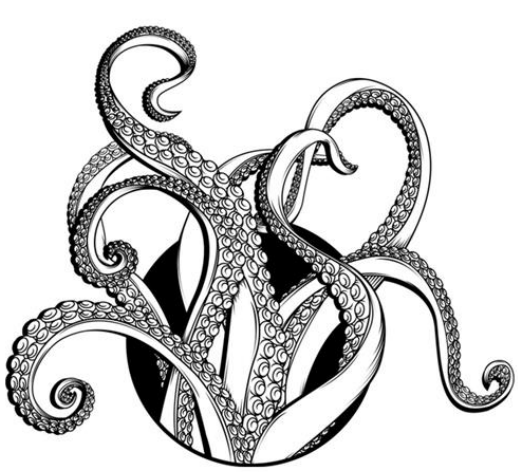

TENTACLE
Rectum

TreatmENT of AnastomotiC LeakagE after RECTAL resection (TENTACLE – Rectum)

Protocol writing committee

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1. SUMMARY

Rationale: Anastomotic leakage occurs in up to 20% after low anterior resection for rectal cancer. It is a severe complication with high associated morbidity, ICU admission, prolonged hospital stay and need for reinterventions and readmissions. Anastomotic leakage is independently associated with the risk of local recurrence and reduced long term survival. Most literature focusses on incidence and predictive factors. Remarkably, there is almost no data on the efficiency of different treatments of anastomotic leakage after low anterior resection.

Anastomotic leakage after rectal cancer resection is generally underreported, mainly due to subclinical leaks below a diverting stoma. However, up to 50% of the leaks do not heal with fecal diversion alone, especially not in an irradiated field, related to a competent sphincter which hampers adequate drainage of the presacral abscess. Late diagnosis of 'reactivated' leaks after stoma reversal is not an infrequent phenomenon. Chronic sinus, gluteal abscess, and fistula formation have been reported in up to 10%, and permanent stoma rates around 20%, both having significant impact on quality of life.

Examples of factors that may influence the severity and chance of healing of the anastomotic leakage are: timing of diagnosis, degree of systemic inflammatory response, etiology (e.g. ischemia of the afferent loop), degree of dehiscence and retraction, location of the leak (e.g. circular staple line, blind loop), whether or not a diverting stoma is in place, and extent of abdominal contamination. However, little is known about to what extent these and other factors contribute to anastomotic leakage severity and chance of healing. In addition, it is not known which anastomoses are likely to be preserved by which type of treatment, and which anastomotic failures require redo surgery at a certain time frame.

Primary study objectives

1. To investigate which factors contribute to anastomotic leakage severity and to compose an evidence based anastomotic leakage severity score, in which clinically relevant subgroups will be explored (e.g. diversion or not), as well as different clinical settings (e.g. leak diagnosis within or beyond 90 days postoperatively).
2. To evaluate the effects of different treatment approaches on all different pre-specified outcome parameters, stratified for severity score, anatomical characteristics of leakages and timing of diagnosis of leakage.

Study design: International multicenter retrospective cohort study.

Study population: Adult patients with anastomotic leakage after low anterior resection for rectal

cancer.

Primary outcome parameter: 1-year stoma-free survival.

Secondary outcome parameters: ICU length of stay, mortality, comprehensive complications index, total number of reinterventions (surgical, radiological, endoscopic) within one year, total number of unplanned readmissions within one year, total hospital stay during one year, total time of having a stoma until one year, stoma present at one year, type of stoma present at one year (diverting, permanent), secondary leakage related complications (extrapelvic abscess, cutaneous fistula, vaginal fistula, bladder fistula, small bowel, ureteric fibrosis with hydronephrosis), hospital related costs.

Sample size calculation: Inclusion of 1097 patients will be sufficient to analyze primary study objective 1 and this is 1246 patients for primary study objective 2. Therefore, the aim is to include at least 1246 patients.

Funding: Radboudumc and Amsterdamumc, location AMC.

2. INTRODUCTION AND RATIONALE

Incidence, definitions and consequences of anastomotic leakage

Rectal cancer resection is associated with considerable morbidity and anastomotic leakage is a severe and frequent postoperative complication. Anastomotic leakage can be defined as “a breach in a surgical join between two hollow viscera, with or without active leak of luminal contents”[Peel 1991]. However, different authors have used various definitions and there is no clear consensus for lower gastrointestinal tract anastomotic leakage definition [Bruce 2001].

Reported rates of anastomotic leakage after low anterior resection are hugely varying, depending on definition, study design, duration of follow-up and case mix factors such as proportion of colo-anal anastomoses [Kingham 2009, Kang 2013, Matsubara 2014, Qu 2015, Shiomi 2015, ESCP group 2018]. In addition, there is a group of patients that experience a late anastomotic leakage, for example after reversal of a protective ileostomy or colostomy that was created during resectional surgery. The late diagnosed leakages comprise around 30% of all diagnosed leaks after low anterior resection. A nationwide cross-sectional study in the Netherlands including 998 low anterior resections revealed that 30-day anastomotic leakage rate was 8.3% based on the original registry data, which increased to 13.4% after retrospective review of original patient files with standardized definition, and the long-term leakage rate was 20.0% after a median follow-up of 43 months [Borstlap 2017].

If anastomotic leakage occurs, mortality rates up to 13% [Buchs 2008, Ashraf 2013, Blumetti 2015] have been published, but are generally low. Most extraperitoneal leakages seal off at the level of the pelvic inlet, thereby preventing abdominal contamination and sepsis. A Dutch population based study showed a 30-day mortality rate after leakage of 1.0% and without leakage of 1.5%, with corresponding 90-day mortality rates of 3.0% and 1.9% (p=0.34), respectively [Borstlap 2017]. Although seldom lethal, anastomotic leakage after low anterior resection is characterized by long lasting clinical consequences, significant impact on long-term functional outcome, and impaired oncological outcome [Wang 2017, Mongin 2014, Hain 2017]. Unintended permanent stoma rates in series of low anterior resection are generally around 20%, with anastomotic leakage as the risk factor with the highest odds ratio [Zhou 2017]. It is often associated with a prolonged ICU treatment, hospital length of stay and multiple reinterventions. The impact of anastomotic leakage on quality of life is high and it is associated with a substantial burden in terms of hospital resources and costs [Ashraf 2013, Hammond 2014].

Current anastomotic leakage treatment strategies

The optimal treatment of an anastomotic leakage after low anterior resection is largely unknown. A wide variety of treatment options have been reported. Construction of a diverting stoma (e.g. ileostomy or transverse double loop colostomy) is usually performed in the treatment of anastomotic leakage. On the other hand, many surgeons already create a diverting ileostomy during primary rectal surgery, which does not necessarily prevent anastomotic leakage, but tends to diminish the severity of the leakage. Especially in patients with a primary diverting ileostomy clinically occult anastomotic leakages might occur [Hain 2016, Song 2018]. Other treatment options include conservative management (antibiotics and supportive care), radiologic drainage, endoscopic treatment and surgical reconstruction of an end colostomy [Buchs 2008, Blumetti 2015, Boyce 2017, Creavin 2019]. However, most studies report data of small cohorts with heterogeneous types of leakages. There are no randomized trials on the efficiency of different treatments of anastomotic leakage, and even no good quality comparative cohort studies. There is a remarkable gap of knowledge for such a frequent and severe clinical problem.

Based on clinical observation and scarce descriptive data, treatment of a low pelvic anastomotic leakage is often conservative in the presence of a primary constructed diverting stoma, with antibiotics, and sometimes combined with any type of passive drainage (e.g. repeated transanal irrigation or percutaneous drainage). In clinically symptomatic leaking anastomoses in the absence of a diverting stoma, relaparoscopy or relaparotomy is performed with often secondary creation of a diverting stoma, cleaning of the abdominal cavity if necessary, placement of drains, and sometimes combined with any transanal intervention (e.g. suture reinforcement, drainage). Anastomotic breakdown and end colostomy formation are less often indicated, and mostly performed in case of complete dehiscence with retraction, or ischaemia of the afferent colon loop. In those cases, the rectal stump is left open, is being cross-stapled at a lower level, or removed by intersphincteric resection. The presacral space can be filled with an omentoplasty after resection of the anastomosis. In highly selected cases, a redo anastomosis is performed after resection of the bowel ends and additional mobilization [Lefevre 2011, Westerduin 2018].

During the last decade, active vacuum-assisted transanal drainage has been introduced, but its use is still restricted with less than 250 cases published worldwide in recent meta-analyses [Popivanov 2019, Shalabi 2018]. A sponge is endoscopically placed through the anastomotic defect into the abscess cavity, which is connected to a transanal drain that is connected to a vacuum bottle [Weidenhagen 2008]. The treatment principle is similar to Vacuum Assisted Closure (VAC) of surgical wounds, with continuous drainage of purulent material, and induction of granulation tissue with improving vascularity. This allows for early control of sepsis, is considered to preserve compliance of the neorectum and prevention of retraction and aims at fastening the healing process. Initially,

endoscopic VAC therapy (EVAC) was continued with gradually reducing the size of the sponge until only a small sinus remained. Subsequently, the approach was aiming at preconditioning the presacral cavity with vital granulation tissue, after which the defect is closed transanally over a suction drain [Bemelman 2018].

Treatment of lately diagnosed leaks is an almost unstudied area in colorectal surgery [Arumainayagam 2009, Fong 2011, Sloothaak 2013]. In those cases, treatment is complicated by extensive fibrosis with a non-compliant neo-rectum. Endosponge treatment does not seem to be effective anymore after already 3-6 weeks from primary surgery. Some authors described marsupialization of the sinus with transanal stapling of the septum that incorporates the sinus into the neorectum [Abild 2012, Alsanea 2010]. Others tried fibrin glue [Swain 2004]. When the chronic sinus is symptomatic and has even resulted in severe secondary complications such as gluteal abscess, fistulas, fasciitis, or osteomyelitis, then extensive salvage surgery is indicated. This consists of resection of the anastomosis with complete debridement of the presacral area and resection of all fibrotic capsules, and subsequent redo anastomosis. When continuity is no longer thrived for, the rectal stump is resected and the presacral cavity filled with healthy, well vascularized tissue, such as an omentoplasty [Musters 2016].

Towards evidence based leakage treatment strategies

Anastomotic leakage severity is currently graded according to how it is treated, according to the Clavien-Dindo scale [Dindo 2004]. Although this scoring system is useful for reporting the consequences of anastomotic leakage, by definition it cannot be used to guide decision making when anastomotic leakage is diagnosed in a clinical setting.

Especially for anastomotic leakages after rectal surgery, grading is often based on the initial early clinical presentation, but does not take into account late consequences that appear after the 30 or 90 day postoperative period. Several leakage related re-interventions might be performed after the initial postoperative period, and this systematically underestimates the severity of mild leakages that eventually develop into severe chronic pelvic sepsis.

Examples of factors that may influence the severity of the anastomotic leakage are timing of diagnosis, degree of systemic inflammatory response, etiology (e.g. ischemia of the afferent loop), degree of dehiscence and retraction, location of the leak (e.g. circular staple line, blind loop), mechanical bowel preparation, placement a transanal drainage tube, whether or not a diverting loop ileostomy is in place, and extent of abdominal contamination. However, little is known about to what

extent these and other factors contribute to anastomotic leakage severity and the short and long-term. In addition, there is a paucity of data on which leakage characteristics dictate the success of a specific treatment.

3. STUDY OBJECTIVES

3.1 Main study objectives

1. To investigate which factors contribute to anastomotic leakage severity and to compose an evidence based anastomotic leakage severity score, in which clinically relevant subgroups will be explored (e.g. diversion or not), as well as different clinical settings (e.g. leak diagnosis within or beyond 90 days postoperatively).
2. To evaluate the effects of different treatment approaches on all different pre-specified outcome parameters, stratified for severity score, anatomical characteristics of leakages and timing of diagnosis of leakage.

3.2 Other study objectives

This is an explorative study and relevant interactions between factors will be investigated. The following other study objectives are predefined. Outcome will be evaluated in terms of preservation of bowel continuity, stoma rate, comprehensive complications index (CCI), number of reinterventions, length of stay and costs.

1. To investigate which factors are associated with 1-year anastomotic failure in patients undergoing low anterior resection for rectal cancer with or without diverting stoma in whom anastomotic leakage is diagnosed at different times within the one year postoperative period (e.g. <2 days versus 2 - 14 days, 14 - 28 days and 28 days - 1 year).
2. To investigate whether time until diagnosis of anastomotic leakage (e.g. <2 days versus 2 - 14 days, 14 - 28 days and 28 days - 1 year) is associated with outcome, stratified for diversion, severity of leakage and other case-mix variables as assessed by explorative regression analysis.
3. To investigate whether time from diagnosis to first active treatment is associated with outcome, stratified for diversion, severity of leakage and other case-mix variables as assessed by explorative regression analysis.
4. To investigate whether initial surgical approach (laparoscopic or open) is associated with severity of the leak, type of treatment, and outcome, corrected for relevant confounders.
5. To compare leakage characteristics, severity, treatment characteristics and outcome in patients with anastomotic leakage that received selective digestive tract decontamination (SDD, including type and timing)/mechanical bowel preparation (including type) versus patients who did not.
6. To investigate whether primary or salvage resection is associated with anastomotic leakage severity and to compare the effectiveness of different anastomotic leakage treatments between primary and salvage resections.

7. To investigate whether the diagnostic modalities that were used to diagnose the leakage (e.g. whether endoscopy was performed) is associated with the choice of treatment and outcome after leakage treatment.
8. To investigate trends in the choice for and effectiveness of different anastomotic leakage treatments throughout the study years.
9. To investigate whether the use of a transanal drain is associated with a reduced severity of anastomotic leakage.

4. STUDY DESIGN

4.1 Study type

International multicenter retrospective cohort study.

4.2 Duration of the study

Data from a recent 5 year cohort of patients who underwent rectal resection with anastomosis from January 1st 2014 until December 31st 2018 will be recorded. The total study duration will be from January 2020 until June 2021 (18 months).

4.3 Study timeline

- January 1st – January 15th 2020: Database building and approval of the first version of the protocol.
- January 16th 2020: Invitation of surgeons by sending first version of protocol and CRF.
- January 2020 – March 2020: study document preparation and pilot in around 5 (inter)national centers. Protocol and CRF refinement based on comments of pilot study results.
- March 16st 2020: Final protocol and CRF is sent to participating centers. All participating surgeons receive a Castor database login.
- April 2020 – December 2019: Data collection.
- January 2021 – June 2021: Analysis and manuscript writing.

4.4 Follow-up of patients

Follow-up duration will be 1 year.

4.5 Study setting

This study will be performed in a multicenter and multinational setting. A large proportion of the group of Dutch hospitals that comprises the Dutch Snapshot Research Group is expected to participate. The rectal TENTACLE study will also be submitted to the European Society of Coloproctology (ESCP) and formal support of the ESCP will be sought (see also *Chapter 5.5 – Feasibility*).

5. STUDY POPULATION

5.1 Population

All consecutive adult rectal cancer patients who have been operated between January 2014-December 2018 will be retrospectively analyzed and evaluated for an anastomotic leakage. All patients with an anastomotic leakage diagnosed within one year from primary surgery are suitable for inclusion.

5.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Aged 18 years or older;
- Cancer located in the rectum, defined according to the international definition of the rectum consensus [D'Souza 2019];
- Rectal cancer resection with primary anastomosis (with or without diverting loop ileostomy) for either primary cancer, completion after local excision or salvage resection for regrowth after watch & wait or local excision;
- Postoperative anastomotic leakage according to the following definition: "a breach in a surgical join between two hollow viscera, with or without active leak of luminal contents" [Peel 1991].

5.3 Exclusion criteria

- Rectal resection for benign disease;
- Rectal resection for recurrent rectal cancer after previous low anterior resection or other primary malignancies;
- Emergency resection;

5.4 Sample size calculation

This is an explorative study and data will be used to investigate to what extent specific characteristics of anastomotic leakages are associated with severity of the leakage and how they relate to the successfulness of different treatments.

For the primary study objective 1, in order to create a risk score with 20 candidate predictors, with a one year stoma-free survival rate of 70% and a root mean square percentage error (rMSPE) of 15%, 1097 patients with anastomotic leakage should be included [Riley 2018].

For comparing the effectiveness of different treatment strategies (primary study objective 2), another power calculation was performed. With a one year stoma-free survival of 70%, a relative difference of 25% (corresponding to an absolute difference of 7%) between treatment strategies is considered to be clinically significant, and with a power of 0.80 and a significance level of 0.05, 1246 patients are needed.

5.5 Feasibility

In order to include the proposed number of patients and to collect data from international patient cohorts, we aim to acquire data from 2 colorectal surgery networks. Although there is some overlap between these groups, we believe that inviting the networks to participate will optimize the chance of obtaining robust results from this study.

1) *The Dutch Snapshot Research group*

   A large group of Dutch hospitals performing colorectal cancer surgery have previously participated in snapshot studies and are expected to participate. During the study period of 5 years, the incidence of rectal cancer according to the IKNL is 4400 per year and we expect that 1350 patients can be screened for eligibility. The 1-year incidence of anastomotic leakage in the Netherlands based on a previous snapshot study of all rectal cancer resections in 2011 is 20%. Therefore, data from 1350 patients with anastomotic leakage (1350*5*0.2) can be included from the Netherlands.

2) *The European Society of Coloproctology group*

   This European group has previously carried out snapshot studies with >1000 collaborators from Europe. The TENTACLE – Rectum protocol will be submitted to this group and the group members could contribute a substantial number of patients to the study. In addition, it would add to the generalizability of our results.

In addition to these scientific groups and societies, collaboration is sought with the following societies to increase patient numbers and to increase generalizability of our results:

3) *Latin American Forum*
4) *American Society of Colorectal Surgeons*
5) *Colorectal Society of Australia and New-Zealand*
6) *Japanese Society for Cancer of the Colon and Rectum*

METHODS

5.6 Primary outcome parameter

- 1-year stoma free survival.

5.7 Secondary outcome parameters

- 30/90/365 day mortality.
- Modified Clavien-Dindo classification.
- Comprehensive complications index (CCI) [Slankamenac 2013].
- Total number of reinterventions within one year (endoscopic, radiologic, surgical).
- Total number of unplanned readmissions within one year.
- Hospital length of stay after index surgery and during one year.
- ICU length of stay.
- Stoma rate at 1 year after index surgery.
- Total time of having a stoma until one year .
- Type of stoma present at one year (diverting, permanent, ileostomy or colostomy).
- Secondary leakage related complications (extrapelvic abscess, cutaneous fistula, vaginal fistula, bladder fistula, small bowel, ureteric fibrosis with hydronephrosis).
- Total hospital costs (estimated by standardized cost list).
- 1-year local recurrence rate.

5.8 List of study parameters

This is a retrospective study and we expect that not all relevant data can be obtained from the patient files. For example, estimation of leak circumference, degree of retraction, and specific location of the leak (circular anastomosis or blind loop) will most often not be possible without an endoscopy and we expect that not all patients underwent an endoscopy. However, it is expected that the large number of patients will provide enough data to analyze whether factors with a lot of missing data are of influence. Therefore, these factors that are likely to have a lot of missing data are taken up in this list, even though missing data may introduce bias.

- *Hospital Characteristics:* hospital type (academic, non-academic teaching, categorial); annual volume of rectal cancer resections 2014-2018, annual volume of restorative low anterior resection 2014-2018, number of restorative low anterior resection for rectal cancer with the

lower border below the sigmoid take-off on sagittal MRI 2014-2018, number of hospital beds; diagnosis & treatment strategy by general surgeon-on-call versus dedicated gastro-intestinal surgeon; ward facilities (e.g. dedicated colorectal nurse / physician assistant); types of diagnostic and treatment modalities that are available in the hospital.

- *Patient and tumor characteristics:* year of surgery, sex; age; length; weight; ASA classification; Charlson comorbidity index; tumor location (distance from anorectal junction to the lower border of the tumor on sagittal MRI); preoperative T-stage; preoperative N-stage; preoperative M-stage.
- *Treatment characteristics:* neoadjuvant therapy (radiotherapy, chemotherapy (with and without bevacuzimab); chemoradiotherapy); interval between end of neoadjuvant treatment and surgery (weeks); perioperative selective digestive decontamination (SDD) received; SDD type and timing; preoperative mechanical bowel preparation; type of mechanical bowel preparation; operation approach (laparoscopic, laparoscopic TaTME, robotic, robotic TaTME, open, TATA); multivisceral resection (if yes: which additional organs); lateral lymph node dissection performed (no, unilateral, bilateral); anastomotic technique (hand sewn, single stapled circular, double stapled circular); anastomotic configuration (E-E, E-S, colopouch, coloplasty); site of anastomosis (cm from anal verge or anorectal junction. In analysis, distance from anal verge is calculated to be 2,5cm extra in females and 3,5cm extra in males. This is used in cases where only one of the two parameters is known.); air leak test performed; complete donuts.
- *Anastomotic leakage diagnosis:* time from surgery to diagnosis of the leakage (days); modality performed to diagnose anastomotic leakage; approximate time from diagnosis to active (e.g. radiologic drainage, endoscopic, surgical) treatment of the leakage (hours).
- *Patient parameters at the time of diagnosis (parameters closest to diagnosis should be used and parameters within 24 hours prior diagnosis can be used):* in-hospital versus out of hospital; altered mental status (GCS<15); respiratory rate; systolic blood pressure; heart rate; temperature; leukocyte count; CRP, serum albumin, kreatinine.
- *Leakage characteristics:* location of the leak (blind loop versus anastomosis, dorsal versus ventral; fistula to vagina, bladder, small bowel, skin, urethra); estimated circumference of the leakage (0-25%, 25-50%, 50-75% and 75-100%, if possible); extent of the contamination (e.g. none, pelvic fluid collections, free abdominal collections); (postoperative) drains in place at time of diagnosis (e.g. abdominal drain, transanal drain).

- *Anastomotic leakage treatment:* (re-)admission to ICU or medium care/high care; transanal drain placement through anastomotic defect; endoVAC/endoSponge placement; percutaneous drainage (transgluteal vs transabdominal); reoperation; reoperation approach; reoperation procedure (drainage only, suturing of the leak; resection of the leak and re-anastomosis; disconnection and end colostomy formation; diverting stoma formation).
- *Anastomotic leak healing:* Anastomotic leak healed (assessed by endoscopy, radiologic imaging and date of healing).
- *Stoma related parameters:* Stoma (re-)reversed and / or (re-)created, including types and dates.
- *Complications with Clavien-Dindo grade:* Pulmonary (pneumonia, pleural effusion requiring drainage, respiratory failure requiring reintubation, acute respiratory stress syndrome (ARDS, Berlin definition[Ranieri 2012]), acute aspiration); cardiac (cardiac arrest requiring CPR, myocardial infarction (WHO definition[Mendis 2011]), atrial dysrhythmia requiring treatment, ventricular dysrhythmia requiring treatment, congestive heart failure requiring treatment); gastrointestinal (ileus (small bowel dysfunction preventing or delaying enteral feeding), small bowel obstruction, clostridium difficile infection, gastrointestinal bleeding requiring intervention or transfusion); urologic (acute renal insufficiency (defined as doubling of baseline creatinine), acute renal failure requiring dialysis, urinary tract infection, urinary retention requiring reinsertion of urinary catheter, delaying discharge or discharge with urinary catheter); thromboembolic (deep venous thrombosis, pulmonary embolism, stroke (CVA); infection (wound infection requiring opening of wound or antibiotics, central intravenous line infection, intra-abdominal abscesses, generalized sepsis (CDC definition [Hall 2011], other infections requiring antibiotics; other complications (abdominal wall dehiscence, multiple organ dysfunction syndrome (Definition: American College of Chest Physicians/Society of Critical Care medicine Consensus Conference Committee [Bone 1992]).
- *The comprehensive complications index (CCI):* The CCI [Slankamenac 2013]) is calculated from all scored complications.
- *Length of stay, readmissions and mortality:* hospital length of stay (total days); total number of unplanned readmissions within one year; ICU length of stay (total days); 30-day mortality; 90-day mortality; 365-day mortality.

6. ANALYSIS

6.1 Analysis strategy – general considerations

The goal of main study objective 1 is to investigate which factors contribute to anastomotic leakage severity and to compose an evidence based anastomotic leakage severity score that reflects the influence of leakage associated parameters on clinical outcome (i.e. 1 year stoma-free survival). This will allow researchers to describe leakage severity in groups of patients and it can be used to correct for differences between groups regarding anastomotic leakage severity. This will therefore aid in performing future comparative effectiveness studies regarding anastomotic leakage treatments.
Only factors that concern treatment characteristics (listed in *chapter 6.3 – List of study parameters / Treatment characteristics*), leakage characteristics (listed in *chapter 6.3 – List of study parameters / Leakage characteristics*) and factors that concern the consequences of the leak for a patient (listed in *chapter 6.3 – List of study parameters / Patient parameters at the time of diagnosis*) are used to compose this score.

Other important parameters that are likely to be predictive for 1 year stoma-free survival (e.g. age, comorbidity index, preoperative performance status, etc.) are not included in the severity score since these can be reported (and corrected for) separately. In addition, it is one of our other study objectives to compose a model that predicts 1-year stoma-free survival in case of anastomotic leakage (see *chapter 3.2 – Other study objectives*) and for this model all predictive factors that are registered in this study will be taken into account.

6.2 Main study objective 1

The first main study objective is to investigate what factors contribute to anastomotic leakage severity and to compose an evidence based anastomotic leakage severity score.
First, univariate analysis is performed on relevant parameters that are described in *chapter 6.3 – List of study parameters.* Relevant parameters are entered into separate binary logistic regression models with 1 year stoma-free survival as outcome parameter in order to explore associations in the data. Second, factors that are considered to be clinically relevant based on literature and/or expert opinion are selected for multivariate analysis. Backwards selection is used to exclude values of $p>0.05$ from the model. Results are presented as odds ratio (OR) with 95% confidence intervals (CI). A 2-tailed $p<0.05$ is considered statistically significant. Third, this multivariate model will be internally validated by bootstrapping, using 5000 bootstrap resamples. Finally, a nomogram is constructed based on the final bootstrapped multivariable regression analysis and this nomogram can be used to calculate the anastomotic leakage severity score.

In order to investigate the relative influence of casemix parameters (e.g. age, comorbidity, etcetera) on the leakage severity score, similar analysis will be performed in which casemix parameters (listed in *chapter 6.3 – List of study parameters / Patient and tumor characteristics)* are also included. If casemix is found to be very strongly associated with outcome relative to the severity score (to the extent that the severity score is of limited additional value in the regression model), latent class analysis is used [Rabe-Hesketh 2008]. The parameters used for the anastomotic leakage severity score (*chapter 6.3 – List of study parameters / Leakage characteristics* and in *chapter 6.3 – List of study parameters / Patient parameters at the time of diagnosis*) are used to create casemix corrected classes of anastomotic leakage severity.

The results obtained by the described analyses will also be performed in subgroups of patients with and without diverting ileostomy and by primary anastomosis height. By performing this sensitivity analysis, we will investigate whether the obtained model is useful for all types of rectal cancer resection or whether different factors are predictive of 1 year stoma-free survival for the different types of rectal cancer resection. If substantial differences are found between the primary analysis and this sensitivity analysis, the possibility of composing different anastomotic leakage severity scoring systems will be considered.

In addition, we will investigate and report whether the anastomotic leakage severity score is also predictive of the other outcome parameters. Together with data from the secondary study objective, (see *chapter 3.2 – Other study objectives*) in which associations between 1 year stoma-free survival and other outcome parameters are investigated, other parameters that can be used as a proxy for 1 year stoma-free survival are being determined.

6.3 Main study objective 2

The second main study objective is to investigate what anastomotic leakage characteristics are associated with success of different treatments and to compare the effectiveness of different anastomotic leakage treatment strategies for leakages classified according to severity and leakage characteristics.

In the first analysis, relevant treatment parameters (listed in *chapter 6.3 – List of study parameters / Anastomotic leakage treatment*) are the exposures. The association between anastomotic leakage characteristics and operation characteristics (see *chapter 6.3 – List of study parameters / Leakage characteristics* and *chapter 6.3 – List of study parameters / Operation characteristics*) and outcome parameters (see *chapter 6.1 – Primary outcome parameter* and *chapter 6.2 – Secondary outcome parameters*) will be evaluated for the exposures in regression analysis. Correction for patient characteristics, tumor characteristics and anastomotic leakage severity score is performed, if

appropriate.

Based on the results of this first analysis, subgroups of patients are created based on individual operation or leakage characteristics or based on combinations of characteristics. The effectiveness of anastomotic leakage treatment strategies is assessed in regression models for the different outcome parameters and corrected for patient characteristics, tumor characteristics and anastomotic leakage severity score, if appropriate. Comparison of the primary outcome parameter and secondary outcome parameters will be expressed in terms of a relative risk and corresponding 95% confidence intervals. A two-tailed $P < 0.05$ is considered statistically significant.

6.4 Other study objectives

Analysis of other study objectives will follow the same principles as described in *chapter 7.2 – Main study objective 1* and *chapter 7.3 main study objective 2*. Detailed and predefined analysis plans will be written during the preparation phase of the TENTACLE – Rectum study (see also *Chapter 4.3 – Study timeline*).

7. ETHICS STATEMENT AND REGULATORY APPROVAL

This study will be conducted in compliance with the principles of the declaration of Helsinki. The study protocol and relevant documents have been approved by the medical ethical committee of the Radboud University Medical Center, Nijmegen, the Netherlands. All participating centers are provided with the study protocol and relevant documents in January 2020, so that participating centers can ask their local ethical committees for approval if needed according to local ethical protocols.

8. DATA HANDLING

8.1 Database system

The Castor database system (www.castoredc.com) will be used. This online medical research database system is certified to meet international security standards and is compliant with all relevant regulations, amongst which are ICH-GCP, GDPR, HIPAA, FDA 21 CFR part 11, ISO 27001 and ISO 9001. More information and individual security certificates can be found on https://www.castoredc.com/security-statement.

8.2 Case report form (CRF)

A detailed CRF is created from the Castor (www.castoredc.com) database and provided to the invited centers (see also appendix 1). The CRF includes info points with definitions and guidelines that aid in adequate scoring of the listed parameters.

8.3 Data collection and data entering

All patient data will be entered anonymously by or under supervision of the treating physician(s). Up to 4 users per participating center will receive a Castor login username and password and these users can enter data into the database. In addition to entering data per patient individually, local study teams can upload their already existing database into the Castor database system and add only the additional data that is required for this study. The TENTACLE – Rectum study team can provide a step-by-step manual and can help the local teams with any data collection issues, if needed.

8.4 Data privacy statement

All anonymous study data will be available to the rectal TENTACLE – Rectum study team. The data of a center will be available to that specific center only through the Castor database system website. The data will not contain identifiable patient parameters (e.g. no date of birth etc.) in compliance with the General Data Protection Regulation (GDPR - EU 2016/679). Each patient will be coded with a unique patient number so that patients in the study are untraceable from the study database. Surgeons that participate in the rectal TENTACLE – Rectum study are asked to keep a password coded file that can identify individual patients locked away in their practice. This file can be accessed by the local investigators if needed, for example in case a relevant new research question requires entering of additional data into the database.

9. PUBLICATIONS

9.1 Main publications

We aim to publish two main manuscripts that cover the investigation of our main study objectives:

1. To investigate which factors contribute to anastomotic leakage severity and to compose an evidence based anastomotic leakage severity score, in which clinically relevant subgroups will be explored (e.g. diversion or not), as well as different clinical settings (e.g. leak diagnosis within or beyond 90 days postoperatively).
2. To evaluate the effects of different treatment approaches on all different pre-specified outcome parameters, stratified for severity score, anatomical characteristics of leakages and timing of diagnosis of leakage.

9.2 Other publications

Other possible publications will cover the secondary study objectives. These manuscripts will be defined at a later stage during the study.

9.3 Publication policy

The TENTACLE – Rectum study embraces corporate authorship and all collaborators that contribute to this study will form the TENTACLE – Rectum collaborative group. This group will co-author all publications in which TENTACLE – Rectum study data is used.

The protocol writing committee is fully involved in conducting this study and will be included as authors in both main publications in which the TENTACLE – Rectum study data is used. If the manuscript is submitted to a journal that does not allow the full number of authors, a number of authors will join the collaborative group instead, based on scientific input during the study, manuscript writing and revising.

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11. APPENDIX 1 – PRINTED CASE REPORT FORM (CRF)

Castor database printed CRF. This printed CRF will not be used in the study, all data is entered online into the Castor database system in which only the applicable data fields for each patient are visible. This can be downloaded from www.tentaclestudy.com.