Experimental evaluation of clinical colon anastomotic leakage

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1. ARTICLES INCLUDED IN THIS THESIS:

Study 1:
Pommergaard HC, Rosenberg J, Schumacher-Petersen C, Achiam MP. Choosing the best animal species to mimic clinical colon anastomotic leakage in humans: a qualitative systematic review. Eur Surg Res 2011;47:173-81.

Study 2:
Pommergaard HC, Achiam MP, Rosenberg J. Colon anastomotic leakage: improving the mouse model. Surg Today 2013 Jun 9. [Epub ahead of print]

Study 3:
Pommergaard HC, Achiam MP, Burcharth J, Rosenberg J. Impaired blood supply in the colonic anastomosis in mice compromises healing without creating clinical leakage. Int Surg. In press.

Study 4:
Pommergaard HC, Achiam MP, Rosenberg J. External coating of colonic anastomoses: a systematic review. Int J Colorectal Dis 2012;27:1247-58.

2. DEFINITIONS

Anastomotic leakage was defined according to the definitions made by the Surgical Infection Study Group [1]. They have defined anastomotic leakage as a leak of luminal contents from a surgical join between two hollow viscera, emerging through the wound or drain site, or collecting in relation to the anastomosis. The leakage may affect the clinical state of the patient, causing fever, abscess, septicemia, metabolic disturbances and/or multiple-organ failure. Any local anastomotic leakage in the absence of clinical symptoms may be regarded as a subclinical leakage [1]. Moreover, anastomotic leakage may be defined according to the degrees of severity.

Radiological leakage is defined as leakage visualized upon radiological examination without the patient having clinical symptoms. Both minor and major clinical leakage are defined as clinical symptoms of anastomotic leakage as described above. However, when a change in surgical and/or medical management is necessary, the leakage is defined as major. For both conditions, radiological leakage may also be present [2].

3. INTRODUCTION

DESCRIPTION OF THE CONDITION

Anastomotic leakage after construction of a colorectal anastomosis is an important clinical problem in gastrointestinal surgery. This condition occurs in 3-7% of all colonic resections with a mortality rate of up to 27%. Anastomotic leakage is even more frequent for the rectal resections with a leakage rate of 13% [3-8]. In addition to this, anastomotic leakage after resection for malignant disease may increase the recurrence rate and impair cancer related survival [9].

PATHOPHYSIOLOGICAL MECHANISMS

When anastomotic leakage occurs in a patient, it may be caused by different pathophysiological mechanisms. Presumably, anastomotic leakage is multifactorial and cannot be attributed to a single mechanism. However, tissue ischemia and insufficient surgical technique are considered two of the most important factors. Regarding surgical technique, it has been shown that experienced surgeons with a high caseload of colorectal resections produce better patient related outcomes, including a reduced anastomotic leakage rate [6, 10], a lower operative mortality and a better 5 year survival [11]. Moreover, the anastomotic leakage rate among the individual surgeons varies greatly, which indicates that the technical abilities of the surgeon play a role for the risk of anastomotic leakage [12].

With regard to ischemia, blood supply and oxygenation are considered important factors for anastomotic healing [13]. It has been shown that low oxygen tension in the perianastomotic tissue is a predictor for colon anastomotic leakage in humans [14] and in the experimental setting [15]. Furthermore, experimental blood loss leading to low tissue oxygen tension reduced collagen content of the anastomosis [16]. Thus, impaired healing in response to ischemia may explain this mechanism.

PREVENTION OF ANASTOMOTIC LEAKAGE

Anastomotic leakage continues to be a major clinical issue, even though researchers have sought to prevent it with various interventions. However, the majority of interventions have only been tested experimentally. Among these are pharmacological interventions such as immunomodulatory drugs (e.g. tacrolimus [17] and TNF-α antagonists [18]), nutrients (e.g. glutamine [19], short chain fatty acids [20] and resveratrol (antioxidant) [21]), hormones (e.g. human growth hormone [22] and insulin-like growth factor 1 [23]) and proteinase inhibitors (matrix metalloproteinases [24]). Most of these studies have found positive results. However, these drugs have not been evaluated clinically.

A promising strategy to prevent anastomotic leakage may be external coating of the anastomosis. With this strategy, possible defects in the anastomosis may be sealed by the coating material, which thereby encapsulates the leak to prevent clinical symptoms. Such defects may be a result of insufficient surgical technique or a result of tissue ischemia and subsequent focal necrosis. These coating materials may be liquid or solid (e.g. mesh). However, common for them are a need for flexibility to allow adaption to the bowel movements as well as an ability to cause minimal tissue reaction.

IMPORTANCE OF EXPERIMENTAL RESEARCH

Whether a novel intervention may be beneficial or even harmful to patients is unknown, hence it is unethical to test such treatments directly in humans. Thus, animal models are necessary to evaluate the safety and efficiency of interventions prior to clinical testing. The optimal animal model should simulate the conditions of a specific disease in humans as accurately as possible. Therefore, the clinical condition of anastomotic leakage in humans with subsequent abscess/peritonitis and sepsis should be simulated if possible. A technical insufficiency model is a relevant choice, in which insufficient surgical technique is simulated by means of too few sutures with subsequent defects in the suture line. When initiating the projects of this thesis, no suitable model of this type was available. The existing models were either unable to simulate the clinical scenario [25], too costly to use [26] or insufficiently described with respect to the methodology to allow reproduction and validation [27]. Therefore, a new animal model of clinical leakage due to technical insufficiency was needed. In addition, the existing models of anastomotic leakage due to ischemia were unsatisfactory as they failed to produce clinical leakage [28-30]. Therefore, a new ischemia model was also needed. Subsequently, these models could be used to test whether anastomotic coating, or other interventions, may decrease the leakage rate in absence of adverse effects.

ETHICAL CONSIDERATIONS

Colorectal anastomotic leakage is a serious condition resulting in multiple deaths worldwide. Thus, with colorectal resection being a common surgical procedure, many patients are at risk for anastomotic leakage. Therefore, it is considered ethical to use experimental animals to evaluate this condition. In order to limit the number of animals in the experiments, inbred animals are used. These animals are genetically similar [31], which causes them to respond in a similar manner in the experiments, and thereby adding less variation to the results. Hence, a smaller number of animals may be used to obtain the same results [31]. Moreover, sample size calculations based on previous studies both avoid unnecessary use of animals and allow proper dimensioning of the experiments to detect a possible effect. Furthermore, to limit the number of animals, the control group from one study may be reused in another study, given that it is not considered a problem for the study design. Strict humane endpoints should be maintained for all the animals throughout all experiments to ensure that no animals are suffering. In collaboration with veterinarians, proper analgesic regimens should be developed to ensure a minimal level of pain in the animals.

OBJECTIVE

The objective of this thesis was:

To identify the best suited animal to model the clinical presentation of colon anastomotic leakage in humans.

To create animal models in which clinical colon anastomotic leakage is induced by two possible pathophysiological mechanisms (technical insufficiency and ischemia).

To determine the best suited coating material for prevention of anastomotic leakage.

4. PRESENTATION OF STUDIES:

STUDY 1: CHOOSING THE BEST ANIMAL SPECIES TO MIMIC CLINICAL COLON ANASTOMOTIC LEAKAGE IN HUMANS: A QUALITATIVE SYSTEMATIC REVIEW

Aim

The aim of this study was to identify the best suited animal to model clinical colon anastomotic leakage in humans.

Methods

This study was a systematic review conducted according to the PRISMA guidelines [32], in which the databases PubMed and Rex were searched up to October 2010. The PubMed search aimed at identifying experimental animal models evaluating clinical anastomotic leakage defined as fecal peritonitis or abscess formation. The Rex database was searched to identify textbooks on animal physiology and anatomy, and surgical aspects of experimental animals. Studies exclusively evaluating healing properties of anastomotic tissue were excluded. For the study selection process, see Figure 1.

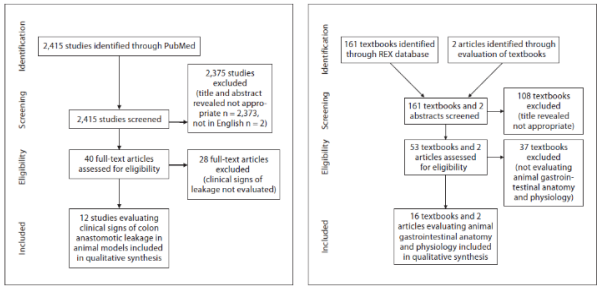

Figure 1
The study selection process (reproduced with permission from European Surgical Research)

Results

Models of clinical anastomotic leakage exist in the mouse, pig, rat, dog and rabbit. The rat is mostly used. However, extreme interventions, such as tissue trauma or a high dose of steroid, are needed to induce anastomotic leakage in this animal. In contrast, the rats may be well suited to evaluate healing properties of anastomotic tissue by methods such as breaking strength, bursting pressure and collagen content measurements. The mouse and

pig are considered the best suited animals to evaluate clinical leakage using technical insufficiency models. The pig is highly comparable to humans, both with respect to gastrointestinal physiology and anatomy. However, the pig model is less validated compared with the mouse model. The pig is also more expensive to use, and housing and surgical procedures are generally more complicated. The mouse is cheap and practical due to easy handling and maintenance, which makes it more suitable than the pig.

Conclusions

The mouse and the pig are considered the best suited animals for models of clinical colon anastomotic leakage created by technical insufficiency. However, the mouse may be preferred for various reasons. Validated models, such as the mouse model, should be used by researchers across centers to allow reproduction and comparison of results.

Limitations

This systematic review was conducted using only one database to identify studies. Other databases, such as Embase and Web of Science, may also have been relevant to use.

The review was conducted according to the PRISMA guidelines [32]. However, these guidelines were not adhered to in every detail. A formal bias assessment of individual studies and across studies were not performed. However, this may be difficult in experimental studies since scoring systems suitable for such studies are lacking. Furthermore, the method section in experimental studies is often insufficiently described, which makes it difficult to evaluate its quality. Reporting of PICO (population, intervention, comparison and outcome) as recommended in the PRISMA guidelines [32], was not done either. However, PICO seems less important when the included studies are not interventional. Moreover, we did not publish a review protocol although this is not mandatory in the guidelines.

The included studies were mostly non-validated models, meaning that the results may be different if the experiment is reproduced in a different setting. Moreover, most models were non-randomized without blinding of the investigator, which may introduce bias in the studies. However, we chose the best available studies and these limitations may be a result of the general study methodology in this field of research.

A large degree of heterogeneity was observed among the studies. The studies were very dissimilar with respect to designs, e.g. non-randomized vs. randomized, use of control group vs. no control group. Moreover, the technique used when constructing the anastomosis was not comparable between studies, e.g. resorbable vs. non-resorbable sutures and continuous vs. interrupted sutures. The place of the anastomosis was also different among the studies with the majority done on the anal part of the colon. Some studies even used additional interventions such as chemotherapy, antibiotic treatment or radiation. The definition of anastomotic leakage was not always clearly defined in the method section, thus it may have been different between the studies. Moreover, the detection of anastomotic leakage was frequently observer dependent and therefore subjected to bias. Considerable differences were noted among the studies with respect to the choice of measurements for healing properties e.g. breaking strength, bursting pressure and collagen measurements. Altogether, these limitations make results of the different studies difficult to compare.

In general, there are many translational limitations of experimental studies, since results obtained in animals are difficult to translate directly into the clinical setting. The use of clinical anastomotic leakage as outcome in the models may reduce this limitation. However, animals and humans are dissimilar on many aspects, e.g. animals used in experiments are often young and healthy compared to the clinical scenario of the old and sick patient with comorbidity. Moreover, little knowledge exists on animal intestinal physiology and anatomy. Altogether, this makes it difficult to compare experimental animals to human.

STUDY 2: COLON ANASTOMOTIC LEAKAGE: IMPROVING THE MOUSE MODEL

Aim

The aim of this study was to improve and validate a model of clinical anastomotic leakage in the technically insufficient colonic anastomosis in mice.

Methods

In this study, experimental anastomoses were performed on the ascending colon with different numbers of sutures in order to mimic the technically insufficient anastomosis. Sufficient anastomoses were compared with insufficient anastomoses, in which the number of sutures was reduced to create a suitable leakage rate. A total of 110 C57BL/6 mice were used in three pilot studies and two experiments in which analgesia, suture numbers and suture materials were modified throughout the studies. The pilot studies were carried out in order to determine how the control anastomosis, with an acceptable low leakage rate, should be created.

Pilot Study 1

In ten animals, the anastomoses were created with twelve 8-0 sutures. Subcutaneously administered Temgesic® was used as analgesia. Prolene® was used as suture material in five mice and Vicryl coated® was used in the remaining five mice.

Pilot Study 2

In six animals, the Pilot Study 1 was repeated. However, in this experiment subcutaneously administrated Rimadyl® (NSAID) was used as analgesia.

Pilot Study 3

In six animals, the Pilot Study 1 was repeated. However, in this experiment the suture material was changed to Vicryl coated®.

Experiment A

Forty-eight animals were randomized to either a twelve (control) or a five (intervention) suture anastomosis. As in the final pilot study, Vicryl coated® was used as suture material and Rimadyl® was used for analgesia.

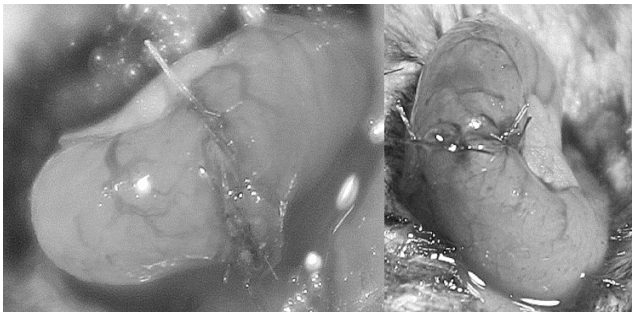

Figure 2
A mouse anastomosis using eight sutures (left picture) and four sutures (right picture), respectively (reproduced with permission from Surgery Today)

Experiment B
Forty animals were randomized to either an eight (control) or a four (intervention) suture anastomosis (Figure 2). In this experiment, voluntarily ingested Temgesic® mixed with Nutella® chocolate spread was used as analgesia and Vicryl coated® was used as suture material.

The primary outcome of this model was clinical anastomotic leakage measured as either abscess formation or fecal peritonitis. The animals were observed for seven days after surgery to evaluate the clinical condition, wellness score (see Table 1) and weight. Animals which were considered too ill were sacrificed and evaluated for signs of anastomotic leakage with re-laparotomy. At the end of the study period all animals were sacrificed and evaluated for signs of anastomotic leakage. The anastomotic breaking strength was determined using a material testing machine (LF+, Lloyd Instruments, Fareham, UK) with an XLC10n loading cell as described earlier [33]. The anastomosis with adjacent bowel was placed in two clamps and pulled apart (10 mm/min). The breaking strength was derived with software from a load-strain curve (Nexygen, Lloyd Instruments, Fareham, UK).

Fischer's exact test was used to compare the anastomotic leakage rates between the groups. Student's t-test was used to evaluate the differences in breaking strength, as the measurements were considered normally distributed. Friedman's test on differences was used to evaluate the course of wellness score and weight loss throughout the study period.

Parameter	Grading	Score
Activity	Normal/medium/low	2/1/0
Fur	Smooth/fluffy/erect	2/1/0
Eyes	Clean and open/clean and closed/dirty and closed	2/1/0
Able to stand straight	Yes/no	1/0
Posture	Normal/modestly curled/fully curled up	2/1/0
Position on feet	Normal/high	1/0
Solitary	Yes/no	0/1
Shivering	Yes/no	0/1

Table 1
Parameters examined for wellness score (reproduced with permission from Surgery Today)

Results

Pilot Study 1
In this study, large bowel obstruction was found in all animals. The animals quickly became very ill and all were sacrificed before post-operative day (POD) 4.

Pilot Study 2
None of the animals had large bowel obstruction and 33.3% had anastomotic leakage presented as small abscesses.

Pilot Study 3
None of the animals had large bowel obstruction and 33.3% had anastomotic leakage, of which 16.6% had abscess formation and 16.6% had fecal peritonitis.

Experiment A
In the control anastomoses (12 sutures), an anastomotic leakage rate of 25% was observed. Among the intervention group (technically insufficient anastomoses) with five sutures, a leakage rate of 67% was observed. This was significantly different compared with controls (p=0.008, Fisher's exact test).

Experiment B
In the control anastomoses (eight sutures), an anastomotic leakage rate of 0% was observed. An anastomotic leakage rate of 40% was observed in the intervention group (four sutures). This was significantly different compared with controls (p=0.003, Fisher's exact test).

The anastomotic breaking strength was not significantly different between the control [0.55 N ±0.09] and intervention anastomoses [0.49 N ±0.15], (p=0.091, Student t-test). The majority of the anastomoses broke in adjacent tissue (only 15.7% and 38.5% broke at the anastomotic line in the control group and intervention group, respectively), which was not different between the groups (p=0.219, Fisher's exact test). The wellness score was lower and the weight loss (Figure 3) was greater in animals with anastomotic leakage compared to the others (p<0.001, Friedman's test).

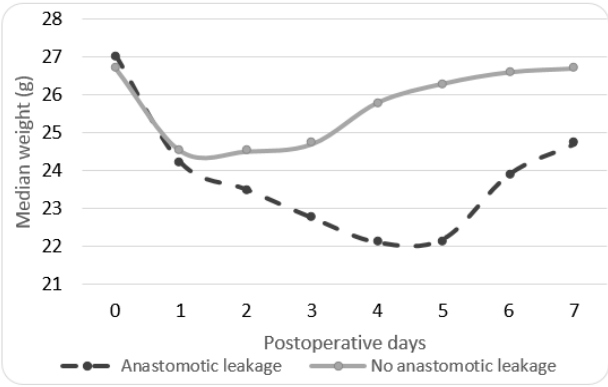

Figure 3
Median weight in animals with or without anastomotic leakage (reproduced with permission from Surgery Today)

Conclusions
The optimal model for creating a technically insufficient anastomosis in the mouse was found to be with four sutures in the

intervention group and eight sutures in the control group. Moreover, the optimal analgesic regimen was considered to include voluntarily ingested Temgesic® mixed with Nutella® chocolate spread. Absorbable sutures may be used instead of non-absorbable sutures making the experiments comparable to the clinical scenario of the interrupted suture anastomoses.

Limitations

The technical difficulties in Pilot Study 1 may have been responsible for the high large bowel obstruction rate. Too large suture bites when constructing the anastomoses may have led to anastomotic stenosis. On the other hand, the large bowel obstructions may have been a result of the subcutaneous Temgesic® regimen leading to paralytic ileus as an adverse effect.

In experiment A, the 12 sutures in the control anastomoses may have been the cause of the high anastomotic leakage rate. Theoretically, the 12 sutures may have induced focal ischemia due to the minimal space between the sutures. On the other hand, NSAIDs per se are suspected to increase the risk of anastomotic leakage [34]. In fact, when changing the suture number from five to four in the intervention group (from experiment A to B) the leakage rate was expected to increase. However, together with the discontinuation of the NSAID, the leakage rate was reduced instead. Thus, it seemed prudent to conclude that Rimadyl® may have had a compromising effect on the healing of the anastomosis.

There may be several explanations for the lack of difference in breaking strength between the sufficient and the insufficient anastomosis. All animals exhibiting signs of illness were removed from the experiment and therefore not included in the breaking strength evaluation. The majority of these animals came from the intervention group. Thus, the animals remaining in the intervention group were not representative for the group. In general, the anastomoses were covered with adhesions around the anastomotic line and these were difficult to remove in a reproducible manner due to the strength of these adhesions and the small size of the anastomosis in the mouse, which may have introduced bias in the experiment. Moreover, the majority of the anastomoses broke in adjacent tissue. Therefore, the breaking strength measurement could be considered a measurement of the integrity of healthy bowel, which may explain the lack of difference between the groups. There were technical difficulties when handling the small anastomosis. Despite using the smallest available instruments, the anastomosis was difficult to position in a reproductive manner in the testing machine. This also may have introduced bias in the experiment.

The wellness score was significantly lower in animals with anastomotic leakage. Thus, it may be a valid indicator of leakage in the animals. However, as with the breaking strength, it may not fully represent all the animals with anastomotic leakage as the very ill animals, with fecal peritonitis, were removed in the early phase of the experiments. Therefore, these animals did not contribute to the wellness score in the main part of the experiment. This might have been accounted for, by carrying forward the last observation of these animals, when performing the wellness score comparison analysis. However, despite this limitation the wellness score was sensitive enough to detect a reduced well-being in the remaining animals with leakage, which primarily may be due to the abscesses affecting the clinical condition.

The wellness score may be used to evaluate the well-being of the animals and to decide when to remove an animal from the experiment. A threshold of 4 or lower (12 represented a normal clinical condition) in the wellness score for removing animals was used by Komen et al. [27]. This may have been suboptimal in our setting, considering that the majority of very ill animals with fecal peritonitis had a wellness score of only 6-7. The animal caretakers, including the veterinarian at the institution, agreed that animals with this score should be removed from the experiments. This difference may be a result of the score being imprecise to evaluate animals with fecal peritonitis or it may be a result of us being inexperienced in using the wellness score. In general, it was uncomplicated to decide which animals should be removed from the experiments. The animals, at least the ones with fecal peritonitis, probably became ill due to a septic condition. As a result, the animals would lie still in the cage making very little effort to escape when being grabbed by the investigator. Therefore, the wellness score may be obsolete for this purpose, when selection of animals to be removed from the experiment can be done this easy, even for investigators with limited experience in handling animals. Although the wellness score may be a more objective method to remove animals from the experiment, in our experience, it was not well suited for this.

STUDY 3: IMPAIRED BLOOD SUPPLY IN THE COLONIC ANASTOMOSIS IN MICE COMPROMISES HEALING WITHOUT CREATING CLINICAL LEAKAGE

Aim

The aim of this study was to evaluate whether reduced blood supply in the colonic anastomosis may induce anastomotic leakage in mice.

Methods

In this experiment, we used 53 C57BL/6 mice in which a sufficient eight-suture anastomosis was created 1 cm distal to the caecum with Vicryl coated® extramucosal sutures. This was a procedure similar to the construction of the anastomoses in the control group of Study 2. In order to create ischemia and subsequent anastomotic leakage, different amounts of blood supply were removed from the anastomoses with bipolar coagulation of mesenteric vessels prior to creating the anastomoses (Figure 4).

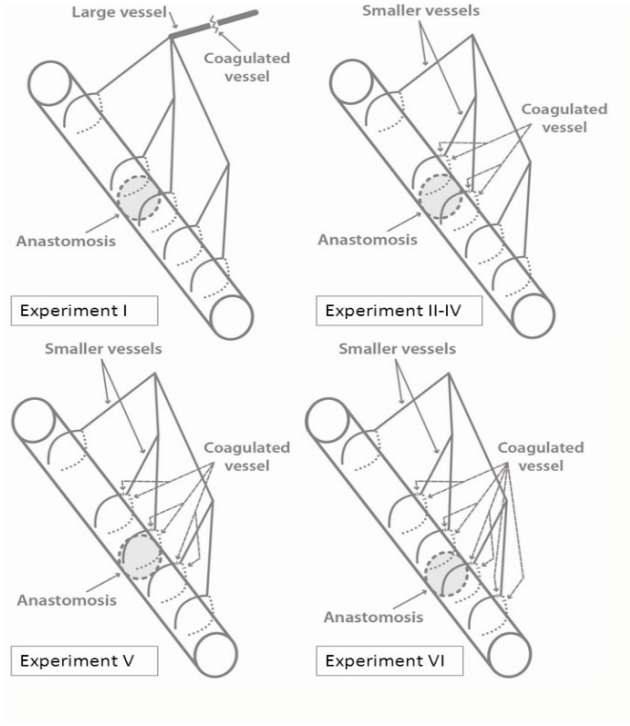

Figure 4
The anatomy of vessels in relation to the anastomotic area and the place of coagulation in the different experiments

The first three experiments were regarded as pilot studies aimed at determining the proper amount of ischemia to induce leakage. The number of coagulated vessel was increased if leakage was not achieved, whereas the number was decreased in case of large bowel obstruction (Table 2). The anastomotic breaking strength was measured in a similar manner as in Study 2 to evaluate anastomotic healing. Moreover, the control group from that study was used as reference when comparing leakage rates, large bowel obstructions rates and breaking strength.

To compare the number of animals with anastomotic leakage or large bowel obstruction between the groups, Fischer's exact test was used. Mann-Whitney's test was used to compare breaking strength (data not normally distributed). Friedman's test on differences was used to compare weight loss and wellness score between the groups.

Experiment	I	II	III	IV	V	VI
Number of mice	4	4	6	19	10	10
Number of vessels coagulated	5	2	2	2	3	4
Site of the anastomosis (cm distal to the cecum)	1	4	1	1	1	1

Table 2
Ischemic intervention and anastomotic site in the different experiments

Results
Summary of results is presented in Table 3.

Experiment	Control group (n=20)	I (n=4)	II (n=4)	III (n=6)	IV (n=19[c])	V (n=10)	VI (n=10)
Clinical leakage rate	0%	0%	**50% (p=0.022*)**	0%	10.5% (p=0.230*)	0%	0%
Large bowel obstruction rate	0%	**100 % (p<0.001*)**	**75% (p=0.002*)**	16.6% (p=0.231*)	5% (p=0.486*)	0%	20% (p=0.103*)
Breaking strength (Newton), median (range)	0.53 (0.40-0.73)	NR	NR	NR	**0.41 (0.36-0.59) (p=0.001[a])**	**0.45 (0.26-0.54) (p=0.009[a])**	**0.38 (0.26-0.46) (p<0.001[a])**
Wellness score	-	NR	NR	NR	→ (p=0.438[b])	→ (p=0.145[b])	→ (p=0.059[b])
Weight loss	-	NR	NR	NR	→ (p=0.397[b])	**↓ (p=0.025[b])**	→ (p=0.059[b])

Table 3
Results from the different experiments
NR: not recorded, significant results are in bold, *: Compared with controls (Fischer's exact test), a: Compared with controls (Mann-whitney test), b: Compared with controls (Friedman's test on differences), c: 20 mice were initially enrolled in this study, however one died during the operation as a result of a failure in the anaesthesia apparatus.

Experiment I (pilot) (n=4)
In this study, one large vessel branching into five smaller vessels was coagulated. All the mice had large bowel obstruction and none had anastomotic leakage.

Experiment II (pilot) (n=4):
In this experiment, two vessels were coagulated. However, in contrast to the other studies the anastomoses were created four centimeters distal to the caecum. In 75% of the animals, large bowel obstruction was observed and 50% had anastomotic leakage.

Experiment III (pilot) (n=6):
This experiment was a repetition of Experiment II (pilot) except for the place of the anastomosis (1 cm distal to the caecum). In total, 16.6% of the animals had large bowel obstruction, whereas none had anastomotic leakage.

Experiment IV (n=19):
This experiment was a repetition of Experiment III (pilot). In total, 10.5% of the animals had large bowel obstruction, whereas 5% had anastomotic leakage. The breaking strength was significantly lower in these animals compared with the control group. The wellness score and weight loss were not significantly different from the control group.

Experiment V (n=10):
This experiment was a repetition of Experiment IV, except that three vessels were coagulated instead of two. This resulted in a large bowel obstruction rate of 0% and anastomotic leakage rate of 0%. The breaking strength was significantly lower in these animals and the weight-loss was significantly higher in this group compared with controls.

Experiment VI (n=10):
This experiment was a repetition of Experiment IV, except that four vessels were coagulated instead of two. This resulted in a large bowel obstruction rate of 20% and an anastomotic leakage rate of 0%. Again, the breaking strength was significantly lower

compared with controls. However, weight loss and wellness score were not different compared with controls.

Conclusions

The reduced blood supply to the colonic anastomosis in mice led to large bowel obstruction and not to anastomotic leakage. However, a relationship between reduced blood supply and reduced breaking strength was evident in this experiment.

Limitations

The lack of anastomotic leakage may be due to the large bowel obstruction in itself. The animals had large bowel obstruction as a result of stenosis in the anastomosis, most likely as a result of tissue ischemia. As the anastomoses were stenotic, no feces were passing the anastomotic line making leakage, at least fecal peritonitis, impossible.

The results from the control group were used from a previous experiment (Study 2). However, this decision was not considered a problem for the study design. The control group was merely used as a comparative reference to evaluate if the reduced blood supply would alter the normal conditions. A new control group would have been introduced at the next level of the model in a randomized setup, if a correlation between ischemia and leakage was found. However, at this point it was considered unethical.

The results may be due to lacking comparability between animals and humans. The experimental animals are young and healthy, compared to old and frail patients, and may generally have a greater ability for neoangiogenesis compared to patients, who may have universal atherosclerosis and co-morbidities. Possibly, the bipolar coagulation may have been done incompletely resulting in reperfusion of the vessels, which may explain the difference between Experiment I and VI, in which a comparable amount of ischemia was induced with completely different outcomes.

As previously stated, anastomotic leakage is a multifactorial condition and therefore pure ischemia may be too simple an approach. When coagulating vessels macrovascular ischemia (necrosis) is induced. However, this may not be comparable to the microvascular disease seen in some humans, which is correlated to anastomotic leakage [35].

In humans, anastomotic leakage generally occurs between day 5 and 8 [36]. However, one study found it to occur at a mean of 12 days after surgery, in which case our model may have been terminated prematurely [37].

In our study, no histological evaluation was performed. Such an evaluation may have offered an explanation for the reduced breaking strength in the ischemic anastomosis and clarified how and if the healing was affected in these anastomoses.

Another limitation was that the place of disruption in the anastomosis was not recorded and therefore we did not know how many anastomoses broke in adjacent tissue.

STUDY 4: EXTERNAL COATING OF COLONIC ANASTOMOSES: A SYSTEMATIC REVIEW

Aim

The aim of this study was to evaluate different coating materials as external coating of colonic anastomoses.

Methods

This study was a systematic review conducted according to the PRISMA guidelines [32]. The databases PubMed, Embase and Cinahl were searched up to September 2011 to identify human or experimental studies evaluating external coating of colonic anastomoses. For the study selection process, see Figure 5. The outcome measures in these studies should be either clinical leakage or indirect measures of reduced anastomotic healing such as breaking strength, bursting pressure, collagen measurements or histological evaluation.

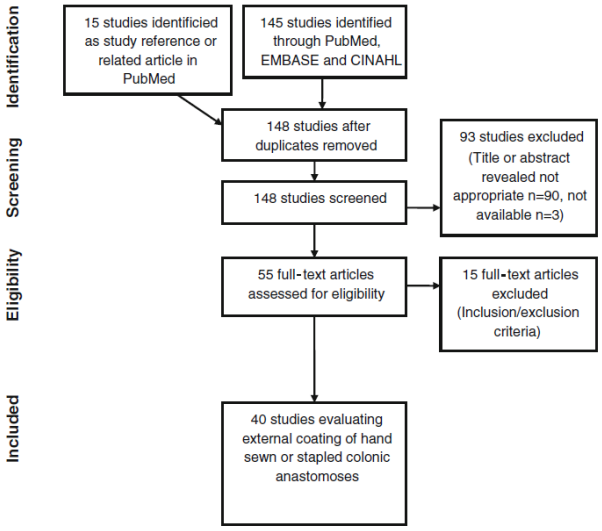

Figure 5

The study selection process (Reproduced with permission from Colorectal Disease).

Results

The majority of the studies were experimental and only fibrin sealant, omental pedicle graft and hyaluronic acid/carboxymethylcellulose were evaluated in humans.
For the human studies, evaluation of omental pedicle graft showed no protective effect against anastomotic leakage. The leakage reduction in humans obtained with fibrin sealant was not significant. However, this may have been a result of too few patients included in the specific study. Hyaluronic acid/carboxymethylcellulose resulted in an increased risk of anastomotic leakage and should therefore be avoided.

The results from the experimental studies are mostly contradictory. When evaluating the same material in a different experimental setting or in different animal species, results are not reproducible and validation of positive results from single studies has generally not been attempted. Despite this, coating with Tachosil®, fibrin sealant and polyethylene glycols (PEGs) show positive results. The experimental studies primarily involved rats in which indirect measures of anastomotic leakage were used to evaluate risk of leakage. However, in contrast to anastomotic coating in humans, mice and pigs, coating in itself may increase the leakage rate in rats.

Conclusions

The evidence is mostly based on experimental studies with non-comparable designs and contradictory results. Validated models with similar design and animal species should be used across research centers in order to make results comparable. Experimental coating with Tachosil®, fibrin sealant and polyethylene

glycols (PEGs) show promising effects. However, these results need confirmation from other studies. Human studies are needed to determine if anastomotic coating is a valuable technique for reducing the leakage rate in the clinical setting.

Limitations

As in Study 1, the PRISMA guidelines were not completely adhered to. Bias assessments of the specific studies and across studies were not performed. However, this may be difficult in experimental studies, as a suited grading system is lacking and the method section of the articles often are insufficiently described. With respect to human studies, a formal quality assessment may have been done. Bias assessment across studies may have been of particular importance in this review, as it may be subjected to substantial publication bias, especially since negative animal studies may be difficult to publish. Thus, the published studies may give a biased analysis, which over-estimates a positive effect of coating. As with Study 1, we did not report PICO and did not publish a review protocol.

Another limitation of this review was that the studies were mainly experimental, which carries the translational limitations as described earlier. There was major heterogeneity among the studies, mainly due to use of different experimental designs, outcomes, surgical techniques and animal species.

5. DISCUSSION

In the studies included in this thesis, we found that mice and pigs may be the preferred animal species to evaluate clinical anastomotic leakage compared with the commonly used rat. On the other hand, the rat is well suited for evaluation of healing in the anastomosis.

We found that it was possible to optimize the model of clinical anastomotic leakage in mice resembling insufficient surgical technique. A relevant analgesic regimen without adverse effects was identified and the optimal number of sutures was determined. Moreover, the use of absorbable suture material was adopted into the model, which more closely resembles the clinical scenario for hand sewn interrupted suture anastomoses.
We found that reduced blood supply creates large bowel obstruction in the mouse unrelated to anastomotic leakage. However, a relationship between reduced blood supply and decreased breaking strength was found.

Evaluating the literature it seems that anastomotic coating may reduce the leakage rate. However, the evidence is scarce and mainly based on experimental studies with contradictory results. Moreover, the studies are difficult to compare since they use different study designs, outcome assessments, animal species and surgical techniques.

THE THEORY BEHIND COLORECTAL ANASTOMOTIC LEAKAGE

The direct pathophysiological mechanisms responsible for anastomotic leakage remain unknown. Some patient related and procedure related risk factors have been determined. However, the most important risk factors remain uncertain. Studies have shown that several patient related risk factors may exist.

Among these are gender, where males seem to have a higher risk of leakage [34, 38-41]. Men have more chronic diseases [42] and a greater tendency to coronary heart disease [43]. This disease may be associated with peripheral and visceral microvascular disease, which has been shown to increase the risk of anastomotic leakage [35]. Moreover, men may have an increased risk of anastomotic leakage as a result of technical difficulties for the surgeon due to a narrow pelvis, at least for the rectal anastomoses [44, 45]. It has been proposed that the amount of intra-abdominal fat is higher in men, which in theory may increase technical difficulties. However, a study found that the amount of intra-abdominal fat was not different between genders [46]. Regarding the rectal anastomoses, several studies found that a low level anastomosis carries an increased risk of leakage compared with a high level anastomosis [38, 41, 47-49]. This may be due to insufficient vascularization in the lower two thirds of the rectum [50]. Furthermore, there may be technical difficulties in the lower pelvis [45]. Lastly, the lack of serosa covering of the lower one third of the rectum, possibly make these anastomoses more prone to leakage [13]. In contrast to the part of the rectum covered with peritoneum, these anastomoses lack the outermost tissue layer, which may reduce the strength of the anastomosis. Some studies have shown that a high ASA (American Society of Anesthesiologists) class [34, 41, 51] may be associated with an increased risk of anastomotic leakage, possibly due to an increased comorbidity rate in these patients, which may impair tissue perfusion and function.

Blood transfusion [34, 40] has been proposed as a risk factor for anastomotic leakage as well. This may serve as a surrogate marker for complicated surgery. Moreover, studies have shown that transfusion in itself may lead to an increased rate of surgical complications as a result of transfusion related immunomodulation (TRIM) [52].

Perioperative radiotherapy has also been shown to increase the risk of anastomotic leakage [38]. This may be a result of reduced healing and increased tissue fibrosis, i.e. micro vascular changes [53].

Furthermore, it has been shown that perioperative use of NSAIDs, and in particular cyclooxygenase (COX)-2 specific antagonists, may be related to anastomotic leakage [34, 54, 55]. This may be due to reduced collagen content and reduced healing properties as a result of inhibition of the COX-2 enzyme [56-58]. Together, this emphasizes that the healing of anastomotic tissue is an important factor related to anastomotic leakage.

While many risk factors for anastomotic leakage have been identified, the true pathophysiological mechanisms behind the factors remain unknown. Most likely, anastomotic leakage is a multifactorial condition with many contributing factors. Animal models, in which single factors such as ischemia are evaluated, may therefore be too simple an approach. This may explain the negative results of Study 3.

EXTRAPOLATION OF EXPERIMENTAL RESULTS TO THE CLINICAL SETTING

In general, it is difficult to extrapolate results from experimental studies to humans due to various reasons. Animals differ from humans with respect to both anatomy and physiology, which makes a specific condition in humans difficult to mimic in animals. It seems that pigs are the most comparable of available animals for surgical experiments. With exception of the spiral colon, which is a unique structure in the pig consisting of the caecum and the ascending colon coiled together, the intestinal physiology and anatomy are highly comparable to humans [59-61]. To a lesser extent, rats and mice are comparable as well [62-65]. The animals used in the models are typically young and healthy, whereas the patients suffering from anastomotic leakage often are old and frail with comorbidity, which makes this comparison difficult. This problem is clearly visualized in animal models where

leakage in a normal/sufficient anastomosis seldom occurs [25, 27, 66]. Therefore, pathophysiological mechanisms must be simulated in animal models in order to create suitable leakage rates [27, 66] and to prevent the sample size from becoming too high to produce an ethically sound experiment with a reasonable cost and duration.

In contrast to mice and pigs, it seems that the rat is not comparable to humans with respect to the clinical response to an insufficient anastomosis. Thus, extreme conditions are needed for the rat anastomosis to leak [67, 68] and, in contrast to the mouse, technical insufficiency is ineffective to create leakage [25]. The rats' higher resistance to infection and/or a more efficient intra-abdominal immune system may explain this [27, 69]. Other researchers have proposed that the fecal consistency in the anal part of the colon in rats, is too high to allow clinical leakage [25]. However, one rat study with insufficient anastomoses done on a more oral part of the colon (transverse colon) still did not result in anastomotic leakage [70]. Moreover, the rationale that the rat may have a more efficient immune system may be supported by the fact that steroid treatment can induce anastomotic leakage in rats [68]. Furthermore, the fact that anastomotic coating may increase the risk of anastomotic leakage may also imply that the intra-abdominal immune system is very strong in the rat. Thus, coating may prevent the intra-abdominal immune system from resolving smaller leakages by e.g. adhesion formation [68, 70]. In addition to the previously described drawbacks of the rat model, this response to coating is not comparable to that of humans [71].

A model of total colectomy has been developed in the pig in which an experimental ileocolic anastomosis was done with a nitinol compression device [72]. This model is interesting as a total colectomy was performed, which more closely resembles the surgical insult in humans compared with an anastomosis created merely on transected bowel. However, the lack of anastomotic leakage and the use of the nitinol compression device limit comparability to the clinical scenario.

THE TECHNICAL INSUFFICIENCY MODEL

The technical insufficiency model created in Study 2 may resemble insufficient surgical technique. However, to a lesser extent it may also be suited to resemble focal anastomotic breakdown because of tissue ischemia/necrosis at the anastomotic line. In both situations, anastomotic coating may be a relevant intervention option.

Initially, the model in the mouse was introduced by Komen et al. [27], who repeated the experiment three times to validate the results. We aimed at improving the model by Komen et al. by introducing a suitable analgesic regimen and by using absorbable instead of non-absorbable sutures. While conducting our experiments the model by Pantelis et al. [66] was published. Their results were in line with ours, suggesting a reduction in the number of sutures in the control as well as intervention anastomoses. Our results along with the results by the two other studies [27, 66], probably makes this model the most validated experimental model of colon anastomotic leakage.

It was difficult to determine whether the analgesic regimen, the suture number (12 vs. 8), or the suture material was responsible for the initial high leakage rate in the control anastomoses in our Study 2. In the study by Komen et al., there was a very low leakage rate of the 12 suture (control) anastomoses. Since Komen et al. did not report the use of analgesic, it might have been our use of NSAID that resulted in the high leakage rate. When changing the suture number in the control anastomoses from 12 to eight and discontinuing NSAID, the leakage rate was reduced. The fact that the leakage rate in the intervention anastomoses was reduced as well, even though the number of sutures was reduced from five to four, indicates that the used NSAID (Rimadyl®) was a cause for the high leakage rate. As discussed earlier, NSAIDs may increase the leakage rate due to reduced healing [56-58]. Thus, Temgesic® may be superior for an animal anastomosis model. When administered orally (mixed with Nutella®), using a validated method for analgesia in the mouse [73, 74], no adverse effects were observed. Thus, the resulting large bowel obstruction in the initial part of the study when Temgesic® was administered subcutaneously may either be a product of a too high dose or a suboptimal surgical technique. It has been shown that the serum concentration in mice receiving voluntarily ingested Temgesic® is more constant compared with when Temgesic® is given subcutaneously [74]. It is known that opioids (e.g. Temgesic®) may contribute to postoperative ileus [75, 76]. However, at that early point in the experiment the anastomoses were not evaluated to conclude whether the large bowel obstruction was a result of anastomotic stenosis. This would have been visualized by a dilation only orally to the anastomosis together with a lack of fecal passage.

The suture material and number of sutures may also have been a factor contributing to the high leakage rates in the control group initially. It has been shown that the anastomotic tissue surrounding sutures has increased collagenolysis (collagen breakdown), which may lead to decreased anastomotic strength [24]. The inflammatory reaction in proximity to the sutures is less for Prolene® compared with Vicryl® [77]. Thus, increased inflammation, in addition to focal ischemia, may have led to increased risk of leakage in the 12 suture anastomoses compared with the eight suture anastomoses. Despite these results, the tissue reaction to Vicryl® is still very limited [78]. Moreover, the use of Vicryl® is comparable to the clinical scenario, where surgeons often use this material when constructing a hand sewn interrupted suture anastomosis. Finally, we showed that control anastomoses with eight sutures led to a leakage rate of 0%, and therefore Vicryl® is considered suited to use in the mouse model.

ISCHEMIA AS A RISK FACTOR FOR ANASTOMOTIC LEAKAGE IN ANIMAL MODELS

As shown in Study 3, it seems that pure ischemia, as induced by compromising blood supply, may result in obstruction/stenosis in the anastomosis. Other studies have found similar results. In a study in pigs, compromised blood supply to the colonic anastomosis resulted in a colonic obstruction rate of 75% [28]. The same tendency has been shown in rats, where ischemia resulted in a colonic obstruction rate of 46% [29]. Therefore, pure ischemia/anoxia may be too simple an approach to use in a model. On the other hand, ischemia may impair healing, as shown in our study by reducing breaking strength. This finding is supported by a study in rats, where ischemia resulted in reduced healing [30]. Pure ischemia failed to create anastomotic leakage, possibly since the scenario is not comparable to the human condition. In humans, it seems that microvascular disease plays a major role for anastomotic leakage [35], which may explain why macrovascular ischemia/total anoxia is not an optimal model. In microvascular disease, tissue perfusion/oxygenation may be impaired but is not completely absent. This may reduce the healing and subsequently create anastomotic leakage, whereas total anoxia may have different effects on the tissue, such as stenosis.

The clinical scenario of microvascular disease may be difficult to produce in animal models. However, genetically modified mice prone to arteriosclerosis may be used as models. Alternatively, to demonstrate the multifactorial causality of anastomotic leakage, ischemia may be used in combination with other risk factors such as technical insufficiency, steroid treatment or irradiation.

Even though ischemia have failed to induce anastomotic leakage in models, the issue is still very relevant in the clinical scenario. As explained earlier, tissue oxygen tension in the anastomotic tissue is a predictor of anastomotic leakage in patients, where low oxygen tension increases the risk of anastomotic leakage [14]. This has also been shown in the experimental setting [15]. Furthermore, it was shown that experimental hypovolemia in rabbits reduced tissue oxygenation and subsequently reduced collagen content [14]. This implies that tissue perfusion and thereby oxygenation may be of major importance for anastomotic healing.

EVALUATION OF HEALING IN THE COLORECTAL ANASTOMOSIS

Reduced healing of anastomotic tissue is considered an important risk factor for anastomotic leakage [13]. Thus, improved healing may subsequently reduce the risk of anastomotic leakage. It is known that the anastomotic tissue is weakest on day 3-5 after surgery, which is a time where collagen degradation dominates over synthesis and the time where the suture holding capacity of the anastomosis is lowest [33, 79]. The activity of matrix metalloproteinases (MMPs), which are involved in the collagen degradation, may be related to the strength of the anastomotic tissue. It has been shown that collagenolysis is increased in anastomotic areas compared with non-resected tissue [80]. Moreover, the levels of the MPPs are highest after three days corresponding to the lowest anastomotic strength at that time [81]. A prospective clinical study involving 119 patients showed that the MMP activity was increased in anastomotic tissue of patients with anastomotic leakage [82]. Furthermore, it has been shown that tissue ischemia increased the activity of MMPs [83]. In experimental studies aiming at improving the anastomotic healing, it has been shown that inhibition of MMPs improved healing of colonic anastomotic tissue [18, 79]. Altogether, it seems that activity of MMPs is important for healing of anastomotic tissue and that inhibiting of these may reduce the risk of leakage. However, to our knowledge, clinical studies evaluating the effect of such MMP inhibitors are still lacking.

Measures such as bursting pressure and breaking strength may be used to evaluate the healing capacity of anastomotic tissue. Bursting pressure is dependent on the diameter of the bowel. Instead, bursting wall tension, which is calculated on the basis of bowel diameter, may be used [84]. However, this diameter may be difficult to measure accurately. Anastomotic bursting pressure may be considered a parameter for anastomotic integrity measuring e.g. degree of adhesions, focal necrosis or technical insufficiency and may not be a direct measurement of the strength of the tissue [85]. Conversely, breaking strength is hypothesized to measure the suture holding capacity of the peri-anastomotic tissue. It is argued that breaking strength is better suited to evaluate healing compared with bursting pressure [85]. However, bursting pressure is a more sensitive measure in the early phase [86] and only after post-operative day (POD) 4, bursting pressure and breaking strength are correlated [85]. Thus, breaking strength may only be a suited measure to evaluate healing after POD 7 [87]. The relevance of bursting pressure and breaking strength are limited when disruption does not occur at the anastomotic line. In a study, in 54% and 42% of the cases for breaking strength and bursting pressure, respectively, the disruptions occurred in adjacent bowel [85]. These results are comparable to our results in Study 1, where the majority of anastomoses broke in adjacent tissue. When evaluating bursting pressure larger forces are applied to the tissue adjacent to the anastomosis due to a larger diameter compared with the anastomotic site [84]. This might explain why the anastomoses tend to disrupt in adjacent tissue and why this measure is not fully representative for the bursting pressure of the anastomosis. The results from our breaking strength measurements may be affected by the degree of adhesions to the anastomosis. If insufficiently removed, these strong adhesions to the anastomotic line may partly be responsible for the break in adjacent tissue. However, they were difficult to remove in a reproducible manner without damaging the anastomosis and therefore may have been insufficiently removed. Anastomotic bursting pressure is normally measured “in situ”, which is considered a strength. This is not possible when evaluating anastomotic breaking strength in which the anastomosis and the adjacent bowel need to be removed from the animal. Bursting pressure and breaking strength have mostly been used for evaluation of anastomotic healing in rats and the mouse may be less suited to evaluate this. The bowel in the mouse is smaller compared with the rat, which may introduce a number of possible biases as explained earlier.

COATING OF COLORECTAL ANASTOMOSES

Coating of colorectal anastomoses seems an alluring interventional strategy. However, it may hold potential problems. Foreign material introduced into the human body may promote abscess formation as the material isolates a potentially contaminated suture line from the peritoneal surface. As seen in experimental studies, anastomotic coating in rats may increase the leakage rate [68, 70]. However, the similar problem is not seen in humans [71].

In theory, a liquid sealant may be superior to a solid sealant, since the liquid may seal smaller defects more tightly. Fibrin sealant has been widely tested [25, 30, 67, 68, 71, 88-91]. However, studies are mostly experimental and clinical evaluation has failed to show a significant effect [71]. Even though not being liquid, Tachosil® seems like a promising coating material [66, 92, 93]. The product is a combination of fibrinogen, thrombin and a collagen sponge. Being flexible, the collagen sponge may induce a more tight sealing without compromising the mobilization of the bowel. Thus, this material may, at least in theory, be superior to fibrin sealant. Two experimental studies found positive effects [66, 92], supporting Tachosil® as a promising coating agent. A novel study in humans has shown positive results, which implies that Tachosil® can be used safely and be easily applied even in laparoscopic colorectal surgery [93]. Other promising coating agents may be polyethylene glycols (PEG’s), which are highly flexible liquid gels that are fully absorbed and excreted through the kidneys. However, to our knowledge, only one experimental study exists, in which a promising effect was found [94] and further studies are needed prior to initiation of clinical trials.

6. CONCLUSION

This thesis has clarified the best suited animal to model clinical colon anastomotic leakage in humans. Moreover, we successfully improved the model of surgical technical insufficiency by determining a relevant analgesic regimen, suture number and suture material. Pure ischemia/anoxia may be too simple a measure to induce clinical leakage in animal models. However, ische-

mia/anoxia seems to impair anastomotic healing. We found that external coating of colorectal anastomoses may be a feasible solution to reduce anastomotic leakage, but experimental evaluation of higher quality and large clinical studies are needed before general clinical use.

7. FUTURE PERSPECTIVES

To elaborate on the experimental research area of anastomotic leakage, the multifactorial origin of this complication may be mimicked in models by using genetically modified animals to mimic chronically ill patients or by combining different pathophysiological mechanisms in models. A future model may possibly combine technical insufficiency and ischemia to mimic more closely the clinical scenario in humans. Alternatively, the existing model could be combined with other risk factors such as steroid treatment, NSAIDs or other medications.

Generally, it seems relevant to identify risk factors for anastomotic leakage in patients. Knowledge on such risk factors may aid the surgeon to select patients not suited for primary anastomosis. However, there is lack of consensus in the literature regarding the most important risk factors for anastomotic leakage and studies are needed to clarify this. Such studies could be large cohort studies, database studies or systematic reviews to quantify the body of evidence on the subject. However, this knowledge may not necessarily clarify pathophysiological mechanisms and experimental research is still important.

A novel technique to reduce the risk of leakage may be the use of indocyanine green fluorescence to evaluate bowel perfusion prior to performing the anastomosis [95]. The proper place on the bowel for the anastomosis may be selected based on an evaluation of tissue perfusion. This may secure proper oxygenation to the anastomosis and thereby reduce the risk of leakage. However, this has only been evaluated in a small selected patient material [95] and larger studies are needed.

A clinical evaluation of the efficacy of colorectal anastomotic coating is highly relevant but needs a large study setup. In order to detect a clinically relevant reduction in the anastomotic leakage rate (e.g. 50 %), a large sample size of approximately 800-1400 patients may be needed. Thus, a large multicenter randomized controlled trial is needed, which requires cooperation between centers as well as substantial financial support. Materials such as fibrin glue, Tachosil® or PEG's could be evaluated. The included patients may be receiving colonic or rectal anastomoses for malignant and/or benign disease. Such a study may be a result of what is practically possible. However, to give a definitive answer to the question of whether or not coating should be used, a sufficient number of patients should be included.

8. SUMMARY

Colorectal anastomotic leakage remains a frequent and serious complication in gastrointestinal surgery. Patient and procedure related risk factors for anastomotic leakage have been identified. However, the responsible pathophysiological mechanisms are still unknown. Among these, ischemia and insufficient surgical technique have been suggested to play a central role. Animal models are valuable means to evaluate pathophysiological mechanisms and may be used to test preventive measures aiming at reducing the risk of anastomotic leakage, such as external anastomotic coating.

The aim of this thesis was to:

Clarify the best suited animal to model clinical anastomotic leakage in humans

Create animal models mimicking anastomotic leakage in humans induced by insufficient surgical technique and tissue ischemia

Determine the best suited coating materials to prevent anastomotic leakage

Study 1:

This study is a systematic review using the databases Medline and Rex. Medline was searched up to October 2010 to identify studies on experimental animal models of clinical colon anastomotic leakage. From the Rex database, textbooks on surgical aspects as well as gastrointestinal physiology and anatomy of experimental animals were identified. The results indicated that the mouse and the pig are the best suited animals to evaluate clinical anastomotic leakage. However, the pig model is less validated and more costly to use compared with the mouse. Most frequently, rats are used as models. However, extreme interventions are needed to create clinical leakage in these animals. The knowledge from this study formed the basis for selecting the animal species most suited for the models in the next studies.

Study 2:

In this experimental study, technically insufficient colonic anastomoses were performed in 110 C57BL/6 mice. The number of sutures in the intervention group was reduced to produce a suitable leakage rate. Moreover, the analgesia and suture material were changed in order to optimize the model. In the final experiment, the four-suture anastomoses resulted in a 40% leakage rate in the intervention groups, whereas the eight-suture control anastomoses had a 0 % leakage rate. Furthermore, the use of absorbable suture together with voluntarily ingested Temgesic® in chocolate spread as analgesic regimen were feasible. This model may be used to test the leakage reducing potential of coating materials.

Study 3:

This experimental study used 53 C57BL/6 mice, in which sufficient eight-suture anastomoses were created. By using bipolar electrocautery, blood supply was reduced in a stepwise manner to create anastomotic leakage as a result of ischemia. The study showed that reduced blood supply led to large bowel obstruction instead of clinical leakage. However, anastomotic breaking strength was reduced in the ischemic anastomoses.

Study 4:

In this systematic review Medline, Embase and Cinahl were searched up to September 2011 to identify studies evaluating external coating of colonic anastomoses. Most studies were experimental, in which designs were not comparable and many results were contradictory.

In a clinical study, a non-significant benefit of fibrin sealant was found. Based on the available clinical and experimental data it was concluded that the fibrin-based sealants, such as Tisseel® and Tachosil®, and polyethylene glycols may be beneficial. However, further experimental and clinical studies are needed before routine clinical use can be recommended.

Discussion

The studies in this thesis may be valuable for the experimental research field of clinical anastomotic leakage. The model of technical insufficiency has been improved and is now thoroughly validated. If used by researchers worldwide, comparison of results is possible. Pure ischemia/anoxia may be too simple an approach to create a clinical leakage model. Thus, future models could focus on multiple risk factors. Conclusively, large-scale clinical multicenter studies are needed to definitively evaluate whether coating of colorectal anastomoses may reduce the leakage rate.

9. REFERENCES

1. Peel AL, Taylor EW. Proposed definitions for the audit of postoperative infection: a discussion paper. Surgical Infection Study Group. Ann R Coll Surg Engl 1991;73:385-8.
2. Bruce J, Krukowski ZH, Al-Khairy G et al. Systematic review of the definition and measurement of anastomotic leak after gastrointestinal surgery. Br J Surg 2001;88:1157-68.
3. Nationwide database for cancer of the colon and rectum - yearly report: Danish Colorectal Cancer Group; 2010 [6. March 2013]. Link: http://dccg.dk/03_Publikation/02_arsraport_pdf/aarsrapport_2010.pdf.
4. Akasu T, Takawa M, Yamamoto S et al. Risk factors for anastomotic leakage following intersphincteric resection for very low rectal adenocarcinoma. J Gastrointest Surg 2010;14:104-11.
5. Boccola MA, Buettner PG, Rozen WM et al. Risk factors and outcomes for anastomotic leakage in colorectal surgery: a single-institution analysis of 1576 patients. World J Surg 2011;35:186-95.
6. Borowski DW, Bradburn DM, Mills SJ et al. Volume-outcome analysis of colorectal cancer-related outcomes. Br J Surg 2010;97:1416-30.
7. Goriainov V, Miles AJ. Anastomotic leak rate and outcome for laparoscopic intra-corporeal stapled anastomosis. J Minim Access Surg 2010;6:6-10.
8. Thornton M, Joshi H, Vimalachandran C et al. Management and outcome of colorectal anastomotic leaks. Int J Colorectal Dis 2011;26:313-20.
9. Mirnezami A, Mirnezami R, Chandrakumaran K et al. Increased local recurrence and reduced survival from colorectal cancer following anastomotic leak: systematic review and meta-analysis. Ann Surg 2011;253:890-9.
10. Smith JA, King PM, Lane RH et al. Evidence of the effect of 'specialization' on the management, surgical outcome and survival from colorectal cancer in Wessex. Br J Surg 2003;90:583-92.
11. Archampong D, Borowski D, Wille-Jorgensen P et al. Workload and surgeon's specialty for outcome after colorectal cancer surgery. Cochrane Database Syst Rev 2012;3:CD005391.
12. Fielding LP, Stewart-Brown S, Blesovsky L et al. Anastomotic integrity after operations for large-bowel cancer: a multicentre study. Br Med J 1980;281:411-4.
13. Thornton FJ, Barbul A. Healing in the gastrointestinal tract. Surg Clin North Am 1997;77:549-73.
14. Sheridan WG, Lowndes RH, Young HL. Tissue oxygen tension as a predictor of colonic anastomotic healing. Dis Colon Rectum 1987;30:867-71.
15. Shandall A, Lowndes R, Young HL. Colonic anastomotic healing and oxygen tension. Br J Surg 1985;72:606-9.
16. Foster ME, Laycock JR, Silver IA et al. Hypovolaemia and healing in colonic anastomoses. Br J Surg 1985;72:831-4.
17. Kiyama T, Tajiri T, Tokunaga A et al. Tacrolimus enhances colon anastomotic healing in rats. Wound Repair Regen 2002;10:308-13.
18. Agren MS, Andersen TL, Andersen L et al. Nonselective matrix metalloproteinase but not tumor necrosis factor-alpha inhibition effectively preserves the early critical colon anastomotic integrity. Int J Colorectal Dis 2011;26:329-37.
19. da Costa MA, Campos AC, Coelho JC et al. Oral glutamine and the healing of colonic anastomoses in rats. JPEN J Parenter Enteral Nutr 2003;27:182-5; discussion 5-6.
20. Netta S, Michalopoulos A, Apostolidis S et al. Enhancement of colonic anastomotic strength in rats by short-chain fatty acids. Tech Coloproctol 2010;14 Suppl 1:S53-5.
21. Cakmak GK, Irkorucu O, Ucan BH et al. The effects of resveratrol on the healing of left colonic anastomosis. J Invest Surg 2009;22:353-61.
22. Christensen H, Oxlund H. Growth hormone increases the collagen deposition rate and breaking strength of left colonic anastomoses in rats. Surgery 1994;116:550-6.
23. Egger B, Inglin R, Zeeh J et al. Insulin-like growth factor I and truncated keratinocyte growth factor accelerate healing of left-sided colonic anastomoses. Br J Surg 2001;88:90-8.
24. Agren MS, Andersen TL, Mirastschijski U et al. Action of matrix metalloproteinases at restricted sites in colon anastomosis repair: an immunohistochemical and biochemical study. Surgery 2006;140:72-82.
25. Van der Ham AC, Kort WJ, Weijma IM et al. Transient protection of incomplete colonic anastomoses with fibrin sealant: an experimental study in the rat. J Surg Res 1993;55:256-60.
26. Nordentoft T, Sorensen M. Leakage of colon anastomoses: development of an experimental model in pigs. Eur Surg Res 2007;39:14-6.
27. Komen N, van der Wal HC, Ditzel M et al. Colorectal anastomotic leakage: a new experimental model. J Surg Res 2009;155:7-12.
28. Hoeppner J, Crnogorac V, Hopt UT et al. The pig as an experimental model for colonic healing study of leakage and ischemia in colonic anastomosis. J Invest Surg 2009;22:281-5.
29. Posma LA, Hendriks T, Verhofstad AA et al. Reduction of oxygenation and blood flow in pedicled bowel segments in the rat and its consequences for anastomotic healing. Dis Colon Rectum 2010;53:93-100.
30. Van der Ham AC, Kort WJ, Weijma IM et al. Healing of ischemic colonic anastomosis: fibrin sealant does not improve wound healing. Dis Colon Rectum 1992;35:884-91.
31. Hau J, Van Hoosier Jr. GL. handbook of laboratory animal science: CRC Press; 2003.
32. Moher D, Liberati A, Tetzlaff J et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. J Clin Epidemiol 2009;62:1006-12.
33. Agren MS, Andersen L, Heegaard AM et al. Effect of parenteral zinc sulfate on colon anastomosis repair in the rat. Int J Colorectal Dis 2008;23:857-61.
34. Klein M, Gogenur I, Rosenberg J. Postoperative use of non-steroidal anti-inflammatory drugs in patients with anastomotic leakage requiring reoperation after colorectal resection: cohort study based on prospective data. BMJ 2012;345:e6166.
35. Fawcett A, Shembekar M, Church JS et al. Smoking, hypertension, and colonic anastomotic healing; a combined clinical and histopathological study. Gut 1996;38:714-8.

36. Daams F, Luyer M, Lange JF. Colorectal anastomotic leakage: aspects of prevention, detection and treatment. World J Gastroenterol 2013;19:2293-7.
37. Hyman N, Manchester TL, Osler T et al. Anastomotic leaks after intestinal anastomosis: it's later than you think. Ann Surg 2007;245:254-8.
38. Eriksen MT, Wibe A, Norstein J et al. Anastomotic leakage following routine mesorectal excision for rectal cancer in a national cohort of patients. Colorectal Dis 2005;7:51-7.
39. Kang CY, Halabi WJ, Chaudhry OO et al. Risk factors for anastomotic leakage after anterior resection for rectal cancer. JAMA Surg 2013;148:65-71.
40. Krarup PM, Jorgensen LN, Andreasen AH et al. A nationwide study on anastomotic leakage after colonic cancer surgery. Colorectal Dis 2012;14:e661-7.
41. Nisar PJ, Lavery IC, Kiran RP. Influence of neoadjuvant radiotherapy on anastomotic leak after restorative resection for rectal cancer. J Gastrointest Surg 2012;16:1750-7.
42. Bird CE, Rieker PP. Gender matters: an integrated model for understanding men's and women's health. Soc Sci Med 1999;48:745-55.
43. Genders TS, Steyerberg EW, Hunink MG et al. Prediction model to estimate presence of coronary artery disease: retrospective pooled analysis of existing cohorts. BMJ 2012;344:e3485.
44. Hida J, Yasutomi M, Maruyama T et al. Anterior resection following posterior transsacral stapling and transection of the anal canal for low-lying rectal cancer in males. Surg Today 1998;28:768-9.
45. Targarona EM, Balague C, Pernas JC et al. Can we predict immediate outcome after laparoscopic rectal surgery? Multivariate analysis of clinical, anatomic, and pathologic features after 3-dimensional reconstruction of the pelvic anatomy. Ann Surg 2008;247:642-9.
46. Koska J, Stefan N, Votruba SB et al. Distribution of subcutaneous fat predicts insulin action in obesity in sex-specific manner. Obesity 2008;16:2003-9.
47. Bertelsen CA, Andreasen AH, Jorgensen T et al. Anastomotic leakage after anterior resection for rectal cancer: risk factors. Colorectal Dis 2010;12:37-43.
48. Chen W, Li Y, Liao Z et al. Active lymphangiogenesis is a major risk factor for anastomotic leakage following sphincter-sparing resection of rectal cancer. J Surg Oncol 2011;104:493-8.
49. Cong ZJ, Fu CG, Wang HT et al. Influencing factors of symptomatic anastomotic leakage after anterior resection of the rectum for cancer. World J Surg 2009;33:1292-7.
50. Foster ME, Lancaster JB, Leaper DJ. Leakage of low rectal anastomosis. An anatomic explanation? Dis Colon Rectum 1984;27:157-8.
51. Choi HK, Law WL, Ho JW. Leakage after resection and intraperitoneal anastomosis for colorectal malignancy: analysis of risk factors. Dis Colon Rectum 2006;49:1719-25.
52. Vamvakas EC, Blajchman MA. Transfusion-related immunomodulation (TRIM): an update. Blood Rev 2007;21:327-48.
53. Iyer S, Balasubramanian D. Management of radiation wounds. Indian J Plast Surg 2012;45:325-31.
54. Holte K, Andersen J, Jakobsen DH et al. Cyclo-oxygenase 2 inhibitors and the risk of anastomotic leakage after fast-track colonic surgery. Br J Surg 2009;96:650-4.
55. Klein M, Andersen LP, Harvald T et al. Increased risk of anastomotic leakage with diclofenac treatment after laparoscopic colorectal surgery. Dig Surg 2009;26:27-30.
56. Cahill RA, Sheehan KM, Scanlon RW et al. Effects of a selective cyclo-oxygenase 2 inhibitor on colonic anastomotic and skin wound integrity. Br J Surg 2004;91:1613-8.
57. Inan A, Koca C, Sen M. Effects of diclofenac sodium on bursting pressures of anastomoses and hydroxyproline contents of perianastomotic tissues in a laboratory study. Int J Surg 2006;4:222-7.
58. Klein M, Krarup PM, Kongsbak MB et al. Effect of postoperative diclofenac on anastomotic healing, skin wounds and subcutaneous collagen accumulation: a randomized, blinded, placebo-controlled, experimental study. Eur Surg Res 2012;48:73-8.
59. Chow PKH. Using Animal Models in Biomedical Research: A Primer for the investigator: River Edge, NJ, USA: World scientific; 2008.
60. Swindle MM, Smith AC, Hepburn BJ. Swine as models in experimental surgery. J Invest Surg 1988;1:65-79.
61. Tumblesom ME. Swine in biomedical research: Plenum press; 1986.
62. Green EL. Biology of the laboratory mouse. 2 ed: Dover publications; 1996.
63. Hem A, Eide DM, Engh E et al. Laboratory Animal Science: Laboratory animal unit; 2001.
64. Jeppsson B. Animal modelling in surgical research: Harwood academic publishers; 1998.
65. Waynforth HB. Experimental and surgical technique in the rat: Academic press; 1980.
66. Pantelis D, Beissel A, Kahl P et al. The effect of sealing with a fixed combination of collagen matrix-bound coagulation factors on the healing of colonic anastomoses in experimental high-risk mice models. Langenbecks Arch Chir 2010;395:1039-48.
67. Byrne DJ, Hardy J, Wood RA et al. Adverse influence of fibrin sealant on the healing of high-risk sutured colonic anastomoses. J R Coll Surg Edinb 1992;37:394-8.
68. Houston KA, Rotstein OD. Fibrin sealant in high-risk colonic anastomoses. Arch Surg 1988;123:230-4.
69. Farris EJ. The rat in laboratory investigation: Hafner publishing company; 1949.
70. Henne-Bruns D, Kreischer HP, Schmiegelow P et al. Reinforcement of colon anastomoses with polyglycolic acid mesh: an experimental study. Eur Surg Res 1990;22:224-30.
71. Huh JW, Kim HR, Kim YJ. Anastomotic leakage after laparoscopic resection of rectal cancer: the impact of fibrin glue. Am J Surg 2010;199:435-41.
72. Kopelman D, Kopelman Y, Peled D et al. Healing of Ileocolic Nitinol Compression Anastomosis: A Novel Porcine Model of Subtotal Colectomy. Surg Innov 2013.
73. Abelson KS, Jacobsen KR, Sundbom R et al. Voluntary ingestion of nut paste for administration of buprenorphine in rats and mice. Lab Anim 2012;46:349-51.
74. Kalliokoski O, Jacobsen KR, Hau J et al. Serum concentrations of buprenorphine after oral and parenteral administration in male mice. Vet J 2011;187:251-4.

75. Kehlet H, Holte K. Review of postoperative ileus. Am J Surg 2001;182:3S-10S.
76. Schang JC, Hemond M, Hebert M et al. How does morphine work on colonic motility? An electromyographic study in the human left and sigmoid colon. Life Sci 1986;38:671-6.
77. Hastings JC, Winkle WV, Barker E et al. Effect of suture materials on healing wounds of the stomach and colon. Surg Gynecol Obstet 1975;140:701-7.
78. Craig PH, Williams JA, Davis KW et al. A biologic comparison of polyglactin 910 and polyglycolic acid synthetic absorbable sutures. Surg Gynecol Obstet 1975;141:1-10.
79. Syk I, Agren MS, Adawi D et al. Inhibition of matrix metalloproteinases enhances breaking strength of colonic anastomoses in an experimental model. Br J Surg 2001;88:228-34.
80. van der Stappen JW, Hendriks T, de Boer HH et al. Collagenolytic activity in experimental intestinal anastomoses. Differences between small and large bowel and evidence for the presence of collagenase. Int J Colorectal Dis 1992;7:95-101.
81. Seifert WF, Wobbes T, Hoogenhout J et al. Intraoperative irradiation prolongs the presence of matrix metalloproteinase activity in large bowel anastomoses of the rat. Radiat Res 1997;147:354-61.
82. Stumpf M, Klinge U, Wilms A et al. Changes of the extracellular matrix as a risk factor for anastomotic leakage after large bowel surgery. Surgery 2005;137:229-34.
83. Savage FJ, Lacombe DL, Boulos PB et al. Role of matrix metalloproteinases in healing of colonic anastomosis. Dis Colon Rectum 1997;40:962-70.
84. Koruda MJ, Rolandelli RH. Experimental studies on the healing of colonic anastomoses. J Surg Res 1990;48:504-15.
85. Ikeuchi D, Onodera H, Aung T et al. Correlation of tensile strength with bursting pressure in the evaluation of intestinal anastomosis. Dig Surg 1999;16:478-85.
86. Mansson P, Zhang XW, Jeppsson B et al. Anastomotic healing in the rat colon: comparison between a radiological method, breaking strength and bursting pressure. Int J Colorectal Dis 2002;17:420-5.
87. Weiber S, Jiborn H, Zederfeldt B. Preoperative irradiation and colonic healing. Eur J Surg 1994;160:47-51.
88. Hulkko OA, Haukipuro KA, Laitinen ST. Fibrin glue protection of primary anastomosis in the obstructed left colon. An experimental study on the rat. Acta Chir Scand 1988;154:49-52.
89. Kanellos I, Christoforidis E, Kanellos D et al. The healing of colon anastomosis covered with fibrin glue after early postoperative intraperitoneal chemotherapy. Tech Coloproctol 2006;10:115-20.
90. Kanellos I, Mantzoros I, Goulimaris I et al. Effects of the use of fibrin glue around the colonic anastomosis of the rat. Tech Coloproctol 2003;7:82-4.
91. Shinohara K, Kobayashi E, Yoshida T et al. Effect of fibrin glue on small and large bowel anastomoses in the rat. Eur Surg Res 1998;30:8-12.
92. Nordentoft T, Romer J, Sorensen M. Sealing of gastrointestinal anastomoses with a fibrin glue-coated collagen patch: a safety study. J Invest Surg 2007;20:363-9.
93. Parker MC, Pohlen U, Borel Rinkes IH et al. The application of TachoSil(R) for sealing colorectal anastomosis: a feasibility study. Colorectal Dis 2013;15:252-7.
94. Giuratrabocchetta S, Rinaldi M, Cuccia F et al. Protection of intestinal anastomosis with biological glues: an experimental randomized controlled trial. Tech Coloproctol 2011;15:153-8.
95. Jafari MD, Lee KH, Halabi WJ et al. The use of indocyanine green fluorescence to assess anastomotic perfusion during robotic assisted laparoscopic rectal surgery. Surg Endosc 2013.