06)</td>
</tr>
<tr>
<td>Indirect effect</td>
<td>0.20 (0.19–0.21)</td>
<td>-0.01 (-0.02–0.00)</td>
<td>0.96 (0.91–1.00)</td>
</tr>
<tr>
<td>Total effect</td>
<td>0.04 (0.03–0.06)</td>
<td>0.15 (0.13–0.16)</td>
<td>4.36 (3.05–5.68)</td>
</tr>
</tbody>
</table>

The extent of mesorectal excision as derived using registry data on tumour height above the anal verge as a proxy (≤10 cm: TME vs 13–15 cm: PME). Patients with rectal cancer located at 11 or 12 centimetres are excluded in the above table due to uncertainty in determining extent of mesorectal resection. All calculations were performed with adjustments for the confounding variables ASA class, age, healthcare region, hospital volume, intraoperative bleeding, extent of mesorectal excision, neoadjuvant treatment, sex and clinical TNM stage. Significant interactions between defunctioning stoma and extent of mesorectal excision, as well as defunctioning stoma and healthcare region, were also included in the statistical models.
The influence of unmeasured confounding in the statistical models was estimated to have had, at most, a limited impact on the calculated effect measures. Specifically, in the direct relationship between a defunctioning stoma and a permanent stoma, any unmeasured confounder would have required a strong correlation of ≥0.5 between the error terms for the exposure and outcome models to result in a null or reversed effect. However, the indirect effect between a defunctioning stoma and anastomotic leakage was more sensitive to residual bias, as overlooked confounding was likely to result in larger effects deviations, with the impact direction depending on the sign of correlation.

**Defunctioning stoma and anastomotic leakage**

For estimates concerning the protective effect of a defunctioning stoma on anastomotic leakage, only patients who could not be classified regarding mesorectal excision (i.e. had a tumour originating at 11 or 12 cm above the anal verge) were excluded, while patients operated in 2016 and those deceased within 90 days of surgery remained. Consequently, 5122 patients were considered in these analyses.

Irrespective of the extent of mesorectal excision, a defunctioning stoma decreased the number of symptomatic anastomotic leakages [TME: RR 0.50 (95% CI 0.38–0.63); PME: RR 0.74 (95% CI 0.60–0.87)] (Table 11). Patients in the TME group had a higher baseline risk of symptomatic anastomotic leakage than those treated with PME [TME: BR 0.18 (95% CI 0.16–0.20); PME: BR 0.11 (95% CI 0.10–0.12)].
Table 11. Displaying estimations of the protective effect of a defunctioning stoma on symptomatic anastomotic leakage in 5122 patients who had anterior resection for rectal cancer in Sweden between 1 January 2007 and 31 December 2016.

<table>
<thead>
<tr>
<th></th>
<th>Baseline risk</th>
<th>Risk difference</th>
<th>Relative risk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Point estimate (95% CI)</td>
<td>Point estimate (95% CI)</td>
<td>Point estimate (95% CI)</td>
</tr>
<tr>
<td>All</td>
<td>0.16 (0.14–0.17)</td>
<td>-0.07 (-0.09–0.05)</td>
<td>0.55 (0.44–0.66)</td>
</tr>
<tr>
<td>TME</td>
<td>0.18 (0.16–0.20)</td>
<td>-0.09 (-0.11–0.07)</td>
<td>0.50 (0.38–0.63)</td>
</tr>
<tr>
<td>PME</td>
<td>0.11 (0.10–0.12)</td>
<td>-0.03 (-0.04–0.02)</td>
<td>0.74 (0.60–0.87)</td>
</tr>
</tbody>
</table>

The extent of mesorectal excision as derived using registry data on tumour height above the anal verge as a proxy (≤10 cm: TME vs 13–15 cm: PME). Patients with rectal cancer located at 11 or 12 centimetres are excluded in the above table due to uncertainty in determining extent of mesorectal resection. All calculations were performed with adjustments for the confounding variables ASA class, healthcare region, hospital volume, intraoperative bleeding, extent of mesorectal excision, neoadjuvant treatment and sex. Significant interactions between defunctioning stoma and extent of mesorectal excision, as well as defunctioning stoma and healthcare region, were also included in the statistical models.
Discussion

One of the main findings in the present thesis was that 20% of patients treated with anterior resection for rectal cancer eventually end up with a permanent stoma. Stoma outcomes vary to a significant degree between different healthcare regions in Sweden, while patients with anastomotic leakage and high tumour stage have a decreased likelihood of a stoma-free outcome. The same holds true if the patient receives a defunctioning stoma – especially in those treated with PME, who in addition seem to draw the least benefits in terms of reducing anastomotic leakage – while reversal of defunctioning stomas also entails a considerable risk of major complications. Finally, novel biomarkers to predict patients at high risk of anastomotic leakage prior to surgery were identified but require validation in larger prospective settings.

Methodological considerations, strengths and limitations

Study design
To evaluate methodological aspects of the present thesis, we must first consider the motives behind the different study designs used, also in the light of plausible options. To begin with, there are two main study types in the field of epidemiology: experimental and observational. The former includes randomised controlled trials, which throughout history have typically been ascribed with the highest level of evidence in the hierarchy of evidence-based medicine, whereas observational reports have often been dismissed as “hypothesis generating” etc. While clinical trials may have evident superiorsities in terms of eliminating selection bias and confounding (i.e. high internal validity), limitations in external validity, i.e. generalisability, are sometimes overlooked (for example, whether the use of defunctioning stomas can be justified in patients treated with PME, based on clinical trials performed exclusively on patients undergoing TME). More importantly, many research questions pose ethical problems that cannot simply be managed with an experimental study design; e.g., we would not allocate surgical complications as anastomotic leakage to different study arms. Accordingly, a non-experimental approach is in many situations the only way to address a certain clinical dilemma and, with this in mind, it should become more evident why most of the research described herein required an observational study design. A pertinent objection to this statement would be the analysis concerning benefits and drawbacks from faecal diversion in patients treated with PME in Paper IV, for which equipoise prevails, and a randomised controlled trial may be motivated. However, as observational reports on the subject have been scarce, it would seem precipitous to overlook decade-spanning data on rectal cancer treatment in Sweden, especially with long-term outcome data.
already at hand. A randomised controlled trial fashioned to evaluate the benefits and drawbacks of faecal diversion in the long term would also require a sizeable study cohort for sufficient statistical power, and would in all likelihood be difficult to complete. Moreover, as was demonstrated with surgical complications, research on anastomotic leakage prediction requires an observational approach; thus, Paper III used a matched case-control study design. Finally, while this section focused on aspects concerning statistical analyses and effect size estimations, the reports also had important descriptive purposes, making an observational study design arguably the most fitting.

Based on the discussed foundations, an observational population-based study design was used to investigate stoma reversal complications, stoma outcomes and underlying mechanisms in Paper I, Paper II & Paper IV, out of which the latter two encompassed nationwide patient cohorts. The main strength was that the studies used well-defined populations with a limited risk of selection bias, while also providing high external validity. To clarify, the opposite would for instance be a study of patients who had treatment at a specific referral centre, thus, with the apparent drawback that treatment allocation might have been due to certain patient characteristics (i.e. selection). For the same reason, results from population-based reports have a high level of generalisability, meaning that the study outcomes are clinically well applicable to the population it represents – in this particular case, almost all patients undergoing anterior resection for rectal cancer in Sweden. In contrast, Paper III had a study population confined to patients who received treatment at the university hospitals of Uppsala and Umeå. Although both centres correspond to somewhat distinct catchment areas, there might be referrals from other hospitals, and the study does not truly fulfil the criteria for a population-based design. Also, Paper III used a matched case-control study design, based on the fact that anastomotic leakage is quite a rare outcome, and that blood sample analyses of all patients in the large sample population (from which studied patients were identified and drawn) would not have been possible. While these features constitute limitations to Paper III, the high quality of the biobank consortium and sample availability offered a unique opportunity to address the issue of anastomotic leakage with one of the statistically best powered translational study attempts to date.

Confounding
As was elaborated previously, the main obstacle in non-experimental research is coping with the issue of confounding and bias. As we have already established that it was not feasible to address most of the research questions posed in the current thesis by means of a randomised clinical trial, observational study designs were inevitable. In these settings, causality in turn becomes difficult to prove. Nevertheless, in an attempt to address these limitations, directed acyclic graphs
were drawn prior to any confounder-adjusted analysis, with the aim to attain unbiased effect estimates in the statistical calculations. Specifically, mediators conveying the effect of interest, as well as collider pathways were identified in relation to the predefined exposures and outcomes, and were managed accordingly. Also, we tried to handle bias by means of study design, e.g. the population-based design in Papers I–II & Paper IV, and the rigorous matching criteria used in Paper III. Furthermore, in Paper IV, effect estimates were evaluated in relation to the probability of residual bias, showing that any overlooked confounder variable would require to have had a considerable impact on the outcome to distort the main results to such degree that they would not remain within the formal statistical significance level. The study (Paper IV) also included numerous sensitivity analyses in an attempt to detect any plausible source of error and test clinical assumptions, e.g. when stoma reversals take place in practice.

To conclude, the principal weakness of the research covered herein is the use of observational study designs. However, as most subject questions for obvious reasons could not be addressed in any other way, we used several methods to address confounding, all of which constitute important methodological strengths of the present thesis.

**Sample size, precision and error**

With methodological strengths and limitations under review, study sample size and related errors also constitute significant aspects. However, some epidemiological principles first require brief mentioning to facilitate understanding of the next section.

Usually, any epidemiological study begins with formulation of an alternative hypothesis ($H_a$); i.e. “permanent stomas after anterior resection for rectal cancer are more common among patients with anastomotic leakage than those without such complication”. The alternative hypothesis is then compared to a contradictory statement, typically referred to as the null hypothesis ($H_0$); i.e. “permanent stomas after anterior resection for rectal cancer are as common among patients with anastomotic leakage as those without such a complication”. To corroborate the $H_a$ (typically referred to as the opposite: reject the $H_0$), we usually collect data to compare the two conflicting hypotheses by means of a statistical test. However, prior to any analysis we must decide what level of uncertainty we are willing to accept – commonly reflected by the precision, that is: size of CIs and p values. As is regularly seen in the medical literature, a 95% CI will indicate that if the study were to be rerun repeatedly, the CI would include the correct point estimate 95% of the times. While the p value also derives from the same equation, it is more frequently used in terms of whether to reject the $H_0$
or not. It corresponds to the probability to observe as large an effect as was actually calculated, or a stronger one, under the assumption that the null hypothesis is true. Thus, if before analysis we postulate a significance level corresponding to a p < 0.05, any test result that meets this criterion would suggest that we reject the $H_0$ in favour of the $H_a$; we will say that the test was “formally statistically significant”.

While the previous is a simplified summary, it is important to remember that a prespecified p value cut-off constitutes an arbitrary significance threshold derived from tradition. What it also means is that we will expect every twentieth test to generate a false positive result. For these reasons, statistical testing, and the term “formally statistically significant” can lead researchers astray and cause serious misinterpretations. What the p value essentially does is to provide us with a rough estimate of the variability of data and an indication of the consistency between the data under study and the $H_0$. Most importantly, the p value is a figure derived from a statistical model, usually under strong assumptions that might not hold true in the real world. These models, along with the p values that are produced, are vulnerable to misclassification of variables, statistical overfitting, multicollinearity, residual confounding, to name but a few examples. Based on this clarification, we refer to any statistical test with a p < 0.05 as “formally statistically significant” (except when stated otherwise), however, with the aforementioned limitations of statistical testing in mind.

**Type I error**

Whenever the $H_0$ is erroneously rejected, we conduct a type I error. Simply put, we proclaim a statistically significant relationship between the exposure and outcome under study, although this conjecture is actually false in reality. The probability of type I error correlates with the predefined level of significance, or alpha level ($\alpha$). In other words, the alpha level is the predefined level of significance that we would expect the estimated p value to be less than, in order to reject the $H_0$.

As we saw earlier on, with an alpha level of 0.05 we would expect every twentieth analysis to give a false positive test result. As a consequence, any study in which multiple tests are performed has an increased risk of conducting type I error. These limitations are particularly relevant in Paper III, where numerous proteins were analysed by means of a predefined biomarker panel. However, to accommodate the risk of false positives, we employed adjustments in terms of the Benjamini-Hochberg procedure for multiple tests at a prespecified level of 0.10. The rationale behind the adjusted probability level was largely attributable to the explorative study design, in which we chose to accept a higher false discovery rate in an attempt to identify novel biomarkers in the Olink analyses specifically, rather than corroborating findings of previous reports, hence once again
illustrating the importance of viewing the statistical test in relation to the study question at hand. Additionally, while more conservative applications, such as the Bonferroni procedure, have been proposed, such methods will indeed substantially reduce the risk of type I error, but at the significant expense of a type II error increase, thus producing an unwanted effect with regard to the explorative study intent.

In contrast to the multiple tests performed in Paper III, only a handful of preselected exposures for a permanent stoma outcome were derived statistically in Paper I & Paper IV. Thus, the risk of type I error was low in these studies, and constitutes a methodological strength.

**Type II error**

A type II error is conducted when we are not able to observe a certain effect that is actually present. In terms of statistical inference, this means we fail to reject the $H_0$ and erroneously draw the conclusion that there is no significant relationship between the exposure and the outcome. The risk of type II error ($\beta$) is related to the concept of statistical power ($1-\beta$), which implies the probability of correctly rejecting the $H_0$. This probability is dependent on $\beta$, indicating that e.g. a level of power of 90% would assume every tenth test will not be able to prove a true association. While poor exposure or outcome data and inappropriate study design can also be contributing factors, the most common cause of a type II error is insufficient sample size.

To avoid type II error, the most formal and statistically correct way to initiate a quantitative study is to perform a power calculation before study start. In summary, what it means is that we calculate the sample size required to detect a predefined minimum effect size between two groups, which is often derived from findings of previous publications or scientific knowledge of the researcher, under the assumption of a set level of statistical power.

None of the studies (Papers I–IV) in the current thesis underwent a formal power calculation prior to study start, which can be considered a weakness, especially in Paper III, in which there was a significant risk of type II error due to the relatively small sample size. However, as we discussed earlier with regard to study design, we included all eligible patients who had been enrolled in the biobank project since the emergence of U-CAN, and a larger sample size was not attainable at that time. Nevertheless, colorectal cancer incidence rates and reported leakage frequencies had been used to give a rough estimate, with an expected ~100 cases possible for inclusion during the study period. We eventually ended up with a mere 41 patients with anastomotic leakage who were matchable to a control and available for inclusion. While this constitutes a major limitation in Paper III, we still managed to derive formally statistically significant results,
even after adjustments for repeated tests. Moreover, we evaluated mostly novel biomarkers, for which a hypothesised effect size estimation in a power calculation would be subject to vast speculation. Additionally, the largest study to date that evaluated biomarkers for postoperative infections in colorectal cancer surgery managed to recruit 30 cases and 30 controls, in which intra-abdominal infections were also included. Furthermore, the known underreporting of leaks to the registry might have resulted in a few leaks not being recognised, which may to some extent have reduced the number of eligible cases identified.

While Paper III had limitations with regard to sample size and the risk of type II error, the remaining studies (Papers I–II & Paper IV) all comprised large cohorts, Paper IV in particular. When discussing statistical power, it is interesting to note that we could not demonstrate a significantly higher risk of a permanent stoma outcome in those with a defunctioning stoma in Paper I, while such a relationship was corroborated in Paper IV. This discrepancy is probably explained by the larger study cohort size that reduced the risk of type II error in Paper IV, a likely culprit in Paper I.

Data quality, validation and missing
Most research questions were addressed through statistical analyses performed with clinical variables derived from registry data. It is therefore essential to review aspects of data quality as a critical source of error in relation to how misclassification may skew calculated outcomes (i.e. information bias). While we will discuss the principal study outcomes separately later on, a more general overview will first ensue, although including review of the validation performed in Paper II as an illustration.

In the event of information bias, misclassification can be either differential or non-differential. The former indicates that misclassification distributions differ between those with and those without the outcome under study as a consequence of their outcome or exposure. These errors may bias estimates in an unpredictable manner and are difficult to adjust for. Conversely, non-differential misclassification means that information bias occurs randomly between subjects regardless of their outcome or exposure status. The latter is an inherent factor of all epidemiological studies and always distorts results towards the $H_0$. In other words, while we expect random misclassification to occur when large registry databases are employed, non-differential bias will, as a product of unsystematic error, dilute effect estimates towards a null result. The main issue therefore arises when we end up with a negative test result, rather than in the event where a significant relationship is derived, for which the estimated association would have been even larger without non-differential misclassification but still be statistically significant.
A way to assess the influence of information bias is by validation, as was performed in Paper II. Noticeably, the primary study objective in Paper II was not to validate the data sources themselves; however, data from the Swedish Colorectal Cancer Registry and the National Patient Registry were crucial to the dichotomous estimations made with regard to long-term stoma outcome. Thus, the registry-based stoma outcomes relied indirectly on the quality of information derived from the registries, while Cohen’s Kappa and nominal agreement were used to report accuracy of the method. The latter two are frequently encountered measures in quality registry validations, and they also occur in studies evaluating similar algorithms, whereas a handbook issued by the executive committee of the Swedish National Quality Registries also referred to several studies reporting these outcomes. Moreover, as was described in the methodological chapter, Cohen’s Kappa is a somewhat arbitrary measure, while it has also been subject to debate due to the influence of prevalence and bias. Although adjusted models have been described, such models are controversial, and we chose to report unadjusted values to make the study more comparable to previous research.

As was demonstrated in Paper II, the registry-based method had almost perfect agreement with the chart-reviewed cohort as reference. Nevertheless, a positive predictive value of 85.1% was calculated and indicated presence of false positive registrations. In other words, these figures gave an information bias estimate, also declaring the one-way direction of this bias (to clarify, the negative predictive value was 100%). Moreover, as this misclassification was not obviously caused by any distribution differences related to subject’s exposure or outcome status, errors were considered to be non-differential. To conclude, we would therefore expect any analysis conducted with registry-based stoma outcomes to be biased towards the null hypothesis.

Based on the earlier clarification, it should also become evident why information bias regarding anastomotic leakage, estimated extent of mesorectal excision, as well as construction of a defunctioning stoma, in most cases would result in similar estimate distortions towards an absence of effect. This is an acknowledged weakness especially relevant to Paper IV and will be interpreted from a clinical viewpoint in the forthcoming chapter. However, an important exception to this statement also arises in the Paper IV main analyses, as misclassification bias in the leakage variable may to some extent have been dependent on the exposure (i.e. construction of a defunctioning stoma). To clarify, patients who received a defunctioning stoma might have experienced a delayed leakage diagnosis that occurred outside the 30 days’ follow-up and was not captured in the reporting to the registry. Hence, differential bias is conceivable, although in a predictable
manner, in which we expect the indirect effect attributable to anastomotic leakage to become attenuated. Conversely, **Paper I & Paper III** were both based on chart-reviewed study cohorts, with a significantly limited risk of information bias as a consequence.

Additionally, to reduce potential bias from registry variables with missing, multiple imputation with chained equations was used. Both **Paper I & Paper IV** reported effect estimates derived from imputed data sets and were supplemented with complete case analyses for comparison, providing information on the amount of bias introduced by missingness. As a consequence of imputation, precision of the effect estimates increased and reduced possible bias from variables with missing, and constitutes a methodological strength.

**Stoma outcomes and permanent stoma prevalence**

Permanent stoma prevalence after anterior resection for rectal cancer in Sweden was established at 20% and varied significantly between different healthcare regions. Although there have been several studies on stoma outcomes after anterior resection throughout the years, large population-based reports that reflect current surgical practice have been scarce. Our figures are, however, in line with recent reports; yet, for the regions and subcohorts presenting in the upper interval, slightly higher

While 24% of patients in the chart-reviewed cohort ended up with a permanent stoma (**Paper I**), the registry-based method likely overestimated the permanent stoma rates in **Paper II & Paper IV**, as a positive predictive value of was 85.1% was computed in **Paper II**. Also, an important difference is that the above-cited studies excluded PME patients, as these have a lower risk of anastomotic leakage, and usually experience better functional outcomes, which in turn suggests a slightly lower permanent stoma frequency would have been expected in the study populations herein (**Paper I, Paper II & Paper IV**). Furthermore, as was corroborated in **Paper IV**, construction of a defunctioning stoma decreases chances of a stoma-free outcome, while recent Swedish reports also showed that stoma reversals are often delayed, possibly due to the low priority given to this type of surgery.

However, although a few patients in **Paper I** only had a 1-year follow-up regarding stoma outcome, a minimum 2-year follow-up time was used in the main analyses in **Paper IV**, while both studies excluded patients with early mortality. Sensitivity analyses in the latter study (**Paper IV**) confirmed that effect estimates did not vary materially between stoma outcomes determined at 2 or 3 years after surgery, while results derived with a 1-year follow-up differed significantly. In other words, while delayed stoma reversals sometimes exceed 12 months, a 2-year follow-up time seems adequate to establish long-term stoma outcomes, with negligible influence of plausible events occurring outside the study period.
As demonstrated with the extent of mesorectal excision, clinical characteristics and events also have a significant impact on long-term stoma outcomes. To illustrate, Paper I had an anastomotic leakage incidence amounting to 17.7% and may suggest a higher permanent stoma rate would be expected compared to studies with lower leakage rates (for instance, Jutesten et al recently found a 65% permanent stoma rate in patients who had anastomotic dehiscence). Nonetheless, leakage figures in Paper I were likely higher due to the longer follow-up time of 90 days, compared to the reported anastomotic leakage incidence of 10% in Sweden based on a 30-day follow-up; add to this the known underreporting of leaks to the registry even in this time frame. Moreover, while the large population-based report by David et al found a 25% permanent stoma rate in patients who received a defunctioning stoma, merely 14.6% of the study population were defunctioned primarily, compared to 72–87% of patients studied in this thesis (Paper I, Paper II & Paper IV). Clearly, the study by David et al differed to a significant degree compared to the patient cohorts investigated in the current thesis, thus likely representing stoma outcomes from two different treatment paradigms with regard to the use of defunctioning stomas. On the other hand, the more recent population-based report by Gadan et al found an 18% permanent stoma rate in the 70% of patients who received a defunctioning stoma primarily, as determined from a chart reviewed cohort with a median follow-up time of 69 months.

While the first study (Paper I) featured a chart-reviewed cohort, a few patients registered with anterior resection had actually had surgery with immediate end colostomy, by means of Hartmann’s procedure (n = 2). Although we were able to exclude these patients during scrutiny of medical records, similar misclassifications may have occurred in patients with registry-based stoma outcomes estimated in Paper II & Paper IV. While these misclassifications are difficult to ascertain, they were most likely few in number and also bidirectional, thus with negligible effect on the estimated stoma outcomes. Moreover, it is a bit puzzling that the Northern healthcare region presented with a significantly higher permanent stoma frequency, as conservative surgery with immediate end colostomy was more common than in the rest of the country, suggesting that the remaining anterior resection patients would be more selected than elsewhere. While long distances to hospitals in the Northern healthcare region was suggested as a hypothetical explanation to this finding in Paper II, a recent Swedish report from the same catchment area demonstrated that there is no such association. Although different policies in the use of defunctioning stomas may speculatively have contributed to this discrepancy, we are not able to draw any firm conclusions about underlying mechanisms for regional differences in the present thesis.
Type of permanent stoma

While the primary aim of the present thesis was not to evaluate different stoma types, it nevertheless warrants comment, in view of the high permanent stoma prevalence found, as a plausible further quality measure. However, as we saw in the introduction, robust evidence from direct comparisons with regard to ideal stoma type is generally lacking; still, we and others find clinical reason to speculate that end colostomy would be the best option in patients where a stoma-free outcome is not achievable. For instance, in patients with major LARS, as well as those with a defunctioning loop-ileostomy left remaining in place, Lindgren et al concluded an end colostomy should be considered, as this “will be a better stoma for the patient and provide more advantageous stoma handling”34. Also, in light of the high permanent stoma rate found in the study by David et al, the authors commented as follows with regard to elderly patients: “…it may be better to perform a low Hartmann’s procedure with permanent stoma, as this will avoid the complications of an ileostomy. Further this will keep the remaining colon in use, improve surveillance and may provide better quality of life.”33.

Thus, of the 75 patients who ended up with a permanent stoma in Paper I, 39 (52.0%) had a colostomy, while the remaining 36 (48.0%) had ileostomies. Similar figures were found in Paper II, in which 445 out of 942 (47.2%) permanent stomas were determined to be loop ileostomies left remaining in place, while 304 (32.3%) were colostomies; it was not possible to specify the remaining 193 (20.5%) stomas to type. While the chart reviewed cohort in Paper I could corroborate that some patients eventually received a permanent stoma due to poor functional outcomes, similar surgical courses were plausible in Paper II; however, the indications for stoma creation could not be identified in the latter study. Theoretically, however, while not all patients seem to be offered conversion to an end colostomy whenever a defunctioning loop ileostomy is left in place, it is conceivable that those who experience severe ileostomy-related complications (e.g. recurrent bowel obstructions and high stoma output) are likely those who eventually are offered such surgery. Nevertheless, end colostomy construction usually requires formal laparotomy, and there may be an unwillingness to operate on patients with severe morbidity34, i.e. the same patients who are likely worst off with a loop ileostomy left in place.

To conclude, while end colostomies may be fashioned eventually in some patients, nearly half of permanent stomas established after anterior resection in Sweden were temporarily intended loop ileostomies that were left in place. From the studies conducted herein, it was not possible to derive the exact figure of how many patients were provided with an end colostomy due to ileostomy-related complications, although our findings corroborate the notion that a considerable number of patients will end up with a permanent ileostomy, for whom there is alarming evidence of frequent hospital readmissions849, risk of chronic kidney
failure\textsuperscript{62, 68} and interference with oncological treatment\textsuperscript{70}. Thus, it is also interesting to note that a recent Swedish report demonstrated an increase in the conversion rate from permanent loop ileostomies to end colostomies, from almost no such conversion surgeries in the earlier study period of 1995 to 2007, to nearly 50\% during more recent years, which likely reflects an increased awareness of this problem\textsuperscript{54}. In addition, the authors of the last-mentioned report seem to concur that end colostomy is the preferable outcome in patients with a permanent stoma: “...loop ileostomies were converted to end colostomies, with the intention to provide the patient with a better permanent stoma, and presumably fewer medical complications and increased quality-of-life.”

**Mechanisms behind stoma non-reversal and permanence**

With the advent of the high permanent stoma prevalence noted after anterior resection for rectal cancer, an increasing number of reports have tried to identify underlying mechanisms\textsuperscript{34, 54, 56, 143, 147, 148}. There have basically been two study subtypes, divided into those aiming to identify causes (most often termed “risk factors”) behind a permanent stoma regardless of faecal diversion, and those, the majority, that were limited to investigating what conditions might decrease chances of stoma reversal in patients primarily defunctioned. We aimed to provide new data mainly concerning causes of stoma permanence regardless of primary faecal diversion, evidenced by the study designs of Paper I & Paper IV. However, Paper I also featured a subgroup analysis performed exclusively on patients who received a defunctioning stoma primarily, in which factors limiting stoma reversal were analysed.

In line with virtually all previous reports, anastomotic leakage was in Paper I found to have a strong effect on the number of patients who eventually ended up with a permanent stoma\textsuperscript{34, 53, 54, 60, 143, 144, 148}. This held true regardless of whether or not the patient had received a defunctioning stoma primarily, and was an expected outcome. However, while David et al found a trend towards more postoperative complications in defunctioned patients who eventually did not undergo stoma reversal, results of their study did not reach formal statistical significance. Notwithstanding, it seems fairly safe to conclude that anastomotic leakage is a strong causative factor behind a permanent stoma outcome, acknowledged by researchers as well as clinicians. Moreover, the pronounced effect attributable to anastomotic leakage noticed in Paper I was likely also strengthened numerically due to the fact that leaks were recorded up to 90 days after surgery.

Also conforming to the results of previous reports, an advanced tumour stage decreased chances of a stoma-free outcome in Paper I\textsuperscript{34, 54, 60}. Stage IV cancer had a strong impact on the permanent stoma rate regardless of whether or not
the patient underwent primary faecal diversion; however, stage III also reduced stoma reversal rates significantly within the first year of surgery in defunctioned patients. While the effect of stage IV was time-independent, reversal rates increased instead for stage III patients 12 months after index surgery, compared to the reference category of patients with stages I–II. This observation is most likely explained by the use of adjuvant treatment in most of patients with stage III, which may postpone stoma reversals, after which reversal rates tend to increase. This has also been corroborated by more recent reports\textsuperscript{54, 147}.

Additionally, a significantly higher risk of a permanent stoma was also found in patients treated with TME compared to PME in Paper I. While this also constitutes a putative relationship, due to the higher risk of anastomotic leakage and worse functional outcomes after TME, a similar positive association between defunctioning stoma and permanent stoma did not reach formal statistical significance in Paper I. Again, the latter was likely due to an inadequate sample size in Paper I, as Paper IV demonstrated a major increase in the number of patients who ended up with a permanent stoma, if they had received a defunctioning stoma. Additionally, the effect of a defunctioning stoma on stoma outcome was especially pronounced in patients treated with PME, for whom the indirect effect attributable to anastomotic leakage was negligible.

To the best of our knowledge, Paper IV is the first study to date to demonstrate that defunctioning stomas significantly decrease chances of a stoma-free outcome. While we have already reviewed some methodological limitations with regard to the use of registry data, it is important from a clinical perspective to once again point out how the indirect effect of anastomotic leakage on stoma outcome was likely underestimated in patients with a defunctioning stoma. On the other hand, the impact of a defunctioning stoma on stoma outcome would likely have been even stronger, had there been no misclassifications in the estimated extent of mesorectal excision. For instance, 51\% of patients estimated with PME had a defunctioning stoma in Paper IV, compared to a previous chart-reviewed Swedish cohort in which only 34\% of these patients received underwent faecal diversion\textsuperscript{50}. To clarify, this observation suggests some patients estimated with PME most likely underwent TME, as faecal diversion is more routinely used in the latter group. We would therefore expect construction of defunctioning stomas to increase the number of permanent stomas in those treated with PME to an even larger extent than was estimated. Together with the lower baseline risk of anastomotic leakage, this suggests that abstaining from faecal diversion seems advisable in PME patients.

Defunctioning stomas also significantly reduced chances of a stoma-free outcome in patients treated with TME in Paper IV; however, anastomotic leakage is also known to occur more often in TME patients (as was also demonstrated in the
Interestingly, Lindgren et al concluded back in 2011 that the risk of a permanent stoma was probably even higher in patients treated with TME if they were managed without a defunctioning stoma primarily (nota bene, numerically but not formally statistically significant)\textsuperscript{34}. Conversely, Blok et al, recently reported several benefits with an institutional shift from routine use of defunctioning stomas, to a highly selective strategy, in which permanent stomas were substantially less common in the group that underwent highly selective diversion\textsuperscript{52}. The Dutch report also concluded that leakage rates did not in fact differ between the two groups, which strengthens the notion that anastomotic leakage most likely occurs irrespective of faecal diversion\textsuperscript{104}. The same observation was made in Paper IV, in which the advantageous effect of a defunctioning stoma was comparable in absolute numbers between patients treated with TME and PME.

To conclude, while the pendulum appears to have swung back in some parts of the Netherlands\textsuperscript{52, 59}, findings of Paper IV suggests that a similar strategical shift in the use of defunctioning stomas, to reduce the high permanent stoma prevalence after anterior resection for rectal cancer in Sweden, might be warranted. To implement such a strategy, however, there is a need for better tools to identify for which patients a defunctioning stoma can be omitted, as has been suggested regarding patients treated with PME in Paper IV.

**Stoma reversal complications**

The complication rate after stoma takedown surgery found in Paper I was considerable, as nearly one in ten of stoma reversals resulted in major complications requiring return to theatre, a need for intensive care or mortality. While a few papers have previously investigated stoma reversal complication rates, most study populations have been heterogeneous and included patients with inflammatory bowel disease and diverticulitis. Also, none of the studies used the Clavien-Dindo classification and are therefore difficult to compare with Paper I\textsuperscript{54, 55, 61, 149, 150}. Nevertheless, studies that reported complication rates for stoma reversals after anterior resection separately had lower incidences than were found in Paper I\textsuperscript{54, 55, 151}. With the heterogeneity of the cited research taken into consideration, the mortality rate of 0.4% found in our population-based study (Paper I) is nevertheless in line with most previous reports\textsuperscript{55, 61, 149, 151} and can therefore likely be employed as a reference standard regarding mortality rates after stoma takedowns in patients previously treated with anterior resection for rectal cancer.
Biomarkers to predict high-risk anastomoses

In Paper III, rectal cancer patients who had anastomotic leakage had distinctly elevated preoperative serum levels of inflammatory proteins CXCL6 and CCL11. Results were derived from a matched case-control study design, in which rigorous matching criteria were used to compare cases to controls with corresponding clinical characteristics who had a complication-free postoperative course. Nevertheless, there was a discrepancy in the use of neoadjuvant therapy within the rectal cancer group – a known risk factor for anastomotic leakage and a possible driver of inflammation. Still, sensitivity analyses with patients divided into subgroups with concordant oncological treatment did not affect calculated outcomes significantly.

While important limitations to Paper III in terms of statistical power and multiple tests must be kept in mind, it is interesting from a clinical viewpoint to note that rectal cancer patients with subsequent leaks had elevated serum concentrations regarding all 15 inflammatory proteins derived from the primary Olink analysis. Conversely, however, preoperative levels of the inflammatory protein hs-CRP were accentuated only in cases with colon cancer but not in patients with rectal cancer. Since hs-CRP is a well-established marker for systemic inflammation, also widely used in clinical settings, these findings are seemingly conflicting when hypothesising an inflammatory mechanism behind leakage in rectal cancer patients, and constitute a weakness of Paper III. Nevertheless, it is crucial to point out that ELISA analyses regarding hs-CRP were barely just statistically significant, and that adjustment for multiple tests was also omitted, which, together with inadequate study size, might be plausible errors that contribute to this disagreement. Also, while hs-CRP is often used in surgical practice to aid detection of anastomotic leakage in the postoperative period\(^1\), previous reports have failed to demonstrate a corresponding correlation in the preoperative period\(^1\). Altogether, while hs-CRP findings in Paper III may suggest that systemic inflammation in the preoperative period had little impact on anastomotic healing, these results were fairly weak. In contrast, CXCL6 and CCL11 both demonstrated a strong correlation to anastomotic leakage in patients with rectal cancer and constitute novel biomarkers not previously investigated in these settings, and therefore require further mention in terms of how they may be involved in processes behind anastomotic healing.

The healing of a colorectal anastomosis basically corresponds to the biological processes involved in the recovery of any surgical wound, in which local inflammation constitutes one of the most critical physiological phases\(^2\). While results in Paper III concerning hs-CRP may suggest that systemic inflammation was not a major driver of anastomotic leakage in patients with rectal cancer, dysregulated local inflammation (i.e. excessively activated or prolonged) may
speculatively have been involved in those with dehiscence, theoretically as denoted by elevated levels of CXCL6 and CCL11. Specifically, impaired wound healing due to dysregulated inflammation has been illustrated for instance in wounds where microbial clearance is incomplete, for which a suggested underlying mechanism has been the increased level of matrix metalloproteases that degrade extracellular matrix\textsuperscript{154}. As CXCL6 is known to stimulate and secrete these proteases from granulocytes\textsuperscript{155}, a high protease activity in patients with leakage might have been involved in patients where the anastomosis failed to heal. CXCL6 also has strong antibacterial properties\textsuperscript{156}, which might suggest that patients with leakage had an unfavourable intestinal microbial milieu to facilitate anastomotic healing. Although these mechanisms may at this point appear somewhat speculative, there is in fact an increasing number of reports that suggest various mechanisms coupled to bacterial activity and composition are likely involved in anastomotic complications and oncological outcomes in patients with colorectal cancer\textsuperscript{157, 158}, and has been demonstrated in several different settings, such as by inhibiting matrix metalloprotease activity by administration of antibiotic agents\textsuperscript{159}.

In light of the fundamental importance of inflammation in wound healing, it is also interesting to note that CCL11, as an increasingly researched novel biomarker, seems to play an important role in some chronic inflammatory conditions\textsuperscript{160, 161}. While elevated levels of CCL11 have been described in several various pathological conditions, CCL11 seems to be especially involved in diseases that affect the gastrointestinal tract\textsuperscript{161, 162}. Specifically, CCL11 has been suggested to play a key role in the development of mucosal inflammation, and conditions affecting the large intestine in particular\textsuperscript{161}. Consequently, the high levels of CCL11 in rectal cancer patients with subsequent leakage noted in Paper III strengthen the notion that local mucosal inflammation was present, and might constitute a suggestive mechanism behind the anastomotic breakdown observed in these patients.

To conclude, while Paper III features several acknowledged limitations, the high levels of CXCL6 and CCL11 noted in rectal cancer patients with anastomotic leakage warrant further study in larger prospective settings. Most interestingly, these biomarkers speculatively correspond to a local inflammation that might not be detectable by means of hs-CRP. Although no firm conclusions can be drawn from these results, plausible mechanisms of action are proposed; the findings are added to the growing body of literature suggesting that inflammation, and its interplay with gut microbiota, deserves a closer look in patients with colorectal cancer\textsuperscript{37, 158}. 

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Conclusions

- Currently, treatment with anterior resection for rectal cancer in Sweden results in a long-term permanent stoma prevalence of 20%. Figures vary significantly depending on the healthcare region in which the patient receives treatment.

- Anastomotic leakage, advanced tumour stage and construction of a defunctioning stoma all contribute independently to the high permanent stoma rate.

- Takedown of a defunctioning stoma after anterior resection for rectal cancer entails a significant risk of major complications, including mortality.

- Inflammatory proteins C-X-C motif chemokine 6 and C-C motif chemokine 11 were clearly elevated in serum before surgery in patients who sustained anastomotic leakage. The association between these proteins and anastomotic leakage needs corroboration in a larger prospective cohort of patients with rectal cancer, but can hopefully be used in the future to identify patients with high-risk anastomoses and in turn aid surgical decisions, e.g. by judicious use of defunctioning stomas and conservative treatment strategies with immediate permanent end-colostomy formation in selected cases.

- Patients with high rectal cancer eligible for partial mesorectal excision draw the least benefit from a defunctioning stoma in terms of reducing leakage, while instead experiencing a clearly elevated risk of a permanent stoma outcome. In these patients, abstaining from faecal diversion seems advisable.
Future Perspectives

The high stoma prevalence in patients treated with anterior resection for rectal cancer in Sweden continues to pose a surgical challenge. This thesis demonstrates that routine use of defunctioning stomas decreases chances of a stoma-free outcome, and that patients treated with PME are particularly susceptible to the negative effects of faecal diversion; they also tend to draw the least benefit in terms of reducing anastomotic leakage. While abstaining from faecal diversion in these patients might be a way to reduce the permanent stoma rate after anterior resection, other ways to identify patients who have the lowest likelihood to become stoma-free are warranted. By means of the long-term stoma outcomes validated in this thesis, a prediction model developed to identify patients with the highest risk of stoma permanence seems like a plausible option. For instance, in patients with characteristics that suggest a stoma-free outcome is highly unlikely, a non-sphincter saving resection might be the judicious option to avoid the morbidity of an anterior resection and, not least, the potential need for a stoma reversal. Additionally, a more selective use of defunctioning stomas also in patients treated with TME would likely help to remedy the situation even further. While such a strategy has already been demonstrated in single institutions, a shift towards highly selective use of defunctioning stomas once again stresses the need for criteria to support either strategy, and should be the focus of future research.

Although a randomised controlled trial on patients treated with PME would be ideal to further clarify the benefits and drawbacks of defunctioning stomas, such a study would be difficult to conduct. As the anastomotic leakage incidence is lower in these patients, the study would require an even greater sample size than the largest clinical trials performed on patients treated with TME to date. Nevertheless, in the event of such a trial, the researchers should include long-term follow-up data regarding functional outcomes and a longer capture period regarding anastomotic leaks and formation of chronic sinuses; previous research efforts have sometimes overlooked functional outcomes, and reports suggest leakage will occur regardless of faecal diversion, but with the leakage diagnosis being delayed.

While preoperative levels of biomarkers CXCL6 and CCL11 were clearly elevated in rectal cancer patients with anastomotic leakage, these associations require validation in a larger prospective cohort. Interestingly, however, these findings fall in direct line with the growing body of evidence that suggests gut inflammation and the microbial milieu likely play an important role in anastomotic healing. Clearly, a closer look at inflammation and its interplay with gut microbiota might be a promising way to decrease leak rates after anterior resection for rectal cancer.
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Appendix

Supplementary Figure 1. Demonstrating the hypothesised causal relationship between different exposures and the outcome (permanent stoma) used in Paper I. Other variables involved in this clinical context were also drawn to distinguish pathways confounding the effect of interest, but also to help identify mediators and collider pathways.
Supplementary Figure 2. Demonstrating the hypothesised causal relationship between a defunctioning stoma, anastomotic leakage and a permanent stoma used in Paper IV. Other variables involved in this clinical context were also drawn to distinguish pathways confounding the effect of interest, but also to help identify mediators and collider pathways.
Supplementary Table 1. Enumerating 15 proteins with titres significantly elevated in serum blood samples drawn preoperatively from patients suffering anastomotic leakage, compared to protein levels of matched controls with a complication-free postoperative course, in patients undergoing curative resection for rectal cancer.

<table>
<thead>
<tr>
<th>Protein</th>
<th>P value*</th>
<th>FDR†</th>
</tr>
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<tbody>
<tr>
<td>C-X-C motif chemokine 6 (CXCL6)</td>
<td>0.0012</td>
<td>Pass</td>
</tr>
<tr>
<td>Eotaxin (CCL11)</td>
<td>0.0028</td>
<td>Pass</td>
</tr>
<tr>
<td>Eukaryotic translation initiation factor 4E-binding protein (4E-BP1)</td>
<td>0.0059</td>
<td>NS</td>
</tr>
<tr>
<td>Natural killer cell receptor 2B4 (CD244)</td>
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<td>NS</td>
</tr>
<tr>
<td>C-X-C motif chemokine 1 (CXCL1)</td>
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<td>NS</td>
</tr>
<tr>
<td>Monocyte chemotactic protein 2 (MCP-2)</td>
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<td>NS</td>
</tr>
<tr>
<td>C-X-C motif chemokine 11 (CXCL11)</td>
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<td>NS</td>
</tr>
<tr>
<td>Tumour necrosis factor ligand superfamily member 14 (TNFSF14)</td>
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<td>NS</td>
</tr>
<tr>
<td>Tumour necrosis factor ligand superfamily member 9 (TNFRSF9)</td>
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<td>NS</td>
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<tr>
<td>CD40L receptor (CD40)</td>
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<tr>
<td>Adenosine Deaminase (ADA)</td>
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<td>C-X-C motif chemokine 25 (CCL25)</td>
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<tr>
<td>STAM-binding protein (STAMBP)</td>
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<tr>
<td>Caspase-8 (CASP-8)</td>
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<tr>
<td>Leukaemia inhibitory factor receptor (LIF-R)</td>
<td>0.0420</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Using multivariable projections, by means of orthogonal projections to latent structures-effect projections for dependent samples, and univariable associations corresponding to a paired t-test of individual variables. †FDR = False discovery rate. Using Benjamini-Hochberg to adjust for false discovery rate of multiple testing. Pass indicating a result remaining significant after controlling for a false discovery rate set at <0.1, with NS denoting non-significance.
Supplementary Figure 3. Demonstrating events denoting a stoma creation, or stoma reversal, in 4529 patients treated with an anterior resection for rectal cancer in Sweden between 1 January 2007 and 31 December 2015, with end of follow-up set at 31 December 2017. SCRCR, Swedish Colorectal Cancer Registry; HA, Hartmann’s procedure; APE, abdominoperineal excision.