New approaches towards risk assessment, diagnosis and prevention strategies of colorectal anastomotic leakage

Niels Komen

De druk van dit proefschrift werd mede mogelijk gemaakt door:

Erasmus Universiteit Rotterdam, Erasmus MC Afdeling Heelkunde, De Financiële Makelaar, Arseus Medical B.V., Covidien

Printing: D. Provo N.V., Gierle, Belgium

Cover image: Associated Press

Rotterdam 2014

New Approaches Towards Risk Assessment, Diagnosis and Prevention Strategies of Colorectal Anastomotic Leakage

Nieuwe benaderingen van risico bepaling, diagnose en

preventie strategieën van colorectale naadlekkage

Proefschrift

ter verkrijging van de graad van doctor aan de

Erasmus Universiteit Rotterdam

op gezag van de

rector magnificus

Prof.dr. H.A.P. Pols

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op

vrijdag 9 mei 2014 om 13:30 uur

door

Nicolaas Antonius Petrus Komen

geboren te Oisterwijk

ERASMUS UNIVERSITEIT ROTTERDAM

Promotiecommissie

Promotor	Prof.dr. J.F. Lange
Overige leden	Prof.dr. C.H.J. van Eijck
	Prof.dr. C. Verhoef
	Prof.dr. J.H.W. de Wilt
Copromotor	Prof.dr. J. Jeekel

Table of Contents

Part 1

General Introduction

The most important and frustrating complication of colorectal surgery is colorectal anastomotic leakage (CAL). An anastomotic defect causes leakage of colonic content into the abdominal and/or pelvic space leading to peritonitis, abscess formation and sepsis that can be fatal. The incidence of CAL varies between 3 % and 19 % [1-4] and mortality rates due to CAL vary between 10 % and 20 % [5-7]. Moreover, CAL is a risk factor for local recurrence of colorectal cancer and is reported to reduce long-term cancer specific survival [8].

The pathological processes leading up to the occurrence of this defect are poorly understood. Even when all patient-, disease- and operation related factors favor proper anastomotic healing, CAL still may occur.

Cause and risk factors

Tissue ischemia at the anastomotic site is considered to be one of the main causes of CAL [9, 10], as illustrated by several studies. CAL has been related to intraoperative hypotension in patients with elevated diastolic blood pressure undergoing complex surgery [11]. By means of Laser Doppler Flowmetry anastomotic perfusion at the proximal loop of the anastomosis has been found to decrease after clamping of either the inferior mesenteric artery or the left colic artery [12]. In addition, perioperative oxygen suppletion has been shown to reduce CAL rates [13]. However, contradicting these ideas and observations, electrically welded anastomoses appear to be healing as well. This technique consists of welding the two bowel endings together by means of bipolar welding tools, creating ischemia [14]. In addition, compression anastomosis techniques have been developed in which both bowel endings are trapped between two opposing rings [15]. These techniques create ischemia too, however, leakage rates are not higher than for sutured anastomoses [16].

Technical failure is considered to be another cause. An anastomosis that is not closed airtight is prone to leak [17]. Therefore, Halsted´s principles of gentle handling of tissues, scrupulous hemostasis, tension-free anastomosis and crush-free dissection aiming to minimize damage to tissue and optimize perfusion are still propagated [18]. Intraoperative air tightness testing is a commonly used technique to evaluate the anastomosis. When positive, additional sutures can be placed or the anastomosis can be taken down allowing construction of a new anastomosis. When the anastomosis cannot be reached a deviating stoma can be constructed. Using this test routinely after colorectal surgery reduces CAL rates and it could therefore be considered a simple and reproducible method to intraoperatively predict the development of anastomotic leakage [17, 19].

An anatomical factor might also play a role in the processes leading to CAL. The superior and inferior mesenteric arteries guarantee the blood supply to the colon. The marginal artery serves as a collateral artery connecting the two arterial systems. However, at the splenic flexure the marginal artery has been shown to be frequently insufficient [20]. This could lead to a compromised blood supply to the anastomosis after left hemicolectomy, sigmoid or rectal resection. Whether this insufficiency is clinically relevant has not yet been shown.

Adding to the poor understanding of CAL is the great variety of reported risk factors. Factors like diverticular disease [21], rectal resection [22], urgent colectomy [23], smoking [24], body mass index (BMI) [25], sex [26], use of steroids [27], radio- and/or chemotherapy [26], American Society of Anesthesiologists (ASA) score [1], a history of cardiac and vascular disease [28], operating time [26] and use of non-steroidal anti-inflammatory drugs (NSAID) [29] are reported to contribute to the development of CAL. Only for a few risk factors level 1 evidence exists. Meta-analyses show no superiority of the hand-sutured technique over the stapled technique [30, 31]. Several meta-analysis show no superiority of mechanical bowel preparation for colorectal surgery in terms of CAL. In contrast, the data seemed to favor patients that did not have bowel preparation [32, 33]. Meta-analysis concerning prophylactic drainage showed no benefit of drainage on CAL [34]. Randomized controlled trials on preoperative radiotherapy [35] and performing an omentoplasty around the anastomosis [36] showed that these factores had no impact on the incidence of CAL.

Considering the great number of reported risk factors and causes, CAL should be considered a multifactorial complication. Moreover, since for each of the aforementioned risk factors confirming as well as contradicting studies have been published it can be assumed not all factors or combinations of factors are known. Therefore more research concerning risk factors for CAL is needed.

Diagnosis

To date, CAL is suspected when certain signs and symptoms are present and confirmation occurs by imaging and/or reoperation [37, 38]. Signs of peritonitis at clinical examination and fecal discharge from a drain or wound are specific signs of CAL. However, more common clinical signs like fever, prolonged ileus, increased amounts of drain fluid, renal failure, leucocytosis, cardiac and respiratory symptoms are not very specific [39-42]. Therefore, confirmation of CAL by means of imaging studies like proctoscopy, CT-scan and/or (water-soluble) contrast enema is needed and is performed six days to two weeks after the operation [2, 39] at which point the patient is generally very ill. Since delay in diagnosis of CAL increases mortality, earlier diagnosis should be strived for [43]. Routine imaging studies might decrease the interval between operation and diagnosis of CAL but are not preferred because of radiation exposure, costs, patient´s discomfort and false positives due to subclinical CAL [43, 44]. In addition, the reported sensitivity of CT-scan in the early postoperative period varies from 15 % to 52 % [45-47] and the false negative rates vary between 35 % and 53 % [45, 47]. For a diagnostic test these rates are rather low.

Alternative imaging consists of contrast radiography. Reported sensitivity and specificity when performed in case of clinical suspicion were 68 % and 94 % respectively [45]. When performed routinely around seven to eight days postoperatively, sensitivity and specificity vary between 20 % and 52 % and 85 % and 87 % respectively [48, 49]. These rates are better than for CT-scan, however the interval between operation and imaging is quite long.

There is a need for additional diagnostic or screening tools to detect CAL in the early postoperative phase. A biomarker reflecting the intra-abdominal milieu surrounding the anastomosis might be an objective tool in addition to current methods, allowing diagnosis of CAL in the early postoperative phase. Such a biomarker might be found in the fluid retrieved from an intra-abdominal drain. Several reports on this matter show promising results, for example higher levels of cytokines, matrix-metalloproteinase and LPS in patients with CAL [50-53].

Treatment

Treatment options of symptomatic CAL contain antibiotic therapy, percutaneous drainage, local transanal drainage and reoperation [61]. The type of treatment depends on the severity of the clinical consequences of CAL. The "International Study Group of Rectal Cancer" has introduced a grading system of CAL [38]. A subclinical leak detected by imaging studies is classified as grade A and requires no intervention. It might, however, result in delayed stoma closure. When the patient's clinical condition requires an active therapeutic intervention that can be managed without operative reintervention, leakage is classified as grade B. Therapeutic interventions include administration of antibiotics and/or radiologic placement of a pelvic drain or transanal lavage. In case of grade C leakage, the patients are ill and require operative management of CAL. This consists of construction of a diverting ileostomy or when already present, the anastomosis is taken down with creation of a descending colostomy (Hartmann's procedure).

An alternative treatment for presacral abscesses due to CAL consists of a combination of negative pressure therapy with minimally invasive, endoscopic closure of the defect [62]. Initially the abscess is drained by means of negative pressure therapy for approximately four days. Afterwards the defect is closed by suturing transanally and drains are left behind in the cavity. So far the technique has been shown to be feasible, however, whether it is better than the aforementioned techniques remains to be investigated.

Prevention

When a patient is not likely to develop CAL based upon lack of risk factors and a negative intraoperative air tightness test, bowel continuity can be restored without additional precautions. However, when a patient is more likely to develop CAL there are a few options. One option is to postpone the operation to allow improvement of the patients´ risk factors.

Factors like BMI, use of steroids or NSAIDs can be influenced preoperatively in order to reduce risk of CAL. Another option is to construct a diverting stoma. This does not decrease CAL rates but it does reduce the number of patients with CAL requiring reoperation [54]. However, as a routine procedure in colorectal surgery it is not very well suited since it has a substantial impact on the quality of life and in up to 19 % of patients the stoma will never be closed [55, 56]. Moreover, stoma closure is associated with high morbidity rates, around 17 % [57]. Small bowel obstruction and wound sepsis belong to the most common complications. Therefore, a diverting stoma should only be constructed in selected, high risk patients.

Next to these commonly used options there are several still experimental methods of which anastomotic sealants are an important one. Products like cyanoacrylate [58], fibrin sealants [59] and platelet rich plasma [60] are a few examples of products that have been studied in animal experiments. For this research, mostly performed on rats, outcome measures like anastomotic bursting pressure (ABP), tensile strength (TS) and hydroxyproline concentration are used. ABP is the peak pressure at which the insufflated bowel segment containing the anastomosis bursts. The force needed to break an anastomosis when pulling both sides is TS. Hydroxyproline is a metabolite of collagen and considered representative for the amount of collagen at the anastomotic site. Several studies conclude that the tested product might improve anastomotic healing because a higher ABP, TS or hydroxyproline concentration were found. However, these endpoints are surrogate endpoints of anastomotic healing and provide us with information about how healing is influenced by the intervention. It provides no information on CAL. In addition, rats are quite resistant against infection, rendering it not the most suited animal for experiments concerning CAL.

Besides these diverting and experimental strategies a means for prevention of an actual anastomotic defect through which fecal content leaks into the abdominal/pelvic space, has not been reported to date.

Purpose and outline

The purpose of this thesis is to investigate several new approaches to research on “the” major complication of colorectal surgery, which might contribute to our knowledge and to reducing morbidity and mortality.

After the general introduction in part 1, part 2 contains different studies concerning risk assessment of CAL.

Chapter 1 describes a retrospective risk factor analysis of patients of a large academic hospital, analyzing risk factors traditionally reported in literature as well as a new factor, being the time of surgery.

In Chapter 2 the patient’s atherosclerotic load, visualized on CT-scans, is retrospectively studied as a new potential risk factor for CAL.

Chapter 3 contains one of few studies on prospectively collected data on the heavily debated role of long-term and perioperative use of corticosteroids as potential risk factors for CAL.

Chapter 4 contains a review on the colon´s vasculature. It deals with Riolan´s arch, supposedly being an anatomical entity, it´s synonyms and relevance. In addition, Griffith´s point is discussed as a potential anatomical factor increasing the risk of CAL.

In Chapter 5 anastomotic perfusion is prospectively compared between high tie versus low tie ligation of the inferior mesenteric artery in order to see whether a difference in blood flow occurs, potentially influencing the risk of CAL.

In Part 3 potential screening methods for CAL are described.

Chapter 6 contains a review of literature concerning screening techniques that have lead to the APPEAL-study (Analysis of Parameters Predictive for Evident Anastomotic Leakage). In Chapter 7 the development of a new real-time PCR for E. coli and E. faecalis on drain fluid is described.

In Chapter 8 the results of a prospective RT-PCR analysis, as part of the APPEAL-study, leading to the first objective screening test for CAL, are presented.

In Chapter 9 the analysis of acute phase proteins in drain fluid as potential biomarkers for CAL is described.

Part 4 deals with an experimental approach to CAL.

Chapter 10 describes a new animal experimental model with anastomotic leakage as outcome measure.

References

1. Alves A, Panis Y, Trancart D, Regimbeau JM, Pocard M, Valleur P. Factors associated with clinically significant anastomotic leakage after large bowel resection: multivariate analysis of 707 patients. World J Surg 2002;26:499-502.

2. Hyman N, Manchester TL, Osler T, Burns B, Cataldo PA. Anastomotic leaks after intestinal anastomosis: it's later than you think. Ann Surg 2007;245:254-8.

3. Matthiessen P, Hallbook O, Rutegard J, Simert G, Sjodahl R. Defunctioning stoma reduces symptomatic anastomotic leakage after low anterior resection of the rectum for cancer: a randomized multicenter trial. Ann Surg 2007;246:207-14.

4. Peeters KC, Tollenaar RA, Marijnen CA, et al. Risk factors for anastomotic failure after total mesorectal excision of rectal cancer. Br J Surg 2005;92:211-6.

5. Fielding LP, Stewart-Brown S, Blesovsky L, Kearney G. Anastomotic integrity after operations for large-bowel cancer: a multicentre study. Br Med J 1980;281:411-4.

6. Alberts JC, Parvaiz A, Moran BJ. Predicting risk and diminishing the consequences of anastomotic dehiscence following rectal resection. Colorectal Dis 2003;5:478-82.

7. Branagan G, Finnis D. Prognosis after anastomotic leakage in colorectal surgery. Dis Colon Rectum 2005;48:1021-6.

8. Mirnezami A, Mirnezami R, Chandrakumaran K, Sasapu K, Sagar P, Finan P. Increased local recurrence and reduced survival from colorectal cancer following anastomotic leak: systematic review and meta-analysis. Ann Surg;253:890-9.

9. Boyle NH, Manifold D, Jordan MH, Mason RC. Intraoperative assessment of colonic perfusion using scanning laser Doppler flowmetry during colonic resection. J Am Coll Surg 2000;191:504-10.

10. Vignali A, Gianotti L, Braga M, Radaelli G, Malvezzi L, Di Carlo V. Altered microperfusion at the rectal stump is predictive for rectal anastomotic leak. Dis Colon Rectum 2000;43:76-82.

11. Post IL, Verheijen PM, Pronk A, Siccama I, Houweling PL. Intraoperative blood pressure changes as a risk factor for anastomotic leakage in colorectal surgery. Int J Colorectal Dis.

12. Seike K, Koda K, Saito N, et al. Laser Doppler assessment of the influence of division at the root of the inferior mesenteric artery on anastomotic blood flow in rectosigmoid cancer surgery. Int J Colorectal Dis 2007;22:689-97.

13. Schietroma M, Carlei F, Cecilia EM, Piccione F, Bianchi Z, Amicucci G. Colorectal Infraperitoneal Anastomosis: The Effects of Perioperative Supplemental Oxygen Administration on the Anastomotic Dehiscence. J Gastrointest Surg.

14. Ho YH, Ashour MA. Techniques for colorectal anastomosis. World J Gastroenterol;16:1610-21.

15. Hardy KJ. A view of the development of intestinal suture. Part II. Principles and techniques. Aust N Z J Surg 1990;60:377-84.

16. Galizia G, Lieto E, Castellano P, et al. Comparison between the biofragmentable anastomosis ring and stapled anastomoses in the extraperitoneal rectum: a prospective, randomized study. Int J Colorectal Dis 1999;14:286-90.

17. Ricciardi R, Roberts PL, Marcello PW, Hall JF, Read TE, Schoetz DJ. Anastomotic leak testing after colorectal resection: what are the data? Arch Surg 2009;144:407-11; discussion 11-2.

18. Imber G. Genius on the Edge: The Bizarre Double Life of Dr. William Stewart Halsted. New York: Kaplan; 2011.

19. Beard JD, Nicholson ML, Sayers RD, Lloyd D, Everson NW. Intraoperative air testing of colorectal anastomoses: a prospective, randomized trial. Br J Surg 1990;77:1095-7.

20. Griffiths JD. Surgical anatomy of the blood supply of the distal colon. Ann R Coll Surg Engl 1956;19:241-56.

21. Platell C, Barwood N, Dorfmann G, Makin G. The incidence of anastomotic leaks in patients undergoing colorectal surgery. Colorectal Dis 2007;9:71-9.

22. Buchs NC, Gervaz P, Secic M, Bucher P, Mugnier-Konrad B, Morel P. Incidence, consequences, and risk factors for anastomotic dehiscence after colorectal surgery: a prospective monocentric study. Int J Colorectal Dis 2008;23:265-70.

23. 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.

24. Sorensen LT, Jorgensen T, Kirkeby LT, Skovdal J, Vennits B, Wille-Jorgensen P. Smoking and alcohol abuse are major risk factors for anastomotic leakage in colorectal surgery. Br J Surg 1999;86:927-31.

25. Nickelsen TN, Jorgensen T, Kronborg O. Lifestyle and 30-day complications to surgery for colorectal cancer. Acta Oncol 2005;44:218-23.

26. Matthiessen P, Hallbook O, Andersson M, Rutegard J, Sjodahl R. Risk factors for anastomotic leakage after anterior resection of the rectum. Colorectal Dis 2004;6:462-9.

27. Konishi T, Watanabe T, Kishimoto J, Nagawa H. Risk factors for anastomotic leakage after surgery for colorectal cancer: results of prospective surveillance. J Am Coll Surg 2006;202:439-44.

28. Makela JT, Kiviniemi H, Laitinen S. Risk factors for anastomotic leakage after left-sided colorectal resection with rectal anastomosis. Dis Colon Rectum 2003;46:653-60.

29. Gorissen KJ, Benning D, Berghmans T, et al. Risk of anastomotic leakage with non-steroidal anti-inflammatory drugs in colorectal surgery. Br J Surg.

30. Lustosa SA, Matos D, Atallah AN, Castro AA. Stapled versus handsewn methods for colorectal anastomosis surgery. Cochrane Database Syst Rev 2001:CD003144.

31. Neutzling CB, Lustosa SA, Proenca IM, da Silva EM, Matos D. Stapled versus handsewn methods for colorectal anastomosis surgery. Cochrane Database Syst Rev;2:CD003144.

32. Slim K, Vicaut E, Panis Y, Chipponi J. Meta-analysis of randomized clinical trials of colorectal surgery with or without mechanical bowel preparation. Br J Surg 2004;91:1125-30.

33. Wille-Jorgensen P, Guenaga KF, Matos D, Castro AA. Pre-operative mechanical bowel cleansing or not? an updated meta-analysis. Colorectal Dis 2005;7:304-10.

34. Jesus EC, Karliczek A, Matos D, Castro AA, Atallah AN. Prophylactic anastomotic drainage for colorectal surgery. Cochrane Database Syst Rev 2004:CD002100.

35. Marijnen CA, Kapiteijn E, van de Velde CJ, et al. Acute side effects and complications after short-term preoperative radiotherapy combined with total mesorectal excision in primary rectal cancer: report of a multicenter randomized trial. J Clin Oncol 2002;20:817-25.
36. Merad F, Hay JM, Fingerhut A, Flamant Y, Molkhou JM, Laborde Y. Omentoplasty in the prevention of anastomotic leakage after colonic or rectal resection: a prospective randomized study in 712 patients. French Associations for Surgical Research. Ann Surg 1998;227:179-86.
37. Bruce J, Krukowski ZH, Al-Khairy G, Russell EM, Park KG. Systematic review of the definition and measurement of anastomotic leak after gastrointestinal surgery. Br J Surg 2001;88:1157-68.
38. Rahbari NN, Weitz J, Hohenberger W, et al. Definition and grading of anastomotic leakage following anterior resection of the rectum: a proposal by the International Study Group of Rectal Cancer. Surgery;147:339-51.
39. Alves A, Panis Y, Pocard M, Regimbeau JM, Valleur P. Management of anastomotic leakage after nondiverted large bowel resection. J Am Coll Surg 1999;189:554-9.
40. Doeksen A, Tanis PJ, Vrouenraets BC, Lanschot van JJ, Tets van WF. Factors determining delay in relaparotomy for anastomotic leakage after colorectal resection. World J Gastroenterol 2007;13:3721-5.
41. Lim M, Akhtar S, Sasapu K, et al. Clinical and subclinical leaks after low colorectal anastomosis: a clinical and radiologic study. Dis Colon Rectum 2006;49:1611-9.
42. Sutton CD, Marshall LJ, Williams N, Berry DP, Thomas WM, Kelly MJ. Colo-rectal anastomotic leakage often masquerades as a cardiac complication. Colorectal Dis 2004;6:21-2.
43. Macarthur DC, Nixon SJ, Aitken RJ. Avoidable deaths still occur after large bowel surgery. Scottish Audit of Surgical Mortality, Royal College of Surgeons of Edinburgh. Br J Surg 1998;85:80-3.

44. Goligher JC, Lee PW, Simpkins KC, Lintott DJ. A controlled comparison one- and two-layer techniques of suture for high and low colorectal anastomoses. Br J Surg 1977;64:609-14.

45. Doeksen A, Tanis PJ, Wust AF, Vrouenraets BC, van Lanschot JJ, van Tets WF. Radiological evaluation of colorectal anastomoses. Int J Colorectal Dis 2008;23:863-8.

46. Nicksa GA, Dring RV, Johnson KH, Sardella WV, Vignati PV, Cohen JL. Anastomotic leaks: what is the best diagnostic imaging study? Dis Colon Rectum 2007;50:197-203.

47. Khoury W, Ben-Yehuda A, Ben-Haim M, Klausner JM, Szold O. Abdominal computed tomography for diagnosing postoperative lower gastrointestinal tract leaks. J Gastrointest Surg 2009;13:1454-8.

48. Hoffmann J, Jensen RH, Shokouh-Amiri MH, Damm P. Clinical value of water-soluble contrast enema in assessing the integrity of left colonic anastomoses. J R Coll Surg Edinb 1988;33:23-4.

49. Akyol AM, McGregor JR, Galloway DJ, George WD. Early postoperative contrast radiology in the assessment of colorectal anastomotic integrity. Int J Colorectal Dis 1992;7:141-3.

50. Herwig R, Glodny B, Kuhle C, et al. Early identification of peritonitis by peritoneal cytokine measurement. Dis Colon Rectum 2002;45:514-21.

51. Bertram P, Junge K, Schachtrupp A, Gotze C, Kunz D, Schumpelick V. Peritoneal release of TNFalpha and IL-6 after elective colorectal surgery and anastomotic leakage. J Invest Surg 2003;16:65-9.

52. 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.

53. Junger W, Junger WG, Miller K, et al. Early detection of anastomotic leaks after colorectal surgery by measuring endotoxin in the drainage fluid. Hepatogastroenterology 1996;43:1523-9.

54. Gastinger I, Marusch F, Steinert R, Wolff S, Koeckerling F, Lippert H. Protective defunctioning stoma in low anterior resection for rectal carcinoma. Br J Surg 2005;92:1137-42.
55. Tsunoda A, Tsunoda Y, Narita K, Watanabe M, Nakao K, Kusano M. Quality of life after low anterior resection and temporary loop ileostomy. Dis Colon Rectum 2008;51:218-22.
56. den Dulk M, Smit M, Peeters KC, et al. A multivariate analysis of limiting factors for stoma reversal in patients with rectal cancer entered into the total mesorectal excision (TME) trial: a retrospective study. Lancet Oncol 2007;8:297-303.
57. Chow A, Tilney HS, Paraskeva P, Jeyarajah S, Zacharakis E, Purkayastha S. The morbidity surrounding reversal of defunctioning ileostomies: a systematic review of 48 studies including 6,107 cases. Int J Colorectal Dis 2009;24:711-23.
58. Bae KB, Kim SH, Jung SJ, Hong KH. Cyanoacrylate for colonic anastomosis; is it safe? Int J Colorectal Dis;25:601-6.
59. van der Ham AC, Kort WJ, Weijma IM, Jeekel H. Transient protection of incomplete colonic anastomoses with fibrin sealant: an experimental study in the rat. J Surg Res 1993;55:256-60.
60. Yol S, Tekin A, Yilmaz H, et al. Effects of platelet rich plasma on colonic anastomosis. J Surg Res 2008;146:190-4.
61. Maggiori L, Bretagnol F, Lefevre JH, Ferron M, Vicaut E, Panis Y. Conservative management is associated with a decreased risk of definitive stoma after anastomotic leakage complicating sphincter-saving resection for rectal cancer. Colorectal Dis;13:632-7.
62. Verlaan T, Bartels SA, van Berge Henegouwen MI, Tanis PJ, Fockens P, Bemelman WA. Early, minimally invasive closure of anastomotic leaks: a new concept. Colorectal Dis;13 Suppl 7:18-22.

Part 2

Risk assessment

Chapter 1

After-hours colorectal surgery: a risk factor for anastomotic leakage

N. Komen, J.W. Dijk, Z. Lalmahomed, K. Klop, W. Hop, G.J. Kleinrensink, H. Jeekel, W. R. Schouten, J. F. Lange

Int J Colorectal Dis. 2009 Jul;24(7):789-95

Abstract

Purpose

This study aims to increase knowledge of colorectal anastomotic leakage by performing an incidence study and risk factor analysis with new potential risk factors in a Dutch tertiary referral center.

Methods

All patients whom received a primary colorectal anastomosis between 1997 and 2007 were selected by means of operation codes. Patient records were studied for population description and risk factor analysis.

Results

In total 739 patients were included. Anastomotic leakage (AL) occurred in 64 (8.7 %) patients of whom nine (14.1 %) died. Median interval between operation and diagnosis was 8 days. The risk for AL was higher as the anastomoses were constructed more distally (p=0.019).

Univariate analysis showed duration of surgery (p=0.038), BMI (p=0.001), time of surgery (p=0.029), prophylactic drainage (p=0.006) and time under anesthesia (p=0.012) to be associated to AL. Multivariate analysis showed BMI greater than 30 kg/m^2 (p=0.006, OR 2.6 CI 1.3–5.2) and “after hours” construction of an anastomosis (p=0.030, OR 2.2 CI 1.1–4.5) to be independent risk factors.

Conclusion

BMI greater than 30 kg/m^2 and “after hours” construction of an anastomosis were independent risk factors for colorectal anastomotic leakage.

Introduction

Anastomotic leakage (AL) is the major complication after colorectal surgery. It is poorly understood as illustrated by the vast body of confirming and contradicting publications on virtually every known potential risk factor. The great variation of reported incidences of AL, between 3 % and 19 % [1, 2], illustrates this as well. The poor understanding of AL may be due to its multifactorial aspect. In addition, studies on potential risk factors are performed in different populations throughout the world and, therefore, show many different independent risk factors for anastomotic leakage, varying from diverticular disease [3] and rectal resection [4] to weight loss [5], urgent operation [6], smoking [7] and BMI [8]. The factor "surgeon" is important as well since it is shown that leakage risk is lower when patients are operated upon by a high-volume surgeon [9]. The group of potential risk factors for AL that is studied in literature is generally quite similar and contains, in addition to the aforementioned factors, mechanical bowel preparation (MBP) [10], prophylactic drainage (PD) [11], ASA-score [4], prolonged operating time [4], use of corticosteroids [12], anastomotic configuration [13], hand-sutured vs. stapled anastomosis [14], neoadjuvant radiotherapy [15], laparoscopic vs. open surgery [16], and gender [8]. Despite the vast body of evidence on these potential risk factors AL remains poorly understood. Therefore, risk factor analysis should not be limited to these factors. Since ischemia is considered to be one of the causes of AL, studying vascular disease, i.e., atherosclerosis, could be a new approach as suggested by Foster et al. [17]. Analysis of known risk factors for atherosclerosis like hypertension [18], dyslipidemia [19], smoking [20], diabetes mellitus [21] could be an interesting addition to the group of potential risk factors.

More and more publications show the danger of after hours medical activity. Gray et al. showed after-hours surgery to be related to higher complications rate [22]. Fechner et al. showed that night time kidney transplantation enhances the risk for complications and graft failure [23]. In addition, several studies have shown that proficiency and situational awareness of physicians is less at night [24–27]. On the matter of colorectal surgery, the time of day at which the patient is operated has never been related to AL.

In short, risk factors for AL vary between different populations and, since it is a multifactorial problem, there are probably several unknown risk factors. This means that more studies on this matter, from different hospitals throughout the world should be performed, published and compared in order to improve understanding of this complication.

This study aims to describe the population of patients with primary colorectal anastomosis treated in a Dutch tertiary referral center and to determine the incidence and risk factors of AL in this population.

Materials and methods

All patients who have undergone surgery on the colon or rectum in the period 1997–2007 were selected in the electronic archive by means of operation code of the relevant operations. In this population, patients whom received a primary anastomosis, involving colon and or rectum, were selected. The electronic- and paper patient records were used to score patient-, surgery-, and disease-related factors for risk factor analysis and description of the patient population. The patient-related factors analyzed in this study were body mass index (BMI), age, gender, diabetes mellitus (DM), smoking, cardiac- and pulmonary comorbidity, history of vascular disease, ASA-score, use of steroids, statins, and anti-hypertensive medication (Table 1).

The surgery-related factors analyzed in this study were prophylactic drainage (PD), time of surgery, type of operation, anastomotic configuration, blood transfusion, surgeon vs. assistant, mechanical bowel preparation (MBP), laparoscopic vs. open operation, duration of surgery, stapled vs. hand-sutured anastomosis, urgent vs. elective operation (Tables 1 and 2). Operations were considered urgent when it was indicated in anesthesiologist's records and the patient's file. Examples of included surgical emergencies were mechanical obstruction due to colorectal cancer and perforated diverticulitis.

The disease-related factors analyzed in this study were the type of neoadjuvant therapy, radio- or chemotherapy or a combination, surgical indication (Table 1). In addition, several outcome parameters like wound infection, pneumonia, urinary tract infection, abdominal wound dehiscence, hospital stay and stay on the intensive care unit were analyzed as well. The primary outcome measure was clinically manifest anastomotic leakage, confirmed by imaging or relaparotomy. In case of imaging, leakage was considered to be present when free air or contrast was visible around the anastomosis. In case of relaparotomy, leakage was considered to be present when a dehiscent anastomosis was visualized. Results are summarized as means and standard deviations (SD) or medians and ranges for the continuous variables, the categorical variables are summarized in frequencies. Median and mean values were compared between groups with and without AL by means of the Mann–Whitney test or Chi-square test in univariate analyses. Multivariate analysis was performed with factors that were significant in univariate analysis and consisted of multiple logistic regression with backwards elimination. In the final model, multiple logistic regression with backwards elimination with all factors, significant or nonsignificant in univariate analysis, was performed. Operation duration was transformed logarithmically in this analysis to reduce the influence of outlying observations. A p-value of 0.05 (two-sided) was considered the limit of significance. Analyses were performed with "SPSS 15.0" statistical software.

Results

Patient population

A total of 739 patients received a primary colorectal anastomosis over the selected period. This number included 90 ileocaecal resections, 27 transverse colon resections, 250 right hemicolectomies, 30 subtotal colectomies, 64 left hemicolectomies, 155 sigmoid resections, 18 rectosigmoid resections and 96 low anterior resections after the total mesenterial excision principle (TME). Nine patients underwent non-traditional resections, consisting of eight anastomosis without resection when intraoperatively, the tumor appeared to have progressed too far and one resection of transverse and descending colon. These nine patients were excluded for further risk factor analysis. A total of 92 (13 %) operations were performed laparoscopically, of which 38 (41 %) were converted. The population is described in Table 1.

Population description and univariate analysis[f]				
Factor		**Anastomotic leakage**	**No anastomotic leakage**	**p-value**
BMI (kg/m²) [a]	<25	33 (9 %)	343 (91 %)	0.001
	25-30	13 (6 %)	217 (94 %)	
	>30	15 (20 %)	62 (81 %)	
Age (years) [b]		61 (22 – 84)	60 (18 – 99)	0.953
Gender (M/F)		32(9 %) / 32 (9 %)	346 (91 %) / 329 (91 %)	0.951
Smoker[c]		Yes: 16 (9 %) No: 44 (8 %)	Yes: 163 (91 %) No: 485 (92 %)	0.918
Use of steroids[c]		Yes: 13 (12 %) No: 51 (8 %)	Yes: 96 (88 %) No: 569 (92 %)	0.277
Use of Anti-hypertensive medication[c]		Yes: 7 (5 %) No: 55 (9 %)	Yes: 137 (95 %) No: 532 (91 %)	0.116
Use of statines[c]		Yes: 6 (9 %) No: 56 (8 %)	Yes: 63 (91 %) No: 608 (92 %)	1.000
ASA-score[c]		I: 13 (20 %) II: 27 (42 %) III: 20 (31 %) IV: 1 (2 %) V: 0 (0 %)	I: 162 (24 %) II: 303 (45 %) III: 125 (19 %) IV: 20 (3 %) V: 1 (0,1 %)	0.203
History of vascular disease[c]		Yes: 11 (9 %) No: 51 (8 %)	Yes: 106 (91 %) No: 564 (92 %)	0.831
Cardiac comorbidity[c]		Yes: 13 (9 %) No: 49 (8 %)	Yes: 134 (91 %) No: 539 (92 %)	0.974
Pulmonary comorbidity[c]		Yes: 8 (9 %) No: 49 (8 %)	Yes: 85 (91 %) No: 563 (92 %)	1.000
Diabetes Mellitus[c]		Yes: 6 (9 %) No: 57 (9 %)	Yes: 60 (91 %) No: 606 (91 %)	1.000
Blood transfusion[c]		Yes: 14 (13 %) No: 48 (8 %)	Yes: 91 (87 %) No: 550 (92 %)	0.114
Mechanical Bowel Preparation[d]		Yes: 37 (8 %) No: 16 (10 %)	Yes: 436 (92 %) No: 142 (90 %)	0.460
Neoadjuvant radiotherapy		Yes: 1 (8 %) No: 63 (9 %)	Yes: 11 (92 %) No: 664 (91 %)	1.000
Anastomotic configuration[c]		E-E 7 (12 %) E-S 2 (2 %) S-E 21 (11 %) S-S 33 (9 %)	E-E 52 (88 %) E-S 90 (98 %) S-E 170 (89 %) S-S 345 (91 %)	0.075
Surgical indication		Cancer: 37 (9 %) IBD: 6 (5 %) Diverticular disease: 3 (5 %) Other: 18 (12 %)	Cancer: 374 (91 %) IBD: 109 (95 %) Diverticular disease: 53 (95 %) Other: 139 (89 %)	0.249
Approach		Laparotomy: 59 (9 %) Laparoscopy: 5 (5 %)	Laparotomy: 588 (91 %) Laparoscopy: 87 (95 %)	0.328
Surgeon vs. Assistant		Surgeon 13 (10 %) Assistant 51 (8 %)	Surgeon 114 (90 %) Assistant 561 (92 %)	0.603
Stapled vs. handsutured[c]		Stapled 5 (8 %) Sutured 57 (9 %)	Stapled 57 (92 %) Sutured 617 (91 %)	1.000
Urgent vs. Elective		Urgent: 19 (12 %) Elective: 45 (8 %)	Urgent: 137 (88 %) Elective: 538 (92 %)	0.110
Prophylactic drainage	Yes	21 (15 %)	121 (85 %)	0.006
	No	43 (7 %)	554 (93 %)	
Time of Surgery[e]	During hours	49 (8 %)	584 (92 %)	0.029
	After hours	15 (14 %)	91 (86 %)	
Duration of operation (minutes)[b]		163 (70 – 365)	144 (49 – 497)	0.038
Time under anesthesia (minutes)[b]		226 (110 – 426)	195 (75 – 630)	0.012

Table 1. Description of operated population and univariate analysis
BMI = Body Mass Index (kg/m²), SD = Standard Deviation, ASA = American Society of Anesthesiologists
[a] BMI missing 47 (6,4 %)
[b] Expressed in median and range
[c] Data do not ad up to 739 due to occasional missing of data
[d] MBP unknown in 108 (14,5 %) patients
[e] During hours is defined as the hours in which a new elective procedure was allowed to start (i.e. between 7.45 h and 15.30 h). After hours is defined as the period in which patients were operated upon by the operating team that is on call (i.e. between 15.30 h and 7.45 h).
[f] The group with nine non-traditional resections ("other" in table 2) was excluded for risk factor analysis

Overall, the mean duration of anesthesia was 217 min (SD=81 min) and the mean duration of operation was 162 min (SD=72 min). A total of 633 (86 %) patients were operated upon during-hours, which was defined as the hours in which a new elective procedure was allowed to start (i.e., between 7.45 h and 15.30 h). This means that generally, the elective program ended around 17.30 h–18.00 h. A total of 106 patients (14 %) were operated upon after-hours, which was defined as the period in which patients were operated upon by the operating team that is on call (i.e., between 15.30 h and 7.45 h). A total of 156 (21 %)

patients were operated upon in an emergency setting, 583 (79 %) patients were operated upon in elective setting. The median hospital stay after the operation was 13 days (range 1–180 days) and the median stay on ICU was 0 days (range 0–72 days). The overall in hospital mortality rate was 4.7 %.

Incidence of anastomotic leakage

Anastomotic leakage was diagnosed in 64 (8.7 %) patients, of which nine patients (14 %) died due to consequences of anastomotic leakage (AL). As depicted in Table 2, the highest incidence of AL occurred after resection of the rectosigmoid (17 %), followed by resection of the transverse colon (15 %) and low anterior resection (13 %). The median interval between operation and diagnosis of anastomotic leakage was 8 days (range 2–61 days).

	Performed operations		
Procedure	**No anastomotic leakage**	**Anastomotic leakage**	**Total**
(Neo)-ileocecal resection	86 (96 %)	4 (4 %)	90
Resection transverse colon	23 (85 %)	4 (15 %)	27
(Extended) Hemicolectomy right	235 (94 %)	15 (6 %)	250
Subtotal colectomy	26 (87 %)	4 (13 %)	30
Hemicolectomy left	56 (88 %)	8 (12 %)	64
Resection sigmoid	142 (92 %)	13 (8 %)	155
Rectosigmoid resection	15 (83 %)	3 (17 %)	18
LAR/TME	83 (87 %)	13 (13 %)	96
Other	9 (100 %)	0 (0 %)	9
Total	675 (91,3 %)	64 (8,7 %)	739

Table 2. The incidence of anastomotic leakage stratified for the performed operations.
“Other” contained eight anastomosis without resection when intraoperatively the tumor appeared to have progressed to far and one resection of transverse and descending colon.
Analysis of the anastomotic leakage rate per operation showed a significant trend (p=0,019), regarding the expected increase in leakage rate going from top to bottom in the table (the group “other” was not included in this calculation).

Risk factor analysis

Analysis of the anastomotic leakage rate per operation showed a significant trend, indicating that higher leakage rates occurred as the anastomosis was constructed further downstream (p=0.019, Table 2). Other potential risk factors for anastomotic leakage that reached significance in the univariate analysis were BMI (p=0.001), PD (p=0.006), time of surgery (p=0.029), duration of operation and anesthesia (p=0.038, p=0.012, Table 1). Multivariate analysis showed body mass index (BMI) and time of surgery, classified in “during-” and “after hours", to be independent risk factors (Table 3). Prophylactic drainage was significantly associated with anastomotic leakage as well (Table 3). Adjusted for these factors, no relation was found between leakage rate and the level of anastomosis.

Factor		Multivariate analysis OR	95 % CI	p-value
BMI (kg/m^2)	< 25	1	-	-
	25 – 30	0.6	0.3 – 1.14	0.115
	> 30	2.6	1.3 – 5.2	0.006
Time of surgery	During hours*	1	-	-
	After hours	2.2	1.1 – 4.5	0.030
Prophylactic drainage	No	1	-	-
	Yes	2.8	1.5 – 5.1	0.001

Table 3. Significant results of multivariate analysis.
The group "other" was excluded for the risk factor analysis, therefore the total number of patients included is 730.
*During hours is defined as the hours in which a new elective procedure was allowed to start (i.e. between 7.45 h and 15.30 h). After hours is defined as the period in which patients were operated upon by the operating team that is on call (i.e. between 15.30 h and 7.45 h).
OR= Odds Ratio, CI = Confidence Interval. Reference categories are indicated by OR = 1.

Patient outcome

Mortality rate amongst the patients with anastomotic leakage (14.1 %) was significantly higher than amongst the patients without AL (3.9 %, p=0.001). The incidence of urinary tract infections, pneumonia, ileus, and wound infections did not differ significantly. Abdominal wound dehiscence (AWD), however, occurred more often in the group of patients with AL (8 % vs. 5 %, p=0.046). The mean hospital stay after operation and the stay at the ICU of patients with AL were 47 days and 7 days, respectively, whereas patients without AL stayed in the hospital 14 days and 1 day at the ICU, respectively (p=0.000).

Discussion

Leakage of a colorectal anastomosis still is a vast problem despite decades of research on this matter. This study was performed in order to improve understanding of this complication and to study new potential risk factors.

Incidence of anastomotic leakage

In this study, an overall incidence of 8.7 % was found over a period of 10 years. In literature leakage rates between 3 % and 6 % are reported for colonic anastomosis [28, 29], while leakage rates between 16 % and 19 % for low colorectal anastomosis are reported [2, 30]. Therefore this finding can be considered “average” considering anastomoses in the colon as well as in the rectum were included. The trend that was found in our study, i.e., higher leakage rates with lower anastomosis, is well-known to [31]. The leakage rate after resection of the transverse colon (four of 27) is remarkably high, which is not confirmed in literature [6]. Possibly the involvement of the watershed area at the splenic flexure and Griffiths’ critical point (insufficient marginal artery at splenic flexure) contribute to this high leakage rate [32]. However, since this group is relatively small and the 95 % confidence intervals for the leakage rate is wide (4–34 %), this finding should be considered only as an indication for further research.

Risk factor analysis

The multivariate risk factor analysis showed BMI, time of operation and prophylactic drainage to be major factors, independently associated with anastomotic leakage. A BMI higher than 30, which is considered obese according to the Centers of Disease Control and Prevention (CDC) [33], increased the risk for developing leakage almost three-fold. As to the explanation of this correlation, it remains unclear whether obesity indicates a defect of tissue structure and healing, whether the increased intraabdominal pressure plays a role or whether construction of an anastomosis simply is technically more demanding because of thick mesenteries and epiploic appendices. A possible solution might be to lower the threshold for construction of a diverting stoma in this population [31]. However, since construction of a stoma is difficult in patients with high BMI, sensibilization and a higher alert for anastomotic leakage in these patients on the ward is a more logical consequence of this finding.

Patients operated upon after-hours had more than a twofold increased risk of anastomotic leakage. This finding was independent of the urgency of the operation, which may be explained by the fact that urgent operations were also performed during-hours and elective operations were performed after-hours (Table 4). The latter occurs regularly due to incoming trauma- or transplantation patients that require immediate operation.

Time of surgery	Urgent vs. Elective Urgent	Elective
During hours	64 (10 %)	569 (90 %)
After hours	92 (87 %)	14 (13 %)

Table 4. Distribution of urgent and elective operations

Possible explanations could be found in the fact that significantly more anastomoses were constructed by residents after-hours (95 %) than during-hours (81 %, p<0.001) and that after-hours, obviously, significantly more urgent operations were performed (86 %) than during-hours (10 %, p<0.001). However, these factors were no independent risk factors in multivariate analysis. Since many potential confounders have been accounted for and are not significant (Table 1), it is reasonable to assume a decreased technical performance of the operating team has contributed to a higher leakage rate at night. This assumption is supported by several studies that have shown medical errors to occur more often at night [24, 34]. In addition, physicians appear to be less proficient at night, which leads to more errors at night than during daytime [25–27].

Decreased non-technical skills of the operating team at night, like teamwork- and management skills and situational awareness, could have contributed to higher leakage rates as well. Situational awareness (SA), defined as the ability of the surgeon to observe, understand and predict events in the operating room (OR), appears to be closely related to technical error rates [35]. In addition, the situational awareness, teamwork- and management skills of the anesthetists and nurses may have an important impact on the outcome of surgical patients as well [27]. Based upon these results, it should be considered to construct a diverting stoma when after-hours construction of a colorectal anastomosis is required. Most studies addressing the problem of anastomotic leakage focus on the urgency of the operation. However, few studies report on the time of surgery in relationship to leakage rate. Higher leakage rates at night may be caused by a decreased technical and/or non-technical performance of the entire operating team. Given the current focus of society on medical errors and its prevention, more research should be done on this aspect of colorectal surgery.

Prophylactic drainage (PD) appeared to be associated with anastomotic leakage, suggesting that it is a risk factor. However, statistical analysis shows that significantly more drains are placed after LAR (54 % of all drains, p=0.001) and that the duration of surgery on average is 70 min longer in the group that received a drain (219 min) than in the group without (148 min, p=0.001). The latter is most likely caused by difficulty of operation and adverse events occurring during operation. This shows that the significant association between drainage and anastomotic leakage reflects the prophylactic drain-policy in our hospital. Additionally, since no significant differences in leakage rates between groups with and without drainage were reported in literature [11], PD cannot be considered as a risk factor for anastomotic leakage.

Patient outcome

Mortality rate due to leakage was 14.1 %, which is comparable to mortality rates reported in literature [4, 29]. The aforementioned 33-day increase in mean hospital stay in case of anastomotic leakage was to be expected and is indicative for the enormous increase in costs that is accompanied by this complication. Concerning the postoperative complications, abdominal wound dehiscence (AWD) occurred significantly more often in patients with anastomotic leakage. In literature, several factors associated with abdominal wound dehiscence, being advanced age, obesity, diabetes mellitus, and steroid use [36], are reported. In this population BMI (p=0.06), presence of diabetes mellitus (p=0.99) and use of steroids (p=0.07) were not associated with AWD. The average age of surgery in the group without AWD was 57 years and in the group with AWD 67 years (p=0.010). However, since age is not a factor associated with anastomotic leakage this finding cannot explain the correlation between AWD and leakage. Another possible explanation may be found in connective tissue disorders. To date, a substantial body of evidence exists correlating aortic aneurysms to incisional hernia, both caused by connective tissue disorders [37]. In addition, Stumpf et al. have shown colorectal anastomotic leakage is associated with disturbances of the extracellular matrix [38]. These findings suggest that a patient population exists that has a higher risk for developing aortic aneurysms, hernia and anastomotic leakage. In addition, this concept may contribute to the previously mentioned misunderstanding and lack of consensus on the matter of anastomotic leakage.

Conclusion

Anastomotic leakage after colorectal surgery remains a major complication. In our hospital, overall leakage rate is 8.7 % and mortality rate is 14.1 % in patients with anastomotic leakage. BMI greater than 30 kg/m2 and “after hours” construction of the anastomosis were independent risk factors for anastomotic leakage.

References

1. Hyman N et al. (2007) Anastomotic leaks after intestinal anastomosis: it's later than you think. Ann Surg 245(2):254–258

2. Matthiessen P et al. (2007) Defunctioning stoma reduces symptomatic anastomotic leakage after low anterior resection of the rectum for cancer: a randomized multicenter trial. Ann Surg 246 (2):207–214

3. Platell C et al. (2007) The incidence of anastomotic leaks in patients undergoing colorectal surgery. Colorectal Dis 9(1):71–79

4. Buchs NC et al. (2008) Incidence, consequences, and risk factors for anastomotic dehiscence after colorectal surgery: a prospective monocentric study. Int J Colorectal Dis 23(3):265–270

5. Veyrie N et al. (2007) Anastomotic leakage after elective right versus left colectomy for cancer: prevalence and independent risk factors. J Am Coll Surg 205(6):785–793

6. Choi HK, Law WL, Ho JW (2006) Leakage after resection and intraperitoneal anastomosis for colorectal malignancy: analysis of risk factors. Dis Colon Rectum 49(11):1719–1725

7. Sorensen LT et al. (1999) Smoking and alcohol abuse are major risk factors for anastomotic leakage in colorectal surgery. Br J Surg 86(7):927–931

8. Kirchhoff P, Dincler S, Buchmann P (2008) A multivariate analysis of potential risk factors for intra- and postoperative complications in 1,316 elective laparoscopic colorectal procedures. Ann Surg 248(2):259–265

9. Borowski DW et al. (2007) Impact of surgeon volume and specialization on short-term outcomes in colorectal cancer surgery. Br J Surg 94(7):880–889

10. Contant CM et al. (2007) Mechanical bowel preparation for elective colorectal surgery: a multicentre randomised trial. Lancet 370(9605):2112–2117

11. Jesus EC et al. Prophylactic anastomotic drainage for colorectal surgery. Cochrane Database Syst Rev 2004(4):CD002100

12. Tresallet C et al. (2008) Effect of systemic corticosteroids on elective left-sided colorectal resection with colorectal anastomosis. Am J Surg 195(4):447–451

13. Simillis C et al. (2007) A meta-analysis comparing conventional endto-end anastomosis vs. other anastomotic configurations after resection in Crohn's disease. Dis Colon Rectum 50(10):1674–1687 794 Int J Colorectal Dis (2009) 24:789–795

14. Fingerhut A et al. (1994) Infraperitoneal colorectal anastomosis: hand-sewn versus circular staples. A controlled clinical trial. French Associations for Surgical Research. Surgery 116(3):484–490

15. Peeters KC et al. (2005) Risk factors for anastomotic failure after total mesorectal excision of rectal cancer. Br J Surg 92(2):211–216

16. Lourenco T et al. (2008) Laparoscopic surgery for colorectal cancer: safe and effective?—a systematic review. Surg Endosc 22:1146–1160

17. Foster ME et al. (1985) Colonic ischaemia and anastomotic healing. Eur Surg Res 17(3):133–139

18. Hansson L et al. (1998) Effects of intensive blood-pressure lowering and low-dose aspirin in patients with hypertension: principal results of the Hypertension Optimal Treatment (HOT) randomised trial. HOT Study Group. Lancet 351(9118):1755–1762

19. Wadwa RP (2006) Cardiovascular disease risk in youth with diabetes mellitus. Rev Endocr Metab Disord 7(3):197–204

20. Zieske AW et al. (2005) Smoking is associated with advanced coronary atherosclerosis in youth. Atherosclerosis 180(1):87–92

21. Stamler J et al. (1993) Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the multiple risk factor intervention trial. Diabetes Care 16(2):434–444

22. Gray A (2000) United Kingdom national confidential enquiry into perioperative deaths. Minerva Anestesiol 66(5):288–292

23. Fechner G et al. (2008) Kidney's nightshift, kidney's nightmare? Comparison of daylight and nighttime kidney transplantation: impact on complications and graft survival. Transplant Proc 40 (5):1341–1344

24. Peberdy MA et al. (2008) Survival from in-hospital cardiac arrest during nights and weekends. JAMA 299(7):785–792

25. Smith-Coggins R et al. (1994) Relationship of day versus night sleep to physician performance and mood. Ann Emerg Med 24 (5):928–934

26. Rollinson DC et al. (2003) The effects of consecutive night shifts on neuropsychological performance of interns in the emergency department: a pilot study. Ann Emerg Med 41(3):400–406

27. Catchpole K et al. (2008) Teamwork and error in the operating room: analysis of skills and roles. Ann Surg 247(4):699–706

28. Mann B, Kleinschmidt S, Stremmel W (1996) Prospective study of hand-sutured anastomosis after colorectal resection. Br J Surg 83(1):29–31

29. Alves A et al. (2002) Factors associated with clinically significant anastomotic leakage after large bowel resection: multivariate analysis of 707 patients. World J Surg 26(4):499–502

30. Pakkastie TE, Luukkonen PE, Jarvinen HJ (1995) Anterior resection controls cancer of the rectum as well as abdominoperineal excision. Eur J Surg 161(11):833–839

31. Rullier E et al. (1998) Risk factors for anastomotic leakage after resection of rectal cancer. Br J Surg 85(3):355–358

32. Lange JF et al. (2007) Riolan's arch: confusing, misnomer, and obsolete. A literature survey of the connection(s) between the superior and inferior mesenteric arteries. Am J Surg 193(6):742–748

33. (CDC), C.o.D.C.a.P. Centers of Disease Control and Prevention (CDC). [Website] 2008 [cited 2008 June 5th, 2008]

34. Neuberger JS et al. (1984) Incidence of needlestick injuries in hospital personnel: implications for prevention. Am J Infect Control 12(3):171–176

35. Mishra A et al. (2008) The influence of non-technical performance on technical outcome in laparoscopic cholecystectomy. Surg Endosc 22(1):68–73

36. Riou JP, Cohen JR, Johnson H Jr (1992) Factors influencing wound dehiscence. Am J Surg 163(3):324–330

37. Fassiadis N et al. (2005) Randomized clinical trial of vertical or transverse laparotomy for abdominal aortic aneurysm repair. Br J Surg 92(10):1208–1211

38. Stumpf M et al. (2005) Changes of the extracellular matrix as a risk factor for anastomotic leakage after large bowel surgery. Surgery 137(2):229–234

Chapter 2

Calcium score: a new risk factor for colorectal anastomotic leakage

N. Komen, P. Klitsie, J.W. Dijk, J.C. Slieker, J. Hermans, K. Havenga, M. Oudkerk, J. Weyler, G.J. Kleinrensink, J.F. Lange

Am J Surg. 2011 Jun;201(6):759-65

Abstract

Background

Anastomotic leakage (AL) is the most feared complication of colorectal surgery. Atherosclerosis is suggested to have a detrimental effect on anastomotic healing. This study aimed to analyze the calcium score, a measure for atherosclerosis, as a risk factor for AL.

Study design

The calcium scores of colorectal patients operated on in 2 Dutch university medical centers were determined using a computed tomography scan and calcium scoring software. The aorta, common iliac arteries, internal and external iliac arteries were studied. Additionally, patient- and operation-related factors were scored.

Results

A total of 122 patients were included. In patients with AL calcium scores were significantly higher in the left common iliac artery (561.4 vs. 156.0, *P* = .028), right common iliac artery (542.0 vs. 144.4, *P* = .041), both common iliac arteries together (1,103.3 vs. 301.9, *P* = .046), and the left internal iliac artery (716.3 vs. 35.3, *P* = .044).

Conclusion

Patients with higher calcium scores in the iliacal arteries have an increased leakage risk.

Introduction

Anastomotic leakage (AL) is the most feared complication in colorectal surgery. Aiming to assess the risk for anastomotic leakage, many studies analyzing potential risk factors have been reported. Factors like diverticular disease [1], rectal resection [2], urgent operation [3], smoking [4], body mass index (BMI)[5], sex [6], use of steroids [7] radio- and/or chemotherapy [6], American Society of Anesthesiologists (ASA) score [8], a history of cardiac and vascular disease [9] sutured or stapled anastomosis [10], prophylactic drainage [11] and operating time [6] are considered risk factors. However, despite this knowledge, leakage rates still are high, varying between 2 % and 24 % [12–16] with high mortality and morbidity rates. This suggests that risk factors for AL remain to be discovered.

Ischemia is considered an etiologic factor for AL. At the anastomotic site, local vascular supply is disrupted because of vessel injury, thrombosis, and tissue compression by sutures or staplers or by mobilization of the intestinal limbs. Surgery activates a cascade of events, including platelet degranulation and the release of complement, kinins, and chemotactic factors, which lead to a migration of neutrophils, lymphocytes, macrophages, and fibroblasts into the anastomotic site. This state of higher metabolic activity and higher oxygen demand combined with a decreased vascular supply causes the anastomosis to be hypoxic compared with normal tissue [17]. Atherosclerosis is a known cause of tissue ischemia [18,19] and is suggested to have a detrimental effect on anastomotic healing [20]. At present, it is possible to quantify the atherosclerotic calcifications on computed tomography (CT) images by means of calcium scoring software. It allows measurement of the calcium mass, calcium volume, number of calcifications, and the calcium score. The latter represents the total atherosclerotic load in the analyzed trajectory [21,22]. With this tool, the study of atherosclerosis as a risk factor for anastomotic leakage is possible. The aim of this study was to analyze the calcium score as a risk factor for anastomotic leakage. It is hypothesized that higher calcium scores are predictive for higher leakage rates.

Methods

All patients who received a primary colorectal anastomosis, irrespective of indication, during elective or emergency surgery at the Erasmus MC between 2002 and 2006 and at the UMCG between 2005 and 2007 were selected by operation code. Patients within this selection whom received a preoperative contrast-enhanced CT-scan with a slice thickness of 5 mm were included. The calcium scores were determined in the following segments of the aortoiliac trajectory: the aorta starting from the T12-L1 level, left and right common iliac arteries, left and right internal iliac arteries, and the left and right external iliac arteries. Scoring was performed with a lower threshold of 500 Hounsfield units using the following software: Siemens Calcium Score (Syngo CT 2006G-W, Siemens, Forcheim, Germany). Together with the calcium score, this software allows measurement of the calcium mass, the calcium volume, and the number of calcifications in a designated trajectory. To describe the studied population, after CT-scan analysis, patient- and operation-related factors were scored (i.e., age, use of antihypertensive drugs and statins, smoking, cardiac comorbidity, history of vascular disease, diabetes mellitus, sex, BMI, use of steroids, type of anastomosis, urgent vs. elective operation, type of operation, prophylactic drainage, ASA score, neoadjuvant therapy, approach, stapled vs. hand sutured, and time under anesthesia respectively). The primary outcome measure was clinically manifest anastomotic leakage, confirmed by imaging or relaparotomy. Whether leakage occurred or not was determined after CT-scan analysis and after the search for patient- and operation-related factors to prevent bias. The median and mean values were compared between groups with and without AL by means of the Mann-Whitney *U* test or chi-square test in univariate analysis. Multiple regression analysis was performed for the calcium score, calcium mass, and calcium volume separately, with factors that were significant in univariate analysis and consisted of multiple logistic regression with backwards elimination. In the final model, multiple logistic regression with backwards elimination of all factors, significant or nonsignificant in univariate analysis, was performed. Statistical analysis was performed with SPSS 12.0 (SPSS Inc, Chicago, IL).

Results

A total of 122 patients were included of whom 11 (9 %) developed anastomotic leakage. In both the group with and without leakage, 3 patients died (27 % vs. 2.7 % [*P* = .004]). None of the scored patient- and operation-related factors were significantly different in univariate analysis as depicted in Table 1.

The number of calcified lesions in the right common iliac artery was significantly different between patients with and without leakage. Differences in calcium volume were significant for the total trajectory, the left common iliac artery, the right common iliac artery, both common iliac arteries together, and the left internal iliac artery. Differences in calcium mass were significant for the total trajectory, the aorta, the left common iliac artery, the right common iliac artery, both common iliac arteries together, and the left internal iliac artery. Differences in the calcium score were significant for the left common iliac artery, the right common iliac artery, both common iliac arteries together, and the left internal iliac artery. The results of the analyses are depicted in Tables 2 to 5.

In some patients, the CT-scan started below the T12-L1 level or ended prematurely. Therefore, not all trajectories could be scored and the number of scored patients does not always add up to 122. Multiple regression analysis as described in the Methods section was performed for the calcium score, mass, and volume in the right and left common iliac arteries together. All 3 parameters were independent risk factors for anastomotic leakage (Table 6).

Comments

AL is the most feared complication of colorectal surgery for which the construction of a diverting stoma (DS) is the most common strategy. Some studies recommend routine construction [14]. However, because DS is associated with considerable morbidity, an impact on quality of life, and mortality after stoma closure [23–27], a more selective approach is warranted. Knowledge of risk factors is required to perform an educated risk assessment preoperatively, allowing a tailor made decision whether or not to construct DS.

Univariate analysis				
Factor		**AL**	**No AL**	**p-value**
Hospital	EMC	7 (9 %)	66 (91 %)	1.000
	UMCG	4 (8 %)	45 (92 %)	
BMI (kg/m^2) *		24.6 ± 2.7	25.5 ± 4.2	0.454
Gender (M/F)	Male	5 (7 %)	66 (93 %)	0.563
	Female	6 (12 %)	45 (88 %)	
Use of steroids†	Yes	3 (27 %)	8 (73 %)	0.099
	No	8 (7 %)	102 (93 %)	
ASA-score†	1	5 (10 %)	45 (90 %)	0.492
	2	4 (8 %)	45 (92 %)	
	3	1 (6 %)	16 (94 %)	
	4	1 (33 %)	2 (67 %)	
Neoadjuvant radiotherapy	Yes	1 (5 %)	19 (95 %)	0.796
	No	10 (10 %)	92 (90 %)	
Anastomotic configuration†	End to End	3 (20 %)	12 (80 %)	0.334
	End to Side	0 (0 %)	9 (100 %)	
	Side to End	2 (6 %)	31 (94 %)	
	Side to Side	5 (10 %)	46 (90 %)	
Type of operation‡	Right sided	3 (9 %)	29 (91 %)	0.974
	Left sided	5 (9 %)	48 (91 %)	
	Rectum	3 (8 %)	34 (92 %)	
Approach	Laparotomy	10 (10 %)	95 (90 %)	0.976
	Laparoscopy	1 (6 %)	16 (94 %)	
Stapled vs. hand sutured	Stapled	2 (4 %)	46 (96 %)	0.237
	Sutured	9 (12 %)	65 (88 %)	
Urgent vs. Elective†	Urgent	1 (6 %)	15 (94 %)	1.000
	Elective	9 (9 %)	96 (91 %)	
Prophylactic Drainage	Yes	3 (7 %)	37 (93 %)	0.943
	No	8 (10 %)	74 (90 %)	
Time under anaesthesia (minutes)		257.7 ± 58.0	250.8 ± 95.7	0.526
Age (years)		60.3 ± 12.4	59.9 ± 16.5	0.841
Use of Anti-hypertensive medication†	Yes	3 (11 %)	25 (89 %)	0.790
	No	6 (7 %)	82 (93 %)	
Use of statins†	Yes	2 (14 %)	12 (86 %)	0.659
	No	7 (7 %)	95 (93 %)	
History of vascular disease†	Yes	3 (14 %)	19 (86 %)	0.600
	No	7 (7 %)	88 (93 %)	
Cardiac comorbidity†	Yes	3 (12 %)	21 (88 %)	0.713
	No	7 (7 %)	86 (93 %)	
Smoker†	Yes	3 (14 %)	18 (86 %)	0.801
	No	7 (9 %)	68 (91 %)	
Diabetes Mellitus†	Yes	1 (10 %)	9 (90 %)	1.000
	No	9 (8 %)	98 (92 %)	

Table 1. Univariate analysis of patient- and operation-related factors.
Age, BMI and time under anaesthesia are expressed in means and standard deviation
* BMI = Body Mass Index (kg/m^2)
† Data do not ad up to 122 due to occasional missing of data.
‡ Right sided includes Ileocecal resection and right hemicolectomy
Left sided includes left hemicolectomy and sigmoid resection
Rectum includes Total Mesorectal Excision (TME) and rectosigmoid resection

Number of lesions					
Trajectory	**AL**	**N**	**Mean**	**SD**	**p-value**
Total trajectory	Yes	11	19,7	17,1	0.067
	No	105	9·2	12·7	
Aorta	Yes	11	10·4	10·0	0.076
	No	109	5·4	8·5	
Left common iliac artery	Yes	11	3·3	3·2	0.072
	No	108	1·7	3·0	
Right common iliac artery	Yes	11	4·7	4·5	**0.036**
	No	108	1·6	2·6	
Left internal iliac artery	Yes	11	1·5	1·6	0.060
	No	106	0·7	1·4	
Left external iliac artery	Yes	11	0·3	0·9	0.963
	No	106	0·1	0·5	
Right internal iliac artery	Yes	11	1·4	1·7	0.184
	No	107	0·6	1·3	
Right external iliac artery	Yes	11	1·3	3·1	0.222
	No	107	0·1	0·4	
Left and right common iliac arteries	Yes	11	8·0	7·6	0.076
	No	108	3·3	5·4	
Left and right internal iliac arteries	Yes	11	2·8	3·2	0.128
	No	106	1·3	2·5	

Table 2. Number of calcified lesions per trajectory.
AL = Anastomotic Leakage; N = the number of patients; Mean = mean number of lesions; SD = standard deviation.

Calcium volume					
Trajectory	**AL**	**N**	**Mean**	**SD**	**p-value**
Total trajectory	Yes	11	1750.6	1902.8	**0.043**
	No	105	520.8	1098.6	
Aorta	Yes	11	1030.8	1351.3	0.057
	No	109	376.2	814.7	
Left common iliac artery	Yes	11	333.0	358.2	**0.025**
	No	108	82.2	169.6	
Right common iliac artery	Yes	11	290.4	287.2	**0.040**
	No	108	75.5	187.9	
Left internal iliac artery	Yes	11	443.4	1360.6	**0.049**
	No	106	16.1	44.5	
Left external iliac artery	Yes	11	0.6	2.1	0.985
	No	106	2.4	12.9	
Right internal iliac artery	Yes	11	35.0	56.5	0.280
	No	107	20.9	78.8	
Right external iliac artery	Yes	11	10.2	31.5	0.275
	No	107	4.8	24.1	
Left and right common iliac arteries	Yes	11	623.5	604.7	**0.041**
	No	108	157.7	335.2	
Left and right internal iliac arteries	Yes	11	478.4	1376.7	0.165
	No	106	36.9	116.2	

Table 3. Calcium volume per trajectory.
AL = Anastomotic Leakage; N = the number of patients; Mean = mean calcium volume; SD = standard deviation.

Calcium mass					
Trajectory	**AL**	**N**	**Mean**	**SD**	**p-value**
Total trajectory	Yes	10	1322.3	1241.4	**0.014**
	No	103	377.0	769.9	
Aorta	Yes	10	755.2	856.0	**0.023**
	No	107	261.7	540.1	
Left common iliac artery	Yes	10	267.8	255.1	**0.010**
	No	107	65.0	133.5	
Right common iliac artery	Yes	10	247.0	224.0	**0.014**
	No	107	57.8	139.8	
Left internal iliac artery	Yes	10	323.9	919.7	**0.022**
	No	105	13.7	34.8	
Left external iliac artery	Yes	10	0.9	2.8	0.946
	No	105	2.1	10.6	
Right internal iliac artery	Yes	10	33.4	48.2	0.155
	No	106	16.3	57.0	
Right external iliac artery	Yes	10	9.9	27.8	0.223
	No	106	3.5	16.9	
Left and right common iliac arteries	Yes	10	514.8	447.7	**0.014**
	No	107	122.9	257.2	
Left and right internal iliac arteries	Yes	10	357.3	937.1	0.083
	No	105	29.8	87.3	

Table 4. Calcium mass per trajectory.
AL = Anastomotic Leakage; N = the number of patients; Mean = mean calcium mass; SD = standard deviation.

Calcium score					
Trajectory	**AL**	**N**	**Mean**	**SD**	**p-value**
Total trajectory	Yes	11	2716.8	2904.5	0.052
	No	105	896.8	1798.1	
Aorta	Yes	11	1489.4	2054.2	0.122
	No	109	618.5	1248.4	
Left common iliac artery	Yes	11	561.4	571.4	**0.028**
	No	108	156.0	318.3	
Right common iliac artery	Yes	11	542.0	538.7	**0.041**
	No	107	144.4	337.2	
Left internal iliac artery	Yes	11	716.3	2116.1	**0.044**
	No	106	35.3	87.4	
Left external iliac artery	Yes	11	2.4	7.9	0.985
	No	106	5.4	25.8	
Right internal iliac artery	Yes	11	78.0	117.8	0.232
	No	107	39.7	132.0	
Right external iliac artery	Yes	11	26.9	79.0	0.267
	No	107	8.3	38.2	
Left and right common iliac arteries	Yes	11	1103.3	1056.6	**0.046**
	No	107	301.9	623.8	
Left and right internal iliac arteries	Yes	11	794.2	2162.0	0.144
	No	106	74.6	209.1	

Table 5. Calcium score per trajectory.
AL = Anastomotic Leakage; N = the number of patients; Mean = mean calcium score; SD = standard deviation.

Multiple regression analyses			
Right and left common iliac arteries	**OR**	**95 % CI**	**p-value**
Calcium score	1.001	1.000 – 1.002	0.003
Calcium mass	1.003	1.001 – 1.004	0.001
Calcium volume	1.002	1.001 – 1.003	0.002

Table 6. Results of multivariate analysis performed for calcium score, -mass and volume in the right and left common iliac arteries together. Each parameter is an independent risk factor for AL. Odds ratio's (OR) show that an increase of one unit of calcium score, -mass or –volume leads to an increased risk for AL of 0.1 %· 0.3 % and 0.2 % respectively.

Atherosclerosis may have a detrimental effect on anastomotic healing because it can lead to ischemia of the intestinal limbs of anastomosis. Therefore, it can be a risk factor that was still unaddressed. In this study, quantification tools for atherosclerosis (i.e., calcium score, mass, and volume) were used to analyze the potential association between anastomotic leakage and atherosclerosis. By means of multivariate analysis, this study has shown that the mean calcium load, expressed as calcium score, mass, and volume, determined in the common iliac arteries is an independent risk factor for AL. Consequently, these tools can and should be used in the decision-making process of whether or not to construct an anastomosis and/or DS. Many risk factors for AL have been reported in the literature, and the majority has been analyzed in this study. No statistically significant differences were found between the groups with and without leakage. The 2 groups are similar except for the calcium score, calcium mass, and volume. This enforces our finding, however, it raises questions on whether the studied population represents the overall population. In addition, univariate analysis has shown the calcium score determined in only the left internal iliac artery to be significantly higher in patients with AL compared with patients without AL. However, this finding cannot be explained anatomically or physiologically. Therefore, multiple regression analysis was not performed for this segment. To address these questions and the small number of patients with AL, a prospective study with larger patient numbers should be performed.

The calcium (Agatson) score is the product of the area of calcification (mm^2) and its radiodensity (Hounsfield units). It is commonly used in studies on atherosclerosis with regard to the coronary arteries. However, controversy remains on whether the total atherosclerotic load is represented. Studies have been published showing the calcium score to represent the entire atherosclerotic load, meaning the noncalcified as well as the calcified plaques [22]. However, a more recent publication suggests that this may not be true and that the calcium score is an underestimation of the real atherosclerotic load [28]. Whether or not the total atherosclerotic load is represented by the calcium score does not affect the results of this study addressing the association between atherosclerotic calcifications and AL. This association indicates that macroangiopathy (i.e., calcifications within the aortoiliac trajectory), is a predictor for AL.

The software used in this study was designed to measure calcium volume and calcium mass parallel to the calcium score. For both parameters, a similar association with leakage was found as for the calcium score (Tables 3 and 4). In addition, differences in calcium mass were significant for the total trajectory and aorta as well. Differences in calcium volume were different for the total trajectory as well. Particularly the association between calcium mass and AL is an interesting finding in view of the interchangeability between different labels of CT-scanners. Because a constant mass of calcium should yield similar results in different CT-scanners, this parameter is best suited to detect differences between systems. The determination of calcium mass allows the comparison of calcium loads quantified with different systems. The results of this study indicate that the calcium mass does not necessarily have to be determined in the common iliac arteries, allowing certain flexibility in scanning protocols.

The number of calcifications does not appear to be a significant factor, the difference is only significant in the right common iliac artery. This is understandable considering a certain score, mass, or volume of calcium can contain multiple small lesions or few larger lesions. Multiple regression analysis was performed for the 2 common iliac arteries together because these were significant for the calcium score, mass, and volume. The odds ratios (Table 6) showed that an increase of 1 U of calcium score, mass, or volume led to an increased risk for AL of 0.1 %, 0.3 % and 0.2 %, respectively. The difference between patients with and without AL for the calcium score was 801.4, calcium mass 391.9, and calcium volume 465.8 (Tables 3–5). This means an 80 % (0.1 x 801.4) increased risk for AL based on the calcium score, 118 % (0.3 x 391.9) increased risk for AL based on calcium mass, and 93 % (0.2 x 465.8) increased risk for AL based on calcium volume.

The quantification of atherosclerotic calcifications as described in this study requires CT imaging of the abdomen. Because abdominal CT-scan still is the principle staging tool for colorectal cancer, obtaining information on atherosclerotic calcifications does not require additional imaging [29,30].

However, for rectal cancer, according to the European guidelines, only an upper-abdominal CT-scan is indicated to search for liver metastasis [31]. This means that for quantification of atherosclerotic calcifications in the (common-) iliac arteries additional imaging is required. However, considering the relatively low cost and speed of CT-scan, this is acceptable.

Conclusions

This study has shown that atherosclerotic calcifications in the left and right common iliac arteries, expressed as calcium score, mass, and volume, are an independent risk factor for colorectal anastomotic leakage. An increase of 1 U of calcium score, mass, or volume leads to an increased risk for AL of 0.1 %, 0.3 % and 0.2 %, respectively. These parameters can be used in a preoperative risk assessment for anastomotic leakage, allowing tailor-made decisions on the construction of an anastomosis and/or a protective or definite stoma.

References

1. Platell C, Barwood N, Dorfmann G, et al. The incidence of anastomotic leaks in patients undergoing colorectal surgery. Colorectal Dis 2007;9:71–9.

2. Buchs NC, Gervaz P, Secic M, et al. Incidence, consequences, and risk factors for anastomotic dehiscence after colorectal surgery: a prospective monocentric study. Int J Colorectal Dis 2007;23:265–70.

3. 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.

4. Sorensen LT, Jorgensen T, Kirkeby LT, et al. Smoking and alcohol abuse are major risk factors for anastomotic leakage in colorectal surgery. Br J Surg 1999;86:927–31.

5. Nickelsen TN, Jorgensen T, Kronborg O. Lifestyle and 30-day complications to surgery for colorectal cancer. Acta Oncol 2005;44:218–23.

6. Matthiessen P, Hallbook O, Andersson M, et al. Risk factors for anastomotic leakage after anterior resection of the rectum. Colorectal Dis 2004;6:462–9.

7. Konishi T, Watanabe T, Kishimoto J, et al. Risk factors for anastomotic leakage after surgery for colorectal cancer: results of prospective surveillance. J Am Coll Surg 2006;202:439–44.

8. Alves A, Panis Y, Trancart D, et al. Factors associated with clinically significant anastomotic leakage after large bowel resection: multivariate analysis of 707 patients. World J Surg 2002;26:499 –502.

9. Makela JT, Kiviniemi H, Laitinen S. Risk factors for anastomotic leakage after left-sided colorectal resection with rectal anastomosis. Dis Colon Rectum 2003;46:653– 60.

10. Lustosa SA, Matos D, Atallah AN, et al. Stapled versus handsewn methods for colorectal anastomosis surgery. Cochrane Database Syst Rev 2001;3:CD003144.

11. Jesus EC, Karliczek A, Matos D, et al. Prophylactic anastomotic drainage for colorectal surgery. Cochrane Database Syst Rev 2004;4: CD002100.

12. Hagmuller E, Lorenz D, Werthmann K, et al. [Uses and risks of drainage following elective colon resection. A prospective, randomized and controlled clinical study] nutzen und

Risiken einer Drainage nach elektiven Colonresektionen. Eine prospektive, randomisierte und kontrollierte klinische Studie. Chirurg 1990;61:266 –71.

13. 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.

14. Matthiessen P, Hallbook O, Rutegard J, et al. Defunctioning stoma reduces symptomatic anastomotic leakage after low anterior resection of the rectum for cancer: a randomized multicenter trial. Ann Surg 2007;246:207–14.

15. Sagar PM, Couse N, Kerin M, et al. Randomized trial of drainage of colorectal anastomosis. Br J Surg 1993;80:769 –71.

16. Komen N, Dijk JW, Lalmahomed Z, et al. After-hours colorectal surgery: a risk factor for anastomotic leakage. Int J Colorectal Dis 2009;24:789 –95.

17. Chang N, Goodson WH 3rd, Gottrup F, et al. Direct measurement of wound and tissue oxygen tension in postoperative patients. Ann Surg 1983;197:470–8.

18. Scharff JR, Longo WE, Vartanian SM, et al. Ischemic colitis: spectrum of disease and outcome. Surgery 2003;134:624 –9; [Discussion: 629–630].

19. Greenwald DA, Brandt LJ, Reinus JF. Ischemic bowel disease in the elderly. Gastroenterol Clin North Am 2001;30:445–73.

20. Foster ME, Brennan SS, Morgan A, et al. Colonic ischaemia and anastomotic healing. Eur Surg Res 1985;17:133–9.

21. Greenland P, Bonow RO, Brundage BH, et al., American College of Cardiology Foundation Clinical Expert Consensus Task Force (ACCF/ AHA Writing Committee to Update the 2000 Expert Consensus Document on Electron Beam Computed Tomography), Society of Atherosclerosis Imaging and Prevention, Society of Cardiovascular Computed Tomography. ACCF/AHA 2007 clinical expert consensus document on coronary artery calcium scoring by computed tomography in global cardiovascular risk assessment and in evaluation of patients with chest pain: a report of the American College of Cardiology Foundation Clinical Expert Consensus Task Force (ACCF/AHA Writing Committee to

Update the 2000 expert consensus document on electron beam computed tomography) developed in collaboration with the Society of Atherosclerosis Imaging and Prevention and the Society of Cardiovascular Computed Tomography. J Am Coll Cardiol 2007;49:378 – 402.

22. Rumberger JA, Simons DB, Fitzpatrick LA, et al. Coronary artery calcium area by electron-beam computed tomography and coronary atherosclerotic plaque area. A histopathologic correlative study. Circulation 1995;92:2157– 62.

23. Engel J, Kerr J, Schlesinger-Raab A, et al. Quality of life in rectal cancer patients: a four-year prospective study. Ann Surg 2003;238: 203–13.

24. Gunnarsson U, Karlbom U, Docker M, et al. Proctocolectomy and pelvic pouch—is a diverting stoma dangerous for the patient? Colorectal Dis 2004;6:23–27.

25. Hallbook O, Matthiessen P, Leinskold T, et al. Safety of the temporary loop ileostomy. Colorectal Dis 2002;4:361– 4.

26. Gastinger I, Marusch F, Steinert R, et al. Working Group ‘Colon/Rectum Carcinoma’. Protective defunctioning stoma in low anterior resection for rectal carcinoma. Br J Surg 2005;92:1137– 42.

27. Guenaga KF, Lustosa SA, Saad SS, et al. Ileostomy or colostomy for temporary decompression of colorectal anastomosis. Cochrane Database Syst Rev 2007;1:CD004647.

28. Henneman MM, Schuijf JD, Pundziute G, et al. Noninvasive evaluation with multislice computed tomography in suspected acute coronary syndrome: plaque morphology on multislice computed tomography versus coronary calcium score. J Am Coll Cardiol 2008;52:216 –22.

29. Van Cutsem EJ, Oliveira J. Advanced colorectal cancer: ESMO clinical recommendations for diagnosis, treatment and follow-up. Ann Oncol 2008;19(Suppl 2):ii33– 4.

30. Van Cutsem EJ, Oliveira J. Colon cancer: ESMO clinical recommendations for diagnosis, adjuvant treatment and follow-up. Ann Oncol 2008;19(suppl 2):ii29 –30.

31. Glimelius B, Oliveira J. Rectal cancer: ESMO clinical recommendations for diagnosis, treatment and follow-up. Ann Oncol 2008;19(Suppl 2): ii31–2.

Chapter 3

Long-term and Perioperative Corticosteroids in Anastomotic leakage: A Prospective Study of 259 Left-Sided Colorectal Anastomoses

The APPEAL-study: Analysis of Parameters Predictive for Evident Anastomotic Leakage.

J. C. Slieker, N. Komen, G. Mannaerts, T. Karsten, P. Willemsen, M. Murawska, H. Jeekel, J.F. Lange

Arch Surg. 2012 May;147(5):447-52

Objective: To determine the risk factors for symptomatic anastomotic leakage (AL) after colorectal resection.

Design: Review of records of patients who participated in the Analysis of Parameters Predictive for Evident Anastomotic Leakage study.

Setting: Eight health centers.

Patients: Two hundred fifty-nine patients who underwent left-sided colorectal anastomoses.

Intervention: Corticosteroids taken as long-term medication for underlying disease or perioperatively for the prevention of postoperative pulmonary complications.

Main Outcome Measures: Prospective evaluations for risk factors for symptomatic AL.

Results: In 23 % of patients, a defunctioning stoma was constructed. The incidence of AL was 7.3 %. The clinical course of patients with AL showed that in 21 % of leaks, the drain indicated leakage, in the remaining patients, computed tomography or laparotomy resulted equally often in the detection of AL. In 50 % of patients with AL, a Hartmann operation was needed. The incidence of AL was significantly higher in patients with pulmonary comorbidity (22.6 % leakage), patients taking corticosteroids as long-term medication (50 % leakage), and patients taking corticosteroids perioperatively (19 % leakage). Perioperative corticosteroids were prescribed in 8 % of patients for the prevention of postoperative pulmonary complications.

Conclusions: We found a significantly increased incidence of AL in patients treated with long-term corticosteroids and perioperative corticosteroids for pulmonary comorbidity. Therefore, we recommend that in this patient category, anastomoses should be protected by a diverting stoma or a Hartmann procedure should be considered to avoid AL.

Anastomotic Leakage (AL) following colorectal resection is a feared complication. The reported incidence of AL is estimated to be between 3 % and 19 % [1-5], and when it occurs, mortality is between 10 % and 20 % [4,6-8]. There are several basic requirements concerning the construction of a colorectal anastomosis: blood flow should be adequate, the anastomosis should be free of tension, and the abdomen should be free of infectious disease and have minimal contamination. Nevertheless, the problem of AL has not diminished over the years [9]. Although accurate prediction of risk is difficult, certain factors are known to contribute to the risk for AL. There is an inverse relationship between the height of the anastomosis from the anal verge and the incidence of AL, with extraperitoneal anastomoses carrying the highest risk [10-15]. For these high-risk anastomoses, studies have demonstrated the important reduction in mortality and morbidity that can be achieved by the construction of a diverting stoma [4,16,17]. Much research has been done to determine additional risk factors for AL. Reported risk factors include male sex [12,13,16], smoking [18], radiotherapy [1,12,19], blood transfusion [1,15,16,20], obesity [5,13] and atherosclerosis [21]. Although corticosteroids are known to impair wound healing, their influence on the healing of colorectal anastomoses is unclear and studies have reported conflicting results [22-25]. The aim of this prospective study was to determine risk factors for clinical AL in patients undergoing left sided colorectal anastomoses, together with a description of diagnostic and therapeutic approaches in patients having developed AL.

Methods

Patients included in this study all participated in the Analysis of Parameters Predictive for Evident Anastomotic Leakage study. In our prospective study, all patients undergoing left sided colorectal resection with construction of an anastomosis were given an intra-abdominal drain. During the first 5 postoperative days, drain fluid was collected for analysis in search of a predictive parameter for AL. Results of drain fluid analysis will be reported separately. Eight centers participated in this study. The study, registered in the Netherlands Trial Register (http://www.trialregister.nl/trialreg/index.asp, study No. NTR1258), was approved by the medical ethical committees of the participating centers in accordance with the ethical standards of the Declaration of Helsinki of 1975, and all patients gave informed consent. Participating centers included patients consecutively treated between January 2007 and December 2009. Centers stopped including patients earlier if their target inclusion was fulfilled.

Inclusion criteria

Surgical procedures included left hemicolectomy, sigmoidectomy, high anterior resection or partial mesorectal excision, low anterior resection or total mesorectal excision, and subtotal colectomy with ileorectal anastomosis. Oncologic resections as well as resections for inflammatory disease were included. Emergency operations and colostomy reversals were excluded.

Surgical Procedure

The surgical procedure was left to the surgeon’s discretion. All patients received preoperative antibiotic prophylaxis and an intra-abdominal drain, but guidelines concerning bowel reparation differed for each center. Patients were operated on by laparotomy or laparoscopy and the anastomosis was stapled or hand sewn, either end to end, end to side, side to end, or side to side. A diverting stoma was constructed according to the surgeon’s preference.

Definitions

Symptomatic AL, the end point of our analysis, was defined as clinically apparent leakage (e.g.: gas, pus, or fecal discharge from the pelvic drain), apparent AL during reoperation, or extravasation of endoluminally administrated water-soluble contrast on computed tomography or contrast enema. Radiologic examination was performed only when there was clinical suspicion of AL. All postoperative fistulas communicating with the surgical anastomosis were classified as leaks. Postoperative abscesses were classified as AL if there was extravasation of enteric contrast on an imaging study, significant perianastomotic air, or communication with the anastomosis noted after radiologic drainage.

Asymptomatic AL was not considered since routine contrast enema was not performed postoperatively.

High anterior resection was defined as a colorectal resection with an intraperitoneal anastomosis.

Low anterior resection was defined as a colorectal anastomosis with an extraperitoneal anastomosis.

Data collection

Patients were followed up from their preoperative admission to the ward until the first postoperative follow-up at the outpatientclinic. Demographic data of the patients, operative details, postoperative events, and follow-up data were obtained through a standardized case record form and entered into a database. We used the *International Classification of Diseases, Tenth Revision* codes to define pulmonary and cardiac comorbidities. The diseases included in pulmonary comorbidity are chronic lower respiratory diseases (J40-J47), lung diseases due to external agents (J60-J70), other respiratory diseases principally affecting the interstitium (J80-J84), other diseases of pleura (J90-J94), and other diseases of the respiratory system (J95-J99). The diseases included in cardiac comorbidity are chronic rheumatic heart diseases (I05-I09), ischemic heart diseases (I20-I25), pulmonary heart disease and diseases of pulmonary circulation (I26-I28), and other forms of heart disease (I29-I52). Use of corticosteroids was subdivided in patients with long-term corticosteroid use for underlying pathology (herein called long-term corticosteroids) and patients having a corticosteroid scheme prescribed by the anesthesiologist or lung specialist for the reduction of postoperative pulmonary complications starting 5 days prior to the surgical intervention (herein called perioperative corticosteroids). In case of AL, the postoperative day of diagnosis was noted along with the clinical manifestation of AL, the diagnostic tool for leak detection, and the treatment. Postoperative mortality included patients who died within 30 days after an operation either at the hospital or after discharge.

Statistical Analysis

Categorical data are presented as numbers with percentages, and numerical data are presented as medians with interquartile ranges. Univariate analysis of the differences between patients having a protective ileostomy or not was performed using X^2-test for categorical data and a Mann-Whitney *U* test for numerical data. Anastomotic leakage binary response (yes or no) was analyzed using a logistic regression model, reporting the odds ratios (ORs) together with 95 % confidence intervals (CIs). The univariate and multivariate analyses were conducted with the following covariates: age (categorized at median = 65 years), sex, body mass index (categorized at 30 kg/m^2), American Society of Anesthesiologists classification (3 or 4 vs. 1 or 2), smoking (yes or no), cardiac comorbidity (yes or no), pulmonary comorbidity (yes or no), corticosteroids (long-term, perioperatively, or none), radiotherapy (yes or no), type of resection (low anterior resection, high anterior resection, sigmoidectomy, left hemicolectomy, or subtotal colectomy), height of anastomosis (>7 cm or <7 cm), anastomosis construction (stapled or hand sewn), configuration (end to end, end to side, side to end, or side to side), and protective ileostomy (yes or no). In the multivariate analysis, the initial model contained all the covariates with the grouped type of resection (low anterior resection vs. others). A backward elimination procedure was applied to remove nonsignificant covariates with $P \geq 0.1$.

Results

A total of 259 patients underwent colorectal resection with left-sided anastomosis. Nineteen patients (7.3 %) developed clinical AL. Mean (SD) age was 64.6 (0.75) years, 144 patients (56 %) were men and 115 (44 %) were women. Thirty-five patients (13.6 %) were treated for inflammatory diseases, 220 (84.9 %), for malignancy, and 4 (1.5 %), for ischemic colitis. Sixty patients (23 %) underwent preoperative radiotherapy, and 89 (34 %) had anastomosis situated below the peritoneal reflection. In 60 patients (23 %) a defunctioning stoma was constructed.

Anastomotic Leakage

Nineteen patients (7.3 %) developed clinical AL. The median postoperative day of diagnosis of AL was 6 (range, 4-10 days). Mortality was significantly increased in patients with AL, compared with patients without AL (15.8 % vs. 2.5 %, p=.02). Four patients (21.1 %) had a diverting stoma at the time of diagnosis. In 3 patients AL manifested with a pelvic or intra-abdominal abscess, 7 had peritonitis and 9 had signs of sepsis. In 8 patients AL was diagnosed through computed tomography, in 7 patients diagnosis was made during laparotomy, and 4 patients had fecal discharge from the drain confirming the diagnosis. In 3 patients treatment consisted of antibiotics and 2 patients had drainage and irrigation of an abscess. The remaining patients were reoperated on: 4 patients had fecal diversion, 2 had reanastomosis, and the remaining 8 had the anastomosis converted into end colostomy. Patients with a diverting ileostomy did not have a clinically lighter presentation of AL or less invasive treatment of the AL.

Univariate and multivariate analysis

Table 1 shows the result of the univariate analysis for the risk for clinical AL. Sex, body mass index, American Society of Anesthesiologists classification, smoking, preoperative radiotherapy, height of the anastomosis, type of resection, and construction of a defunctioning stoma were not associated with an increased risk for AL. Two factors were significant in the univariate analysis: pulmonary comorbidity and use of corticosteroids. The incidence of AL was significantly higher in patients taking corticosteroids as long-term medication (50 % AL, p=.002) and those taking corticosteroids perioperatively (19 % AL, p= .001), compared with patients not taking corticosteroids (5.2 % AL). In patients known to have pulmonary comorbidity, the incidence of AL was 22.6 %; this was 5.3 % in patients without pulmonary comorbidity (p=.002). Multivariate analysis was performed, showing a significant result for perioperative corticosteroids (OR 26.98, standard error 30.71, p=.004, 95 % CI 2.89-251.10) but a wide CI because of a relatively small quantity of cases, therefore, we decided to perform a multivariate analysis with long-term corticosteroids and perioperative steroids combined. From that final multivariate model, it can be concluded that taking corticosteroids increased the risk for AL by more than 7 times (OR 7.52, standard error 4.47, p=.001, 95 % CI 2.35-24.08).

Patients with low anastomosis had a nearly 3 times greater risk for AL, compared with patients with high anastomosis (OR 2.98, standard error 1.65, p=.049, 95 % CI 1.01-8.83). There was no difference in the risk for AL in patients taking corticosteroids between high and low anastomoses. The characteristics of patients with and without a defunctioning ileostomy are demonstrated in Table 2. Significantly more patients with preoperative radiotherapy and low anastomoses or low anterior resections had a diverting stoma. A diverting stoma was equally constructed in patients receiving corticosteroids or not receiving corticosteroids.

Comment

Risk assessment for AL is important because it could lead to better management of high-risk patients. For extraperitoneal anastomoses, which often involve extensive surgical resection and neoadjuvant radiotherapy, it is known that the risk for AL is increased but that the construction of a diverting stoma reduces morbidity and mortality in cases of AL.[4,16,17]. The benefit of a diverting stoma outweighs the morbidity it involves because the important complications of clinical AL can be avoided in high risk groups. However, risk assessment and knowledge of risk factors are essential in deciding on a diverting stoma. In this prospective study, the incidence of clinical AL was 7.3 % among 259 left-sided colorectal anastomoses, and the mortality rate in patients with AL was 15.8 %. These high incidences are in line with percentages of AL and associated mortality reported in literature [1,4,5,12] and again emphasize the importance of this complication in colorectal surgery.

Variable	No AL (n=240)	AL (n=19)	OR (95 % CI)	SE	p-value
Age, years, mean (range)	65 (56-73)	68 (53-76)	1.02 (0.40-2.60)	0.48	0.963
Gender					
Male	134 (93.1 %)	10 (6.9 %)			
Female	106 (92.2 %)	9 (7.8 %)	1.14 (0.44-2.90)	0.54	0.787
BMI (kg/m^2)	26.3 (0.29)	25.6 (1.1)	0.96 (0.85-1.06)	0.06	0.50
ASA					
1	64 (97.0 %)	2 (3.0 %)			
2	119 (91.5 %)	11 (8.5 %)			
3	54 (90.0 %)	6 (10.0 %)			
4	2 (100 %)	-			
- 1 vs 2			2.96 (0.64-13.8)	2.32	0.167
- 1 vs 3			3.56 (0.69-18.3)	2.98	0.130
- 1 vs 4			-	-	-
Smoking					
Yes	53 (94.6 %)	3 (5.4 %)			
No	181 (92.3 %)	15 (7.7 %)	0.68 (0.19-2.45)	0.44	0.558
Cardiac comorb.					
Yes	70 (92.1 %)	6 (7.9 %)			
No	168 (92.8 %)	13 (7.2 %)	1.09 (0.39-2.98)	0.56	0.864
Pulm. comorb.					
Yes	24 (77.4 %)	7 (22.6 %)			
No	214 (94.7 %)	12 (5.3 %)	4.99 (1.80-13.85)	2.59	0.002
Steroids					
Yes - chronic	3 (50.0 %)	3 (50.0 %)			
Yes - peri-op	17 (81.0 %)	4 (19.0 %)			
No	218 (94.8 %)	12 (5.2 %)			
- Chronic Y vs N			4.29 (1.25-14.76)	2.70	0.021
- Peri-op Y vs N			18.25 (3.32-100.15)	15.85	0.001
Radiotherapy					
Yes	55 (91.7 %)	5 (8.3 %)			
No	184 (92.9 %)	14 (7.1 %)	1.20 (0.41-3.48)	0.65	0.736
Type of resection					
LAR	82 (92.1 %)	7 (7.9 %)			
HAR	57 (95.0 %)	3 (5.0 %)			
Sigmoidectomy	73 (90.1 %)	8 (9.9 %)			
L hemicolectomy	24 (96.0 %)	1 (4.0 %)			
Subt colectomy	4 (100 %)	-			
- HAR vs LAR			0.61 (0.15-2.48)	0.44	0.497
- Sigm vs LAR			1.28 (0.44-3.71)	0.69	0.645
- Left hemicolectomy vs LAR			0.48 (0.06-4.16)	0.53	0.512
- Subt col vs LAR			-	-	-
Height anast					
> 7cm	158 (94.0 %)	10 (6.0 %)			
< 7cm	80 (89.9 %)	9 (10.1 %)	1.79 (0.69-4.58)	0.86	0.225
Stapled	187 (93.0 %)	14 (7.0 %)			
Handsewn	51 (91.1 %)	5 (8.9 %)	0.78 (0.27-2.26)	0.42	0.464
Configuration					
ETE	60 (87.0 %)	9 (13.0 %)			
ETS	17 (94.4 %)	1 (5.6 %)			
STE	128 (94.1 %)	8 (5.9 %)			
STS	26 (96.3 %)	1 (3.7 %)			
- ETS vs ETE			0.41 (0.05-3.42)	0.44	0.407
- STS vs ETE			0.26 (0.03-2.20)	0.28	0.219
- STE vs ETE			0.43 (0.16-1.16)	0.22	0.095
Prot. ileostomy					
Yes	56 (93.3 %)	4 (6.7 %)			
No	180 (92.3 %)	15 (7.7 %)	0.86 (0.27-2.70)	0.50	0.799

Table 1. Univariate analysis of risk factors for anastomotic leakage
Abbreviations: AL, anastomotic leakage; ASA, American Society of Anesthesiologists; ETE, end to end; ETS, end to side; HAR, high anterior resection; LAR, low anterior resection; OR, odds ratio, SE, standard error; STE, side to end; STS, side to side.

Variable	Without ileostomy (n=195)	With ileostomy (n=60)	p-value
Age, years, mean (range)	65 (57-74)	66 (53-72)	0.45
Gender			
Male	104 (73.2 %)	38 (26.8 %)	0.17
Female	91 (80.5 %)	22 (19.5 %)	
BMI (kg/m^2)			
< 30	154 (77.4 %)	45 (22.6 %)	0.17
> 30	29 (67.4 %)	14 (32.6 %)	
ASA			
1 - 2	149 (76.8 %)	45 (23.2 %)	0.82
3 – 4	46 (75.4 %)	15 (24.6 %)	
Smoking			
Yes	40 (72.7 %)	15 (27.3 %)	0.52
No	150 (76.9 %)	45 (23.1 %)	
Pulmonal comorbidity			
Yes	26 (89.3 %)	5 (16.1 %)	0.30
No	169 (75.4 %)	55 (24.6 %)	
Corticosteroids			
Yes - home medication	6 (100 %)	0 (-)	.12
Yes - peri-operatively 5days	19 (90.5 %)	2 (9.5 %)	
No	170 (74.6 %)	58 (25.4 %)	
Radiotherapy			
Yes	20 (33.9 %)	39 (66.1 %)	
No	175 (89.3 %)	21 (10.7 %)	< 0.01
Type of resection			
TME	38 (43.7 %)	49 (56.3 %)	< 0.01
HAR	51 (86.4 %)	8 (13.6 %)	
Sigmoidectomy	78 (96.3 %)	3 (3.7 %)	
Left hemicolectomy	25 (100 %)	-	
Subtotal colectomy	4 (100 %)	-	
Height anastomosis			
> 7cm	154 (92.8 %)	12 (7.2 %)	< 0.01
< 7cm	40 (45.5 %)	48 (54.5 %)	

Table 2. Distribution of variables between patients with and without defunctioning ileostomy
Abbreviations: ASA, American Society of Anesthesiologists; HAR, high anterior resection; TME, total mesorectal excision.

The clinical course of patients with AL showed that most patients had signs of sepsis or peritonitis. In 21 % of leaks, the drain indicated leakage, in the remaining patients, computed tomography or laparotomy resulted equally often in the detection of AL. In 50 % of patients with AL, a Hartmann operation was needed. In 26 % of patients in this series, a diverting stoma was constructed, significantly more often in high-risk patients who underwent radiotherapy and had low anastomosis after a total mesorectal excision surgery. In univariate analysis, low anastomoses did not have an increased risk for AL, however, in the multivariate analysis, low anastomoses did increase the risk for AL (p=.049). Having a nonsignificant effect in a univariate analysis and a significant effect in a multivariate analysis is because of an interaction effect among factors. First, there is an interaction between the height of an anastomosis and a protective ileostomy, with 80 % of protective ileostomies in low anastomoses. Second, there is also an interaction with steroids; more than 90 % of patients with stoma did not take steroids. Therefore, when looking at the effect of the height of anastomosis in patients not taking steroids in the multivariate analysis, the risk of leakage will be higher in low anastomoses. In patients taking corticosteroids (long-term use of corticosteroids or perioperative use of corticosteroids), the risk for AL was significantly increased. It is known that corticosteroids impair wound healing by decreasing activation and infiltration of inflammatory cells. These inflammatory cells, macrophages and polymorph leucocytes, are essential in the first phase of wound healing [26]. Additionally, corticosteroids inhibit the expression of growth factors and matrix proteins such as collagen synthesis [26]. Known complications of glucocorticoids include gastrointestinal bleeding, peptic ulcer perforation, and sigmoid diverticular perforation [27,28], as well as postoperative complications such as wound infection and wound dehiscence [29-31]. Despite these effects on wound healing in general, conflicting results are found in literature concerning the effect of corticosteroids on the healing of colorectal anastomoses. Most experimental studies have shown less breaking strength and collagen concentrations in the anastomosis of animals treated with steroids [32-37], although some have found equal healing [38,39]. On the contrary, few clinical studies have found corticosteroids to be a risk factor for AL [22,23,40,41]. Among these is 1 prospective study of patients with colorectal carcinoma; other studies are retrospective, only included patients with Crohn disease, or looked at postoperative complications in general and not specifically AL. However, most clinical studies have failed to demonstrate a significant relationship between corticosteroids and impaired wound healing after colorectal surgery [14,24,25,42-47]. Unfortunately, most studies were retrospective, had broad inclusion criteria, and often only included patients with Crohn disease. In a substantial part of these studies, postoperative complications in general were taken as outcome measures and AL was not specifically addressed. To our knowledge, this is the first study to search for risk factors for AL that not only included long-term use of corticosteroids but also separately focused on corticosteroids prescribed perioperatively for the reduction of postoperative pulmonary complications. In a systematic review, Smetana et al. [48] showed that chronic obstructive pulmonary disease is an important independent risk factor for postoperative pulmonary complications, as are specific surgical interventions including abdominal surgery and operations lasting longer than 2.5

hours. Therefore, the Dutch guidelines for the prevention of postoperative pulmonary complications recommend perioperative corticosteroids for patients having newly diagnosed, unstable, or severe chronic obstructive pulmonary disease, as well as undergoing extensive surgical interventions [49]. In these patients, it is advised to start 5 days prior to surgery administration of prednisone, 30 mg daily, which can be ended or gradually reduced 2 to 3 days postoperatively. In our study, perioperative corticosteroids were prescribed to 8 % of patients, and to our knowledge, this is the first study to demonstrate a significant relationship between patients undergoing perioperative corticosteroid treatment and clinical AL. However, perioperative use of steroids is not considered a substantial risk factor for AL according to surgeons because these patients did not receive a diverting stoma more often than patients who did not take corticosteroids. In conclusion, our results show that the construction of a diverting stoma in patients with extraperitoneal anastomoses, who have often undergone preoperative radiotherapy and extensive total mesorectal excision surgery, is effective in reducing the risk for AL. However, in this prospective study, we did find a significantly increased incidence of AL in patients taking long-term corticosteroids and perioperative corticosteroids for pulmonary comorbidity. Therefore, we recommend that in this patient category, anastomoses should be protected by a diverting stoma or, in the case of chronic corticosteroids, a Hartmann procedure should also be considered to avoid the morbidity and strongly increased mortality associated with AL.

References

1. Alves A, Panis Y, Trancart D, Regimbeau JM, Pocard M, Valleur P. Factors associated with clinically significant anastomotic leakage after large bowel resection: multivariate analysis of 707 patients. *World J Surg*. 2002;26(4):499-502.

2. Hyman N, Manchester TL, Osler T, Burns B, Cataldo PA. Anastomotic leaks after intestinal anastomosis: it's later than you think. *Ann Surg*. 2007;245(2):254-258.

3. Matthiessen P, Hallbo¨o¨k O, Rutega°rd J, Simert G, Sjo¨dahl R. Defunctioning stoma reduces symptomatic anastomotic leakage after low anterior resection of the rectum for cancer: a randomized multicenter trial. *Ann Surg*. 2007;246(2):207- 214.

4. Peeters KC, Tollenaar RA, Marijnen CA, et al. Dutch Colorectal Cancer Group. Risk factors for anastomotic failure after total mesorectal excision of rectal cancer. *Br J Surg*. 2005;92(2):211-216.

5. Komen N, Dijk JW, Lalmahomed Z, et al. After-hours colorectal surgery: a risk factor for anastomotic leakage. *Int J Colorectal Dis*. 2009;24(7):789-795.

6. Alberts JC, Parvaiz A, Moran BJ. Predicting risk and diminishing the consequences of anastomotic dehiscence following rectal resection. *Colorectal Dis*. 2003;5(5):478-482.

7. Branagan G, Finnis D; Wessex Colorectal Cancer Audit Working Group. Prognosis after anastomotic leakage in colorectal surgery. *Dis Colon Rectum*. 2005; 48(5):1021-1026.

8. Fielding LP, Stewart-Brown S, Blesovsky L, Kearney G. Anastomotic integrity after operations for large-bowel cancer: a multicentre study. *Br Med J*. 1980; 281(6237):411-414.

9. den Dulk M, Marijnen CA, Collette L, et al. Multicentre analysis of oncological and survival outcomes following anastomotic leakage after rectal cancer surgery. *Br J Surg*. 2009;96(9):1066-1075.

10. Detry RJ, Kartheuser A, Delriviere L, Saba J, Kestens PJ. Use of the circular stapler in 1000 consecutive colorectal anastomoses: experience of one surgical team. *Surgery*. 1995;117(2):140-145.

11. Mann B, Kleinschmidt S, Stremmel W. Prospective study of hand-sutured anastomosis after colorectal resection. *Br J Surg*. 1996;83(1):29-31.

12. Matthiessen P, Hallböök O, Andersson M, Rutega°rd J, Sjo¨dahl R. Risk factors for anastomotic leakage after anterior resection of the rectum. *Colorectal Dis*. 2004;6(6):462-469.

13. Rullier E, Laurent C, Garrelon JL, Michel P, Saric J, Parneix M. Risk factors for anastomotic leakage after resection of rectal cancer. *Br J Surg*. 1998;85(3): 355-358.

14. Vignali A, Fazio VW, Lavery IC, et al. Factors associated with the occurrence of leaks in stapled rectal anastomoses: a review of 1,014 patients. *J Am Coll Surg*. 1997;185(2):105-113.

15. Yeh CY, Changchien CR, Wang JY, et al. Pelvic drainage and other risk factors for leakage after elective anterior resection in rectal cancer patients: a prospective study of 978 patients. *Ann Surg*. 2005;241(1):9-13.

16. Law WL, Chu KW. Anterior resection for rectal cancer with mesorectal excision: a prospective evaluation of 622 patients. *Ann Surg*. 2004;240(2):260-268.

17. Tan WS, Tang CL, Shi L, Eu KW. Meta-analysis of defunctioning stomas in low anterior resection for rectal cancer. *Br J Surg*. 2009;96(5):462-472.

18. Sørensen LT, Jørgensen T, Kirkeby LT, Skovdal J, Vennits B, Wille-Jørgensen P. Smoking and alcohol abuse are major risk factors for anastomotic leakage in colorectal surgery. *Br J Surg*. 1999;86(7):927-931.

19. Eriksen MT, Wibe A, Norstein J, et al. Norwegian Rectal Cancer Group. Anastomotic leakage following routine mesorectal excision for rectal cancer in a national cohort of patients. *Colorectal Dis*. 2005;7(1):51-57.

20. Ma¨kela¨ JT, Kiviniemi H, Laitinen S. Risk factors for anastomotic leakage after left-sided colorectal resection with rectal anastomosis. *Dis Colon Rectum*. 2003; 46(5):653-660.

21. Komen N, Klitsie P, Dijk JW, et al. Calcium score: a new risk factor for colorectal anastomotic leakage. *Am J Surg*. 2010;201(6):759-765.

22. Konishi T, Watanabe T, Kishimoto J, Nagawa H. Risk factors for anastomotic leakage after surgery for colorectal cancer: results of prospective surveillance. *J Am Coll Surg*. 2006;202(3):439-444.

23. Post S, Betzler M, von Ditfurth B, Schu¨rmann G, Ku¨ppers P, Herfarth C. Risks of intestinal anastomoses in Crohn’s disease. *Ann Surg*. 1991;213(1):37-42.

24. Trésallet C, Royer B, Godiris-Petit G, Menegaux F. Effect of systemic corticosteroids on elective left-sided colorectal resection with colorectal anastomosis. *Am J Surg*. 2008;195(4):447-451.

25. Jex RK, van Heerden JA, Wolff BG, Ready RL, Ilstrup DM. Gastrointestinal anastomoses: factors affecting early complications. *Ann Surg*. 1987;206(2):138-141.

26. Schäcke H, Döcke WD, Asadullah K. Mechanisms involved in the side effects of glucocorticoids. *Pharmacol Ther*. 2002;96(1):23-43.

27. Weiner HL, Rezai AR, Cooper PR. Sigmoid diverticular perforation in neurosurgical patients receiving high-dose corticosteroids. *Neurosurgery*. 1993;33(1): 40-43.

28. Mpofu S, Mpofu CM, Hutchinson D, Maier AE, Dodd SR, Moots RJ. Steroids, non-steroidal anti-inflammatory drugs, and sigmoid diverticular abscess perforation in rheumatic conditions. *Ann Rheum Dis*. 2004;63(5):588-590.

29. De Vivo A, Mancuso A, Giacobbe A, Priolo AM, De Dominici R, Maggio Savasta L. Wound length and corticosteroid administration as risk factors for surgicalsite complications following cesarean section. *Acta Obstet Gynecol Scand*. 2010; 89(3):355-359.

30. Steingrimsson S, Gottfredsson M, Kristinsson KG, Gudbjartsson T. Deep sterna wound infections following open heart surgery in Iceland: a populationbased study. *Scand Cardiovasc J*. 2008;42(3):208-213.

31. Busti AJ, Hooper JS, Amaya CJ, Kazi S. Effects of perioperative anti-inflammatory and immunomodulating therapy on surgical wound healing. *Pharmacotherapy*. 2005;25(11):1566-1591.

32. Cali RL, Smyrk TC, Blatchford GJ, Thorson AG, Christensen MA. Effect of prostaglandin E1 and steroid on healing colonic anastomoses. *Dis Colon Rectum*. 1993;36(12):1148-1151.

33. Del Rio JV, Beck DE, Opelka FG. Chronic perioperative steroids and colonic anastomotic healing in rats. *J Surg Res*. 1996;66(2):138-142.

34. Furst MB, Stromberg BV, Blatchford GJ, Christensen MA, Thorson AG. Colonic anastomoses: bursting strength after corticosteroid treatment. *Dis Colon Rectum*. 1994;37(1):12-15.

35. Phillips JD, Kim CS, Fonkalsrud EW, Zeng H, Dindar H. Effects of chronic corticosteroids and vitamin A on the healing of intestinal anastomoses. *Am J Surg*. 1992;163(1):71-77.

36. Polat A, Nayci A, Polat G, Akso¨yek S. Dexamethasone down-regulates endothelial expression of intercellular adhesion molecule and impairs the healing of bowel anastomoses. *Eur J Surg*. 2002;168(8-9):500-506.

37. Mantzoros I, Kanellos I, Demetriades H, et al. Effects of steroid on the healing of colonic anastomoses in the rat. *Tech Coloproctol*. 2004;8(suppl 1):s180-s183.

38. Mastboom WJ, Hendriks T, de Man BM, de Boer HH. Influence of methylprednisolone on the healing of intestinal anastomoses in rats. *Br J Surg*. 1991; 78(1):54-56.

39. Eubanks TR, Greenberg JJ, Dobrin PB, Harford FJ, Gamelli RL. The effects of different corticosteroids on the healing colon anastomosis and cecum in a rat model. *Am Surg*. 1997;63(3):266-269.

40. Golub R, Golub RW, Cantu R Jr, Stein HD. A multivariate analysis of factors contributing to leakage of intestinal anastomoses. *J Am Coll Surg*. 1997;184(4): 364-372.

41. Yamamoto T, Allan RN, Keighley MR. Risk factors for intra-abdominal sepsis after surgery in Crohn's disease. *Dis Colon Rectum*. 2000;43(8):1141-1145.

42. Allsop JR, Lee EC. Factors which influenced postoperative complications in patients with ulcerative colitis or Crohn's disease of the colon on corticosteroids. *Gut*. 1978;19(8):729-734.

43. Bruewer M, Utech M, Rijcken EJ, et al. Preoperative steroid administration: effect on morbidity among patients undergoing intestinal bowel resection for Crohns disease. *World J Surg*. 2003;27(12):1306-1310.

44. Colombel JF, Loftus EV Jr, Tremaine WJ, et al. Early postoperative complications are not increased in patients with Crohn's disease treated perioperatively with infliximab or immunosuppressive therapy. *Am J Gastroenterol*. 2004; 99(5):878-883.

45. Heimann TM, Greenstein AJ, Mechanic L, Aufses AH Jr. Early complications following surgical treatment for Crohn's disease. *Ann Surg*. 1985;201(4):494-498.

46. Knudsen L, Christiansen L, Jarnum S. Early complications in patients previously treated with corticosteroids. *Scand J Gastroenterol Suppl*. 1976;37:123-128.

47. Lipska MA, Bissett IP, Parry BR, Merrie AE. Anastomotic leakage after lower gastrointestinal anastomosis: men are at a higher risk. *ANZ J Surg*. 2006;76(7): 579-585.

48. Smetana GW, Lawrence VA, Cornell JE; American College of Physicians. Preoperative pulmonary risk stratification for noncardiothoracic surgery: systematic review for the American College of Physicians. *Ann Intern Med*. 2006;144(8): 581-595.

49. Dutch Society for Anesthesiology. Guideline: Prevention of Peri-Operative Pulmonary Complications in Non-Pulmonary Surgery in Patients With an Increased Risk of Pulmonary Complications. Utrecht, the Netherlands: Nederlandse Vereniging voor Anesthesiologie; 2007.

Chapter 4

Riolan's arch: confusing, misnomer, and obsolete. A literature survey of the connection(s) between the superior and inferior mesenteric arteries

J. F. Lange, N. Komen, G. Akkerman, E. Nout, H. Horstmanshoff, F. Schlesinger, J. Bonjer, G.J. Kleinrensink

Am J Surg. 2007 Jun;193(6):742-8. Review

Abstract

Background

There are 2 interpretations of Riolan's arch: (1) Riolan's arch is identical to a central part of the marginal artery (MA), connecting the superior (SMA) and the inferior mesenteric (IMA) arteries; and (2) Riolan's arch represents a rare artery, connecting the SMA and the IMA. The current review aims to emphasize the clinical importance of the colon's vasculature and to show the feasibility of abolishing the terms "Riolan's arch" and "meandering mesenteric artery".

Methods

A literature survey was performed.

Results

It appears that no distinct identity can be ascribed to Riolan's arch and that the "meandering mesenteric artery" represents an angiographically hypertrophied MA and/or the ascending branch of the left colic artery. However, a rare, centrally located, communicating artery has been described. Generally, the MA is sufficient for left colic circulation after ligation of the IMA, but at the splenic flexure, patency of the ascending branch of the left colic artery can be primordial.

Conclusion

As connections between the SMA and the IMA can be adequately described using structures mentioned in *Terminologica Anatomica*, the terms "Riolan's arch" and "meandering mesenteric artery" should be abolished.

Introduction

Jean Riolan the Younger (1580 –1657), a famous 17th century French anatomist, was a great admirer of the views of Galenus, which, at that time, had survived for 14 centuries. As a great dissector he greatly contributed to anatomical knowledge, as one could conclude from the eponyms carrying his name like Riolan's muscle, Riolan's bouquet, and Riolan's arch. The latter anatomical term has remained, despite the attempt of the Paris Anatomical Conference (1955) to refute all eponyms in anatomy, a well-known entity in radiology, aortic, and colon surgery [1].

Interpretations and Synonyms of Riolan's Arch

In general, "Riolan's arch" refers to a connection between the superior (SMA) and inferior mesenteric (IMA) arterial systems. This connection is held responsible for collateral perfusion after, for example, ligation of the IMA during aortic and colon surgery and after atherosclerotic stenosis or occlusion of SMA or IMA [2,3]. However, there is no consensus on which anatomical structure is represented by this eponym [4]. This is confirmed by the large number of synonyms that can be found in literature (Table 1).

Two different interpretations of Riolan's arch can be found in literature: (1) a synonym for the marginal artery (MA) of the colon, also known as the MA of Drummond, arcus paracolicus, or paracolic arcade; and (2) a rare distinct anatomical entity connecting the SMA with the IMA. Several authors do not mention Riolan's arch and consider the MA to be the crucial connection between the SMA and the IMA [5–15]. Others do mention Riolan's arch but are merely considering it to represent the MA, with or without the ascending branch of the left colic artery (ALCA) (Fig. 1A) [2,16–27]. However, many authors consider Riolan's arch to be a distinct anatomical entity, additional to the MA and ALCA (Fig. 1B) [2,28–44]. This interpretation has been supported by the original radiological interpretation of the angiographic "meandering mesenteric artery," located between the middle colic artery (MCA) and the left colic artery (LCA), as a distinct anatomical entity [43] (Fig. 2).

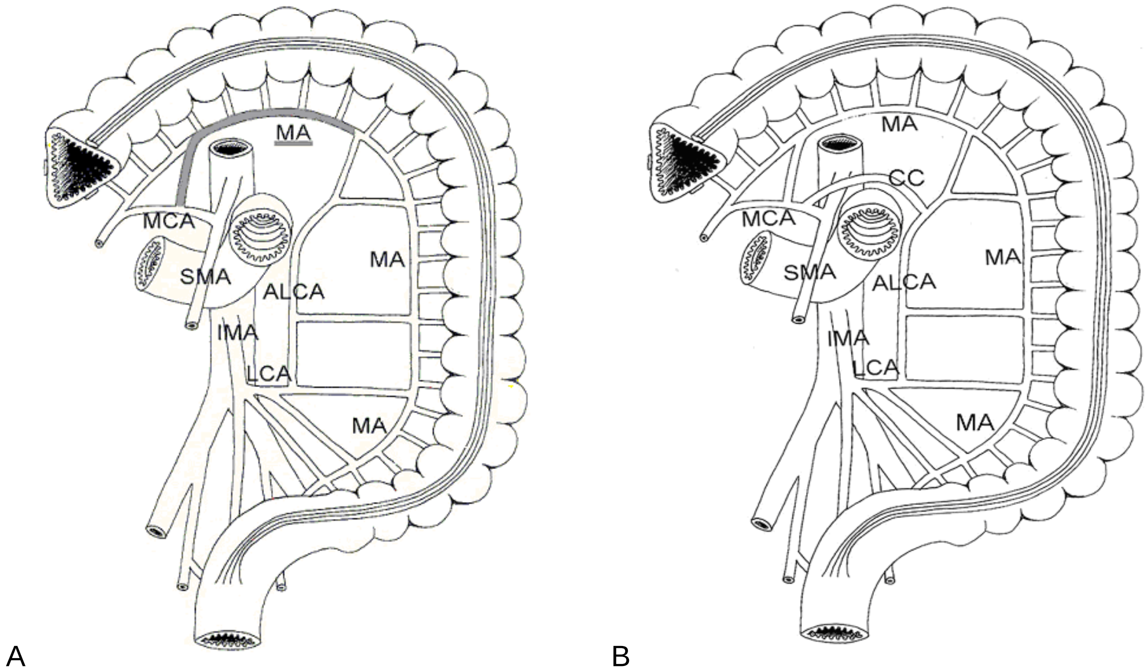

Fig. 1.
(A) Schema of colic arterial circulation in which Riolan's arch is interpreted as the marginal artery of colon (of Drummond), marked arterial segment. ALCA = ascending branch of left colic artery; IMA = inferior mesenteric artery; LCA = left colic artery; MA = marginal artery; MCA = middle colic artery; SMA = superior mesenteric artery.
(B) Schema of colic arterial circulation in which Riolan's arch is interpreted as a centrally communicating, additional collateral artery (CC).

Synonyms of Riolan's arch
central anastomotic artery of colon
mesomesenteric artery
middle-left colic collateral
intermesenteric artery or arcade
meandering mesenteric artery
anastomosis (magna) of Riolan
meandering artery of Riolan
great colic artery of Riolan
arch of Treves
artery of Moskovitch
artery of Gonzalez
anastomosis maxima of Haller
arcus magnus mesentericus

Table I. Synonyms of Riolan's arch. Data from Ernst [3], Bertelli et al [16], Moneta [27], Davis [33], Van Gulick and Schoots [45], and Lanz and Wachsmut [47].

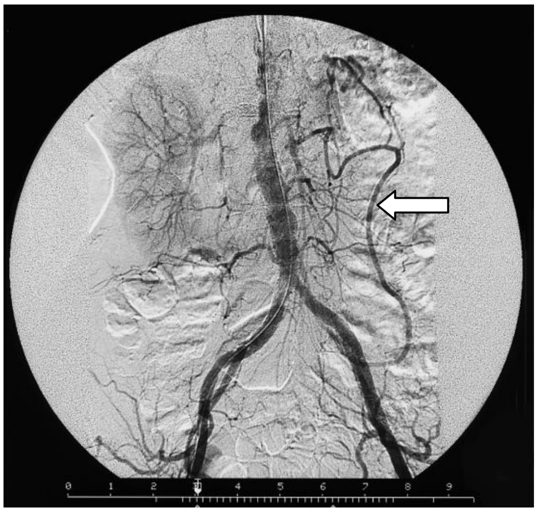

Figure 2. The angiographic "Meandering Mesenteric Artery", indicated by the arrow, in case of occlusion of the superior mesenteric artery

To determine which anatomical structure Jean Riolan the Younger referred to by Riolan's arch, Riolan's texts were extensively explored by other groups, but no description of the arterial circulation of the colon was found [16,28]. Additionally, the authors of the current review were unable to find a description in Riolan's "Opera Anatomica." One chapter is devoted to the intestinal vascularization, only describing the celiac trunk (Fig. 3).

DE ARTERIA COELIACA.

CAPVT XVIII.

PRIVSQVAM Inteſtina extrahas, Arterias quæ venæ Portæ ramos comitantur, obſeruabis. Tanta eſt venarum & arteriarum ſocietas, vt pauciſſimæ venæ in toto corpore reperiantur, arteriâ coniuge deſtitutę. Quia inter ſe communicant per venarum Anaſtomoſes. Itaque venę Portæ ramos, duæ Arteriæ à trunco Aortæ deſcendentis prodeuntes comitantur, nempe *arteria Cæliaca, & Meſenterica inferior*, iuxta Os ſacrum enata. Supra venæ Portæ truncum arteriæ Cœliacæ truncus conſidet, & inde perſequenda illius propagatio ac diſtributio in Lienem & Meſenterium. *Cæliaca* in tot ramos & ſurculos diuaricatur, quot vena Porta; qui nominibus iiſdem inſigniuntur, & in eaſdem partes feruntur. Non eſt igitur opus ſingulatim propagines arteriarum exprimere, ſufficiet diſcrimen aſſignare vtriuſque vaſis; quod Arteria candidior, craſſior, & inanior appareat, quàm vena. Arteria Cœliaca, vt referunt omnes *Anatomici*, ſupra venæ Portæ truncum in duos Ramos Meſentericum & Splenicum finditur: ſed habet Lien ſuam arteriam propriam, de qua videbis *Arantium in Obſeruat. Anatom.* In hypochondriacis & inflammatoriis diſpoſitionibus, micantis arteriæ Cœliacæ palpitationem ſupra Vmbilicum appreſſa manu non licet manifeſtò percipere, quum habeat ſupra ſe poſita Inteſtina, niſi magnum intus conceptum fuerit incendium, vel abdomen macilentum fuerit, ac depreſſum.

Figure 3. Chapter of Riolans's "Opera Anatomica", Leiden, 1650, p. 115, the only chapter about intestinal vasculature, with its translation, which follows (Courtesy of the library of the Leiden University Medical Centre):
On the Arteria Coeliaca. Chapter XVIII: Before taking out the intestines you may observe the arteries, which accompany the branches of the vena portae. There is such a close association between veins and arteries that within the whole body only very few veins may be found which lack an accompanying artery. Accordingly they communicate by means of venous anastomoses. [We call them arterio-venous anastomoses.] And so there are 2 arteries originating from the trunk of the aorta descendens, which accompany the branches of the portal vein; these are: the celiac artery and the inferior mesenteric artery, which issues forth near the os sacrum. The trunk of the celiac artery is situated above the main-stem of the portal vein and from there its further continuation and distribution directed towards the spleen and the mesenterium can be followed. The celiac artery splits up into just as many branches and bifurcations as the vena porta; they [ie, the arterial branches] are indicated by the same terms [the portal branches] and they lead into the same parts. Therefore it is not necessary to describe the offshoots of the arteries one by one; it serves the purpose to label both kinds of vessels with their characteristic features, [Which means] that an artery has a lighter colour, is thicker and seems less filled than a vein. According to all anatomists the celiac artery is just above the stem of the vena portae divided into 2 branches, ie, the ramus mesentericus and the ramus splenicus; but the spleen has also its own proper artery, about which you may consult the *Anatomical Observations of Arantius*. [Arantius was a contemporary fellow-anatomist.] In the management of abdominal inflammations it is not permitted to ascertain the palpitation of the pulsating celiac artery by applying pressure with the hand above the umbilicus. The reason is, that the [inflamed] intestines are situated above the artery. [Therefore the pressure of the hand could be very painful.] There is an exception to this rule if there is a great motivation to gain knowledge of the internal situation and also if the abdomen is emaciated and flattened.

Consequently, considering the fact that Riolan never specifically wrote about the colon's vasculature, it has to be assumed that the arch is named after him out of respect for this great anatomist [16,45], which was a common phenomenon at that time. Probably, Albrecht von Haller, one of Riolan's coworkers, was the first to refer to the collateral arterial anatomy of the colon and MA in 1743 [6,16,45,46].

The aim of the current report is to review the literature related to the vasculature of the colon, to emphasize its clinical importance, and to show the feasibility of abolishing the terms "Riolan's arch" and "meandering mesenteric artery."

Anatomy of Connections Between the SMA and IMA

Many dissection, angiographic, and arterial cast and corrosion studies concerning colic arterial anatomy have been published. Unfortunately, no reports could be found in which angiographic and/or cast and corrosion studies were systematically correlated to dissection of the arterial system. Another misfortune is the vast variety of specimen numbers and methods, even in the same study, which makes interpretation of the results extremely difficult. Therefore, all principal arterial structures between the SMA and the IMA will be discussed in subsequent order.

Middle colic artery

The MCA, representing the terminal arm of the SMA system, is absent in 2 % to 22 % of patients [6,16,28,29,46,47], in which case the transverse colon will be supplied by the right colic artery (RCA) and a pronounced "marginal Riolan anastomosis", according to Lanz and Wachsmuth [47]. The latter term is defined as the part of the MA, between the left branch of the MCA and the ALCA [47]. When the LCA is absent, the MCA can extend to the splenic flexure (7 %) [23,48].

Marginal artery of colon (of Drummond)

Although angiographic evidence of a direct SMA–IMA connection is lacking, according to most authors, MA is always present, connecting the MCA with the LCA [3,6,16,23,41,46,47,49,50]. The MA–ALCA connection (Cannon-Böhm point) at the transverse colon is a common phenomenon as well, representing the boundary between the 2 autonomic nerve supplies and corresponding to the embryonic primary colic flexure at the fusion level of the midgut and hindgut [38].

Ascending branch of left colic artery

ALCA, representing the left arm of SMA–IMA connection and constituting an arch from the distal transverse colon down to the sigmoid colon secondary to the MA, is reported to be present in 63 % to 100 % of patients [6,29,46,50]. When present, it parallels the inferior mesenteric vein and extends up to the splenic flexure. When absent, the left branch of MCA is reported to extend to the splenic flexure [23,48]. In a few cases (14 %), the medial branch of the ALCA's terminal bifurcation, localized at a few inches from the splenic flexure, is lacking, leaving the splenic flexure with a single arcade, constituted by the MA [29]. When the medial branch of the ALCA's terminal bifurcation is connected to the MA at the transverse colon, the importance of the ALCA is related to the functional patency of the MA at the splenic flexure and/or the descending colon [20].

Marginal artery at splenic flexure

Although clinical involvement of the splenic flexure ('watershed area') in ischemic colitis is a familiar syndrome, its prevalence is unknown [51,52]. Some authors consider the splenic flexure a predilection area for ischemic colitis, compared to the descending or sigmoid colon [53]. However, Keighley et al. showed these segments to be equally involved [54]. Inoue et al. related the localization of colon ischemia to differences in dominance of the SMA, IMA, or the internal iliac arteries (IIA) [55]. It was found that splenic flexure ischemia after ligation (or atherosclerotic occlusion) of the IMA occurred more often in patients with an IIA dominant collateral colic circulation. From the perspective of the IIAs, the splenic flexure is the most remote colon segment, possibly explaining this finding. This logic was confirmed by the finding that in the majority of studied patients with a dominant SMA, ischemia of the rectosigmoid was more common. There are anatomical arguments for a clinical role of the MA at the splenic flexure. For example, Steward et al. reported 100 % MA patency at the splenic flexure by injecting contrast into the SMA [46]. Binns et al. confirmed these findings in their cadaver angiography study [56]. Contradicting these findings, Griffiths et al. reported a frequent insufficiency of the MA at the level of the splenic flexure, currently referred to as Griffiths' critical point [6], which is confirmed by several other authors [7,29,57].

An explanation for this contradiction could be the fact that Steward and Binns did not ligate the 2 branches of the ALCA's terminal bifurcation, leaving the possibility that filling of the IMA system by the ALCA and thus "bridging" of a possibly insufficient MA at the splenic flexure could have occurred.

Considering these results, it is recommended to respect both terminal branches of the ALCA while resecting the left colon and/or rectum. As such, the possibility of bridging the MA at the splenic flexure remains [6,8,58]. However, its clinical importance is unknown, since the prevalence of an insufficient MA at the splenic flexure is unknown.

Central communications between the SMA and IMA

Quénu et al. frequently observed tiny intermesenteric arteries at the level of the duodenojejunal flexure, running along the cranial part of the inferior mesenteric vein [17]. However, in general, such "central" communications between the SMA and the IMA have been described only in low frequencies (0 % to 18 %) [6,16,23,28,29,42,46,59]. They can be compared with the artery of Bühler (ramus anastomoticus) between the celiac trunk and the SMA [29,30]. In addition to the MA and the ALCA, a central artery between the SMA and the IMA might serve as a third pathway of collateral arterial circulation of the colon [16]. Such an artery is mentioned in *Terminologica Anatomica* as "arteria ascendens" [60]. Bertelli et al., denominating this communication by "intermesenteric trunk," discerned 3 different types, with a total incidence of 18 % [16]: (1) the direct type (arteria ascendens), representing an extremely rare direct communication between the SMA and the IMA; (2) the indirect type, representing a connection between the MCA and the LCA (prevalence 9 %); and (3) a communication between 1 of the 2 mesenteric arteries and 1 of the main branches, usually the LCA (prevalence 9 %). Van Damme et al. observed a small central intermesenteric arcade, running at the level of the duodenojejunal angle, in 12 % of cadavers [23]. In their opinion, this shunt was unreliable in acute vascular occlusion. A middle (third) mesenteric artery with a direct connection to the LCA, has been described in extremely rare instances [61]. To date, an additional, protective role of these rare central connections has never been demonstrated.

Other pathways between the SMA and IMA

Arterial connections between the retroperitoneal and intestinal vascularisation have been recognized for a long time [23]. Pereira et al. have shown that several, although anatomically not defined, arterial channels must add to the collateral circulation of the left colon [4]. Michels et al. have described minute parieto-visceral connections at the level of the left renal capsula, which might hypertrophy in atherosclerotic occlusive disease [26,29].

Summary of Connection(s) Between the SMA and IMA

Considering the previously reviewed studies, it can be stated that, to date, there is no evidence for the presence of any regular arterial entity, other than the MCA, MA, and ALCA and their anastomosis at the distal transverse colon. This was already supported in Orr's classical *Operations* (1944) by the introduction of the concept of "Riolan's space", in which no vessels can be encountered [62]. Subsequently, the ALCA can be regarded as the only common secondary arch, bridging the MA at the splenic flexure [6,20,26,29,41,46]. The importance of the ALCA is illustrated by the nomenclature of "arteria intermesenterica," as represented in Sobotta's *Atlas of Anatomy* [13].

Clinical importance of connection(s) between the SMA and IMA

The clinical importance of a functional arterial communication between the SMA and the IMA has been recognized for many decades. However, the results from clinical, mostly intraoperative, experiments on collateral colic circulation are not unequivocal. As with the anatomical studies, methodological variety allows only general conclusions.

In chronic atherosclerotic occlusive disease, intestinal ischemia generally only becomes manifest if all 3 main axes (celiac trunk, SMA, and IMA) show occlusive signs on angiography [3]. However, complete absence of intestinal necrosis in case of occlusion of all 3 main axes also has been described [19]. In atherosclerotic obstructive disease, for example, occlusion of the SMA or infrarenal aorta (Leriche's syndrome), the angiographic finding of a hypertrophic "meandering mesenteric artery" (Fig. 2), as introduced by Moskowitz et al. in 1964, is a well-known phenomenon [2,33,43,44]. The meandering mesenteric artery supposedly is a thick, tortuous, uniform vessel connecting the proximal segments of the MCA and LCA, representing a central anastomosis. It can be distinguished from a normal MA, while this vessel is not tortuous, runs along the descending colon and is rarely visualized on angiography [43,44]. Besides the aforementioned features, a precise anatomical definition of the meandering mesenteric artery cannot be found. Some authors consider Riolan's arch and the meandering mesenteric artery to represent the same structure [44]. Others consider the meandering mesenteric artery to represent the MA together with the ALCA, anastomosing at the level of the distal transverse colon [18,63,64]. Considering arterial perfusion of the greater part of the colic collateral vascular bed up to the descending colon, the SMA is more important than the IMA. Acute occlusion of the SMA will lead to intestinal necrosis, comprising the splenic flexure [55,65]. The IMA, however, can be ligated ("high-tie") in left colon and rectum resections without development of necrosis of the afferent colic loop [66,67]. In aortic surgery as well, it is an old adagium to ligate the IMA at its origin, respecting the LCA for collateral arterial circulation of the distal colon and rectum [68]. In the rare case of necrosis of the colon, it can be attributed to underdeveloped collaterals or intraoperative hypotension and possibly to absence of the MCA or occlusions of the IIA [4,67,69–72]. This is confirmed in a clinical study in which the IMA was clamped intraoperatively in patients undergoing colon surgery [73]. After an immediate MA-pressure drop, the authors observed collateral arteries taking over colic blood supply within 30 seconds and partial restoration of MA-pressure. The IIAs also play an important role in collateral rectosigmoidal arterial circulation, as ischemia of the colon and/or rectum is more frequent in case of occlusion of these vessels [4,74]. Pereira et al. intraoperatively clamped the IMA, MCA, and MA at the transverse colon, without a significant drop of stump pressure in non-atherosclerotic subjects with sigmoid carcinoma, underlining the dominance of the IIAs [4]. It is suggested that the SMA– IMA collateral pathway does not play an important role for the left colon and rectum in non-atherosclerotic subjects. In congruence with these findings, a significant persistent decreased perfusion, up to 50 %, of the afferent colic loop after rectosigmoid resection was found by laser Doppler flowmetry [75,76]. In addition, Fasth et al. measured significant arterial pressure decreases in the MA at the left colon after clamping of the IMA, suggesting that postoperative systemic systolic pressures should be monitored in order to prevent anastomotic dehiscence [73]. A "high-tie" strategy (ligation of the IMA stem) in oncological rectal surgery will not result in ischemia of the proximal loop, provided the sigmoid colon is at least partially resected in view of an incomplete MA at sigmoid level, and no signs of SMA stenosis are present [58,73,77–81]. Hall et al. observed that oxygenation of the descending colon was maintained or even improved after IMA ligation in distal colorectal resections, provided the sigmoid was resected [80]. Compared to the high-tie IMA ligation, Corder et al. did not observe any improvement of anastomotic leak rate after selective preservation of the ALCA in low anterior resection, questioning the importance of this vessel as a collateral and underlining the role of the MA [78]. However, in both groups the terminal bifurcation of the ALCA at the splenic flexure was respected, so bridging, as mentioned before, could have occurred. Unfortunately, necrosis of the afferent limb after colon resection has never, with certainty, been ascribed to an insufficient MA at the level of the splenic flexure. This renders the clinical value of the anatomical and angiographic findings, concerning the MA at this location, as yet unproven. As for the functional role of the connection between the SMA and IMA systems, it can be stated that it is important with regard to viability of the afferent colic loop in oncological high-tie rectosigmoid resections. As a rule, the sigmoid colon should be at least partially resected. However, the individual capacity of the arterial collateral system of the left colon and rectum, being largely dependent on the anatomical and atherosclerotic status of the arteries involved, is unpredictable.

Until modalities for pre-, intra-, and post-operative monitoring of colic perfusion in colon and aortic surgery are validated, ischemia of the left colon in colic and aortic surgery will continue to occur.

Conclusion

In literature, including surgical textbooks and atlases, there is ongoing confusion about the identity of Riolan's arch. This can be explained by 3 main factors: (1) the absence of publications by Jean Riolan himself on colic collateral arterial circulation; (2) the large interindividual variety of colon arterial anatomy; and (3) the application of many different methods of anatomical investigations on colon anatomy with often conflicting results.

Authors who appoint high prevalences (>50 %) to Riolan's arch generally refer to the MA. Low prevalences (<20 %) are reported by authors who, in some instances at least, refer to the rarely occurring more central connections [41,42]. From the present literature review it can be concluded that Riolan's arch is a misnomer. Because in all anatomical studies no other regular structures are reported than already mentioned in *Terminologica Anatomica*, a distinct identity cannot be ascribed to "Riolan's arch" [60]. Consequently, we propose to completely abolish the entity of Riolan's arch. In addition, the radiological denomination of "meandering mesenteric artery" also must be abandoned, as this term too does not reflect a distinct anatomical entity, but represents an angiographically hypertrophied MA and/or ALCA. Although clinically the additional role of the ALCA to the MA with regard to the viability of the left colon in aortic and colon surgery has never been established with certainty, the connection of the MA with the ALCA at the level of the left transverse colon might be of importance in chronic atherosclerotic obstructive disease of the SMA, the IMA, and/or celiac trunk. Therefore, a hypertrophic ALCA must not be ligated during colon surgery. In addition, considering that the MA might sometimes be incomplete at the level of the splenic flexure and/or descending colon, respecting the ALCA's terminal bifurcation, bridging the MA at the splenic flexure is primordial under those circumstances. In abdominal aortic surgery, after IMA ligation, collateral arterial flow with regard to the left colon and rectum is at least as dependent on patent IIAs as on the SMA arterial system. Mere consciousness in aortic and colon surgery of the importance of evaluating and respecting the MA, connecting the SMA and the IMA, and being bridged at the splenic flexure by the ALCA's terminal bifurcation, must replace ongoing confusion on Riolan's arch.

References

1. Haubrich WS. Riolan of the arc of Riolan. Gastroenterology 2003; 124:607.

2. Connolly JE, Kwaan JHM. Prophylactic revascularisation of the gut. Ann Surg 1979; 190: 514–22.

3. Ernst CB. Colon ischemia following aortic reconstruction. In: Rutherford RB, ed. *Vascular Surgery*. 5th ed. Philadelphia: WB Saunders; 2000:1542–9.

4. Pereira AH, Cutin J, Eggers E. Inferior mesenteric artery backpressure and collateral circulation to the distal colon. Cardiovasc Surg 1993;1:536–40.

5. Drummond H. The arterial supply of the rectum and pelvic colon. Br J Surg 1913;1:677–85.

6. Griffiths JD. Surgical anatomy of the blood supply of the distal colon. Ann R Coll Surg Eng 1956;19:241–56.

7. Anson BJ, McVay CB. *Surgical Anatomy*. 5th ed. Philadelphia: WB Saunders; 1971.

8. Siddharth P, Ravo B. Colorectal neurovasculature and anal sphincter. Surg Clin North Am 1988;68:1185–200.

9. Nogueras JJ, Jagelman DG. Principles of surgical resection. Influence of surgical technique on treatment outcome. Surg Clin North Am 1993;73:103–16.

10. Skandalakis JE, Skandalakis PN, Skandalakis LJ. *Surgical Anatomy and Technique*. New York: Springer Verlag; 1995.

11. Olson TR. *ADAM Student Atlas of Anatomy*. 1st ed. Baltimore: Williams & Wilkins; 1996.

12. Nyhus LM, Baker RJ, Fischer EJ. *Mastery of Surgery*. 3rd ed. Boston: Little Brown; 1997.

13. Sobotta J. *Atlas der Anatomie des Menschen*. München, Germany: Urban & Fischer; 1999.

14. Moore KL, Dalley AF. *Clinically Oriented Anatomy*. 4th ed. Philadelphia:Lippincott Williams & Wilkins; 1999.

15. Morris PJ, Wood WC. *Oxford Textbook of Surgery*. 2nd ed. Oxford, UK: Oxford University Press; 2000.

16. Bertelli L, Lorenzini L, Bertelli E. The arterial vascularisation of the large intestine. Anatomical and radiological study. Surg Radiol Anat 1996;18(suppl 1):S1–59.

17. Quénu L, Chabrol J, Herelemont P. Le colon, ses variations, ses artères. CR Assoc Anat 1954;41:760 –9.

18. Kountz SL, Laub DR, Connolly JE. 'Aortioiliac steal' syndrome. Arch Surg 1966;92:490 –7.

19. Reinhart K. Verschlu_ der drei unpaaren, viszeralen Aortenäste mit Ausbildung eines erweiteren Riolanischen Kollateralkreislaufes. ROFO Fortsch Rontgenstr Nuklearmed 1978;129:699 –703.

20. Welch JP, Welch CE. Operative management of cancer of the colon. In: Maingot R, ed. *Abdominal Operations.* 7th ed. New York: Appleton- Century-Crofts; 1980:2103–50.

21. Goligher J. *Surgery of the Anus, Rectum and Colon*. 5th ed. London: Bailliere Tindall; 1984.

22. Platzer W, ed. *Pernkopf Anatomy*. 3rd ed. Baltimore: Urban & Schwarzenberg; 1989:232.

23. VanDamme JP. Behavioural anatomy of the abdominal arteries. Surg Clin North Am 1993;73:699 –725.

24. Keighley MRB, Williams NS. *Surgery of the Anus, Rectum and Colon*. 2nd ed. London: Saunders; 1997:2293.

25. Hussmann J, Russell R. *Memorix Surgery*. Newe York: Chapman & Hall; 1997.

26. Rohen JW, Yokochi C, Lütjen-Drecoll E. *Color Atlas of Anatomy*. 4th ed. Baltimore: Williams & Wilkins; 1998.

27. Moneta GL. Diagnosis of intestinal ischemia. In: Rutherford RB, ed. *Vascular Surgery*. 5th ed. Philadelphia: WB Saunders; 2000:1501–11.

28. Garcia-Ruiz A, Milsom JW, Ludwig KA, et al. Right colic arterial anatomy. Dis Colon Rectum 1996;39:906 –11.

29. Michels NA, Siddharth P, Kornblith PL, et al. The variant blood supply to the small and large intestines: its import in regional resections. J Int Coll Surg 1963;39:127–70.

30. Netter FH. Lower digestive tract. In: Oppenheimer E, ed. *The Ciba Collection of Medical Illustrations, Digestive System Part II*. Summit, NJ: Ciba; 1973:67–70.

31. Berk JE. Imaging (instruments, technique, normal displays)-angiography. In: Haubrich WS, Schaffner F, Berk JE, eds. *Bockus Gastroenterology*. 4th ed. Philadelphia: WB Saunders; 1985:500 –1.

32. Fischer DF Jr, Fry WJ. Collateral mesenteric circulation. Surg Gynecol Obstet 1987;164:487–92.

33. Davis JH. Aorta and peripheral arteries. In: van Schaik T, Manning TA, eds. *Clinical Surgery*. 1st ed. Toronto: Mosby; 1987:2147–53.

34. Grendell JH, Ockner RK. Vascular diseases of the bowel. In: Sleisenger MH, Fortran JS, eds. *Gastrointestinal Diseases*. 4th ed. Philadelphia: WB Saunders; 1989:1903–5.

35. MacDonald PH, Beck IT. Ischemic disease of the intestine. In: Thomson ABR, Shaffer EA, eds. *First Principles of Gastroenterology*. Canadian Association of Gastroenterology; 263–86. Available at: http://www.gastroresource.com/GITextbook/En/Default.htm. Accessed April 20, 2007.

36. Brandt LJ, Boley SJ. Ischemic and vascular lesions of the bowel. In: Sleisenger MH, Fortran JS, eds. *Gastrointestinal Disease*. 5th ed. Philadelphia: WB Saunders; 1993:1927–9.

37. Hansen KJ, Murray SP, Stoney RJ. Visceral ischemic disease. In: Loscalzo J, Craeger MA, Dzau VJ, eds. *Vascular Medicine. A textbook of vascular biology and diseases*. Boston: Little Brown; 1996; 871.

38. Thiel W. *Photographic Atlas of Practical Anatomy*. 1st ed. Springer: Berlin, 1997.

39. Glasby MA, Owen WJ, Kristmundsdottir F. *Applied Surgical Anatomy. A Guide for the Surgical Trainee*. 1st ed. Oxford, UK: Butterworth Heinemann; 1998.

40. Sternbach Y, Perler BA. Acute mesenteric ischemia. In: Yeo C, ed. *Shackleford's Surgery of the Alimentary Tract*. Philadelphia: WB Saunders; 2001; 5:17–31.

41. Gray H. Cardiovascular: Inferior Mesenteric Artery and Superior Mesenteric Artery. In: *Gray's Anatomy*. 38th ed. Edinburgh, UK:Churchill & Livingstone; 1999:1553– 6.

42. Cheng B, Chen K, Gao S, et al. Colon interposition. Recent Results Cancer Res 2000;155:151– 60.

43. Mosckowicz M, Zimmerman H, Felson B. The meandering mesenteric artery of the colon. Am J Roentgenol 1964;92:1088 –99.

44. Gourley EJ, Gering SA. The meandering mesenteric artery: a historic review and surgical implications. Dis Colon Rectum 2005;48: 996–1000.

45. Van Gulik TM, Schoots I. Anastomosis of Riolan revisited; the meandering mesenteric artery. Arch Surg 2005;140:1225–9.

46. Steward JA, Rankin FW. Blood supply of the large intestine. Its surgical considerations. Arch Surg 1933;26:843–91.

47. Lanz T, Wachsmut W. *Praktische Anatomie, Bauch*. Berlin: Springer- Verlag; 2004:379–83.

48. Sierocinski W. Anastomoses of the arteries supplying the descending and sigmoid colon in man. Folia Morphol Warsz 1976;35:467–79.

49. Ventemiglia R, Khalil KG, Frazier OH, et al. The role of preoperative arteriography in colon interposition. J Thorac Cardiovasc Surg 1977; 74:98 –104.

50. Peters JH, Kronson JW, Katz M, et al. Arterial anatomic considerations in colon interposition for oesophageal replacement. Arch Surg 1995;130:858–63.

51. Marston A. Ischemic enterocolitis. In: Maingot R, editor. *Abdominal Operations*. 7th ed. New York: Appleton-Century-Crofts; 1980: 1803–19.

52. Yamazaki T, Shirai Y, Tada T, et al. Ischemic colitis arising in watershed areas of the colic blood supply: a report of two cases. Surg Today 1997;27:460 –2.

53. Guttormsen NL, Bubrick MP. Mortality from ischemic colitis. DisColon Rectum 1989;32:469 –72.

54. Keighley MRB. Colic ischemia and ischemic colitis. In: Keighley MRB, Williams NS, eds. *Surgery of the Anus, Rectum and Colon*. 2nd ed. London: Saunders; 1997:2289 –312.

55. Inoue Y, Iwai T, Endo M. Determining variations in colic circulation during aortic surgery. Cardvasc Surg 1997;5:626 –33.

56. Binns JC, Isaacson P. Age-related changes in the colic blood supply: their relevance to ischemic colitis. Gut 1978;19:384 –90.

57. Meyers MA. Griffiths' point: critical anastomosis at the splenic flexure. Significance in ischemia of the colon. Am J Roentgenol 1976; 126:77–94.

58. Phillips RKS. *Colorectal Surgery*. 1st ed. London: Saunders; 1998: 82–3.

59. Stearns MW Jr. Benign and malignant neoplasms of colon and rectum. Surg Clin North Am 1978;58:605–18.

60. Federative Committee on Anatomical Terminology (FACT) and the International Federation of Associations of Anastomists (IFAA). *Terminologica Anatomica*. Stuttgart, Thieme: 1998.

61. Lawdahl RB, Keller FS. The middle mesenteric artery. Radiology 1987;165:371–2.

62. Orr TG. *Operations of General Surgery*. 4th ed. Philadelphia: WB Saunders; 1946.

63. Wind GG. *Applied Laparoscopic Anatomy: Abdomen and Pelvis*. Baltimore: Williams & Wilkins; 1997.

64. Gonzales LL, Jaffe MS. Mesenteric arterial insufficiency following abdominal aortic resection. Arch Surg 1966;93:10 –20.

65. Leung FW, Su KC, Pique JM, et al. Superior mesenteric artery is more important than inferior mesenteric artery in maintaining colic mucosal perfusion and integrity in rats. Dig Dis Sci 1992;37: 1329–35.

66. Wojczys R, Bednarz W. Effect of inferior mesenteric artery ligation on the healing of colic anastomosis in the rat. Eur J Surg 1997;163:215–7.

67. Karmody AM, Jordan FR, Zaman SN. Left colon gangrene after acute inferior mesenteric artery conclusion. Arch Surg 1976;111:972–5.

68. Siddharth P, Smith NL. An anatomic basis to prevent ischemia of the colon during operations upon the aorta. Surg Gynecol Obstet 1981; 153:71–3.

69. Young JR, Humphries AW, Dewolfe VG, et al. Complications of abdominal surgery. Arch Surg 1963;86:51–9.

70. Bast TJ, van der Biezen JJ, Scherpenisse J, et al. Ischaemic disease of the colon and rectum after surgery for abdominal aortic aneurysm: a prospective study of the incidence and risk factors. Eur J Vasc Surg 1990;4:656 –7.

71. Meissner MH, Johansson KH. Colon infarction after ruptured aortic aneurysm. Arch Surg 1992;127:979–85.

72. Mitchell KM, Valentine RJ. Inferior mesenteric artery reimplantation does not guarantee colon viability in aortic surgery. J Am Coll Surg 2002;194:151–5.

73. Fasth S, Hultén L, Hellberg R, et al. Blood pressure changes in the marginal artery of the colon following occlusion of the inferior mesenteric artery. Ann Chir Gynaecol 1978;67:161–4.

74. Shigematsu H, Nunokawa M, Hatakeyama T, et al. Inferior mesenteric and hypogastric artery reconstruction to prevent colic ischemia following aortic aneurysmectomy. Cardiovasc Surg 1993;1:13– 8.

75. Boyle NH, Manifold D, Jordan MH, et al. Intraoperative assessment of colic perfusion using scanning laser Doppler flowmetry during colic resection. J Am Coll Surg 2000;191:504 –10.

76. Dworkin MJ, Allen-Mersh TG. Effect of inferior mesenteric artery ligation on blood flow in marginal artery-dependent sigmoid colon. J Am Coll Surg 1996;183:357– 60.

77. Schiedler MG, Cutler BS, Fiddian-Green RG. Sigmoid intramural pH for prediction of ischemic colitis during aortic surgery. A comparison with risk factors and inferior mesenteric artery stump pressures. Arch Surg 1987;122:881– 6.

78. Corder AP, Karanjia ND, Williams JD, et al. Flush aortic tie versus selective preservation of the ascending left colic artery in low anterior resection for rectal carcinoma. Br J Surg 1992;79:680 –2.

79. Karanjia ND, Corder AP, Bearn P, et al. Leakage from stapled low anastomosis after total mesorectal excision for carcinoma of the rectum. Br J Surg 1994;81:1224–6.

80. Hall NR, Finan PJ, Stephenson BM, et al. High tie of the inferior mesenteric artery in distal colorectal resections: a safe procedure. Int J Colorectal Dis 1995;10:29 –32.

81. Hida J, Yasutomi M, Maruyama T, et al. Indication for using high ligation of the inferior mesenteric artery in rectal cancer surgery. Examination of nodal metastases by the clearing method. Dis Colon Rectum 1998;41:984 –7.

Chapter 5

High tie versus low tie in rectal surgery: comparison of anastomotic perfusion

N. Komen, J. Slieker, P. de Kort, J. H. W. de Wilt, E. van der Harst, P.P. Coene, M. Gosselink, G. Tetteroo, E. de Graaf, T. van Beek, R. den Toom, W. van Bockel, C. Verhoef, J. F. Lange

Int J Colorectal Dis. 2011 Aug;26(8):1075-8

Abstract

Purpose

Both “high tie” (HT) and “low tie” (LT) are well known strategies in rectal surgery. The aim of this study was to compare colonic perfusion after HT to colonic perfusion after LT.

Methods

Patients undergoing rectal resection for malignancy were included. Colonic perfusion was measured with laser Doppler flowmetry, immediately after laparotomy on the antimesenterial side of the colon segment that was to become the afferent loop (measurement A). This measurement was repeated after rectal resection (measurement B). The blood flow ratios (B/A) were compared between the HT group and the LT group.

Results

Blood flow was measured in 33 patients, 16 undergoing HT and 17 undergoing LT. Colonic blood flow slightly decreased in the HT group whereas the flow increased in the LT group. The blood flow ratio was significantly higher in the LT group (1.48 vs. 0.91, p=0.04), independent of the blood pressure.

Conclusion

This study shows the blood flow ratio to be higher in the LT group. This suggests that anastomoses may benefit from better perfusion when LT is performed.

Introduction

To date, 100 years after the introduction of the low tie and high tie techniques for colorectal surgery by Miles and Moynihan, respectively [1, 2], the discussion on which is the best technique continues, as illustrated by two recently published reviews [3, 4]. Titu et al. have summarized literature comparing low tie and high tie techniques for curative colorectal surgery [3]. They concluded that no undisputable evidence favoring one technique exists. Nevertheless, they propagate the high tie technique since it allows better lymph node retrieval and therefore a more accurate tumor staging. In another review comparing the low tie with the high tie technique, Lange et al. distinguished three aspects in the discussion: oncological, anatomical and technical [4]. They concluded that for each aspect the evidence is insufficient to favor one technique. Nevertheless, they favor the low tie technique since it is less invasive, also with regard to colonic innervation and motility, and it would be beneficial for anastomotic perfusion compared to the high tie technique.

Adequate anastomotic perfusion is considered essential for anastomotic healing. Performing a high tie (HT) technique allows anastomotic perfusion only through the marginal artery, which may lead to a decrease in anastomotic perfusion [5, 6]. When a low tie (LT) technique is performed, anastomotic perfusion is allowed not only through the marginal artery, but through the left colic artery and its ascending branch as well. This anatomical reality suggests that anastomotic perfusion is higher after low tie, however, no evidence exists supporting this hypothesis. The aim of this study is to compare the high tie technique to the low tie technique with regard to anastomotic perfusion.

Patients and methods

Patients planned for elective rectal resection for malignancy in four participating hospitals, with nine participating surgeons, were eligible for this non-randomized, prospective study. The procedure was represented by a Total Mesorectal Excision with or without anastomosis.

Blood flow was measured with the O2C system. The O2C system (“oxygen to see”, Lea Medizin Technik, Giessen, Germany) is a laser Doppler flowmetry system that has often been used to measure intestinal blood flow for research purposes [7, 8]. Blood flow, expressed in arbitrary units, is determined by analyzing the Doppler frequency shifts in laser light (820 nm) reflected from moving red blood cells. The laser light is emitted into the tissue, and the backscattered light is detected with a flat probe with a measurement depth of 4–6 mm (Lea Medizin Technik, Giessen, Germany). The O2C measurement frequency is 30 Hz. Measurements were performed at two moments during the operation, being (a) right after median laparotomy and (b) just before construction of the anastomosis or colostomy, in case of abdominoperineal resection. The measurements were performed on the antimesenterial, serosal side of the colon segment that was to become, or was after resection (at moment b), the proximal loop. For all measurements, after placement of the flat probe, the flow measurement was allowed to stabilize until a constant flow was measured. Afterwards, the flow was recorded for 30 s, obtaining 15 values. The mean of these 15 measurements was used to calculate the blood flow ratio (BFR), B/A. During the measurements, the blood pressure was measured as well, and the mean arterial pressure (MAP) was calculated.

The high tie technique was defined as ligation of the inferior mesenteric artery (IMA) at its origin. The low tie technique was defined as ligation of the superior rectal artery (SRA), just below the branching of the left colic artery (Fig. 1). The surgeon decided which technique was used.

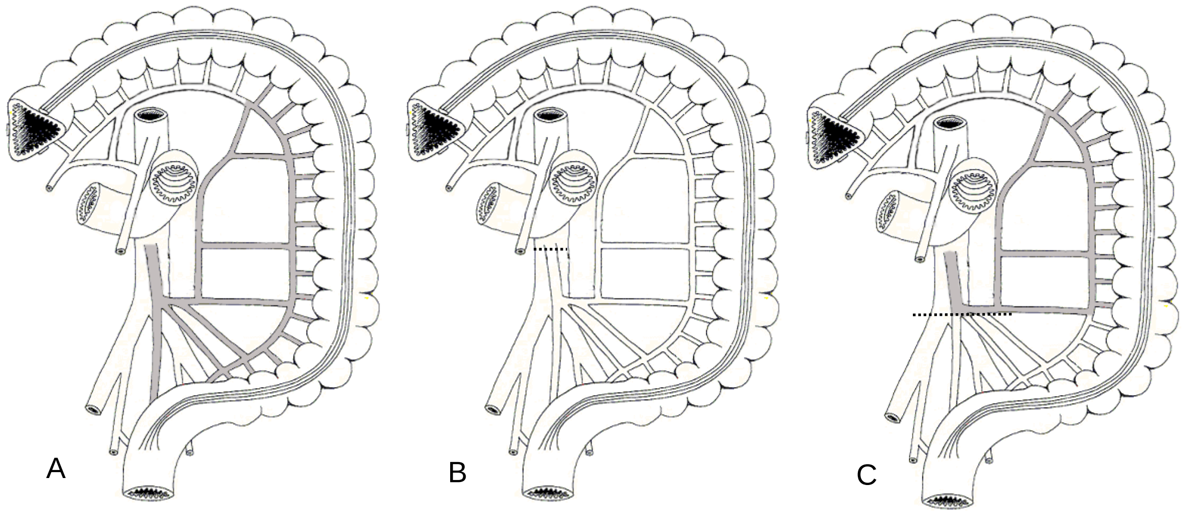

Figure 1. The vasculature of the colon.
A) Grey indicates the flow area of IMA, B) The dashed line indicates the level of ligature in HT, leaving no flow in the inferior mesenteric artery and its branches C) The dashed line indicates the level of ligature in LT, grey indicates the flow area of IMA after LT

The BFR distribution was normalized by a logarithmic transformation and compared between the HT and LT groups by means of an unpaired t test. MAP was compared between moments A and B with a paired sample t-test.

Statistical analysis was performed with SPSS 15.0.

Results

During 1 year, 33 patients were included in four different medical centers. A HT was performed in 16 patients (48 %) of whom 12 (75 %) received a primary anastomosis. A LT was performed in 17 patients (52 %) of whom also 12 (71 %) received a primary anastomosis. In all patients receiving a primary anastomosis, the splenic flexure was mobilized. Nine patients (75 %) in the high tie group and ten patients (83 %) in the low tie group received a defunctioning stoma. The mean number of lymph nodes harvested in the high tie group was 11 (range, 6–23), in the low tie group 12 (range, 6–33) (p=0.35). The mean number of positive lymph nodes harvested in the high tie group was 3 (1–5), in the low tie group 4 (1–9) (p=0.32). Two patients developed anastomotic leakage, one in the HT group and one in the LT group. In the HT group, significantly more patients received neoadjuvant radiotherapy.

No significant differences were found in the remaining baseline characteristics (Table 1).

The mean BFR was significantly higher in the LT group as depicted in Table 2, whereas the blood pressure during measurements was not significantly different as depicted in Table 3.

Discussion

To date, the discussion on the matter of high tie versus low tie continues. This study focuses on the colorectal vasculature and the flow change after HT or LT. Seike et al. found the colonic blood flow to vastly decrease after ligation of IMA or SRA [5], with the subsequent conclusion that this could be an unavoidable factor in the pathophysiology of colorectal anastomotic leakage. However, this study shows otherwise. After a HT procedure, only a

Baseline characteristics	High Tie	Low Tie	p-value
Gender (M/F)	11/5	12/5	1,000
Age	55 ± 17	61 ± 13	0,363
BMI	25 ± 3	27 ± 7	0,473
Operation			
APR	4	5	1,000
LAR	12	12	
Neoadjuvant therapy			
RT	14	8	0,024
No RT	2	9	
ASA-score			
I	7	5	0,170
II	8	6	
III	1	6	
Cardiovascular comorbidity	2 (13 %)	4 (24 %)	0,656
Operating time (minutes)	160 (100–340)	145 (45–225)	0.450
Tumour stage			
0	1	0	0,250
I	4	4	
IIa	2	4	
IIb	1	0	
IIIa	2	3	
IIIb	1	3	
IIIc	0	1	
IV	4	0	

Table 1. Patient characteristics of included patients.
M/F = Male / Female; BMI = Body Mass Index (kg/m^2); APR = Abdomino-Perineal Resection; LAR = Low Anterior Resection; RT = Radiotherapy; ASA-score = American Society of Anaesthesiologists score

Ratio	HT / LT	Mean Ratio	Std. Error Mean	p-value
B/A	HT	0,91	0,24	0,04
	LT	1,48	0,32	

Table 2. Comparison of blood flow ratios between the high tie (HT) and the low tie (LT) technique.

Group	MAP A/B	Mean	Std. Error Mean	p-value
HT	A	67,1	2,2	0,473
	B	64,2	3,2	
LT	A	69,8	4,4	0,075
	B	75,7	2,0	

Table 3. Mean Arterial Pressures (MAP) measured during the blood flow measurements at time points A and B, respectively.

slightly decreased blood flow was observed at the end of the operation (BFR 0, 91), whereas an increased blood flow was measured after LT (BFR 1, 48). The blood flow changes occurred independently from the systemic blood pressure (Table 3). These different findings may be explained by the time interval between arterial ligation and measurement. Seike et al. performed their measurements immediately after clamping of the artery. In this study the first measurement was performed immediately after laparotomy, and the second measurement, just before construction of the anastomosis or colostomy, i.e. at the end of the operation. Therefore, the interval between ligation and measurement is much longer in this study compared to the aforementioned study. This suggests that over time, a recruitment of colonic arteries occurs, allowing recovery of blood flow. In order to study whether these blood flow changes are permanent or not, blood flow measurements in the postoperative period would be interesting. In addition, since anastomotic leakage is generally detected around the eighth postoperative day [9], it could provide important information on the pathophysiological processes concerning blood flow leading to AL. The BFR was significantly higher after LT which means LT allows better perfusion of the proximal anastomotic loop at the end of the operation. Most likely, this is due to the preservation of the left colic artery and its ascending branch. In addition to the marginal artery, these arteries allow a second pathway for blood supply and faster and/or a more extensive recruitment of colonic arteries. Therefore, since good perfusion is essential for proper anastomotic healing, LT would be the preferred technique for this aspect of the high tie–low tie comparison.

The average BFR after LT shows an increase in blood flow compared to the initial value at the end of the operation. This has been described before by Karlicek et al. [10] and could be due to reactive hyperaemia as a result of colon manipulation. However, it could also be due to a variety of ischaemia reperfusion injuries (IRI). These injuries have been well described in animal models in which an IRI leads to visible hyperaemia and decreased anastomotic strength [11]. This response is probably also present after HT, however, it is more outspoken after LT most likely due to preservation of the left colic artery. Whether these findings have an impact on the incidence of anastomotic leakage should be evaluated by analyzing the blood flow during the postoperative period or in a similar but larger study.

The O2C allows non-invasive measurement of blood flow, however, the measurements are sensitive to several variables. First, it depends on placement of the probe. The probe has to be placed on the exact same spot for perfect comparability. Since it is virtually impossible to mark a spot on the colon without influencing the local blood flow or without hindering the progress of the operation, placement of the probe will be slightly variable. Second, the measurements are sensitive to different pressures applied on the probe when holding it in the right position. Higher pressures are likely to lead to more compressed arteries and a lower blood flow. In order to limit the influence of these variables on the outcome, measurements were performed by the same surgeon, allowing reproducibility of the measurement. In addition, the blood flow ratio was calculated for which the first measurement served as a control. The use of a ratio also allowed standardizing intrinsic, patient-related differences like microangiopathy due to atherosclerosis and diabetes mellitus. Table 1 shows baseline characteristics to be comparable between HT and LT except for radiotherapy. Significantly more people in the HT group received neoadjuvant radiotherapy. This is, however, unlikely to have an effect on the blood flow in the proximal anastomotic loop since this loop is located outside the radiation field. In addition, the high tie group contained a higher number of patients with metastasized disease (stage 4 present in 25 % in the HT group vs. 0 % in the low tie group). This difference most likely illustrates the participating surgeons having preferred to perform a high tie technique in patients with metastasized disease.

Conclusion

When comparing high tie ligation to low tie ligation, this study shows the perfusion of the proximal loop of the anastomosis to be better after low tie ligation. Considering neither of both techniques is favorable on the oncological or technical aspect, low tie ligation may be the technique of choice in patients undergoing rectum resection.

References

1. Miles WE (1971) A method of performing abdomino-perineal excision for carcinoma of the rectum and of the terminal portion of the pelvic colon (1908). CA Cancer J Clin 21(6):361–364

2. Moynihan B (1908) The surgical treatment of cancer of the sigmoid flexure and rectum. Surg Gynecol Obstet 6:463–466

3. Titu LV, Tweedle E, Rooney PS (2008) High tie of the inferior mesenteric artery in curative surgery for left colonic and rectal cancers: a systematic review. Dig Surg 25(2):148–157

4. Lange MM et al. (2008) Level of arterial ligation in rectal cancer surgery: low tie preferred over high tie. A review. Dis Colon Rectum 51(7):1139–1145

5. Seike K et al. (2007) Laser Doppler assessment of the influence of division at the root of the inferior mesenteric artery on anastomotic blood flow in rectosigmoid cancer surgery. Int J Colorectal Dis 22(6):689–697

6. Dworkin MJ, Allen-Mersh TG (1996) Effect of inferior mesenteric artery ligation on blood flow in the marginal artery-dependent sigmoid colon. J Am Coll Surg 183(4):357–360

7. De Bruin AF et al. (2009) The impact of chronic smoking on rectal mucosal blood flow. Tech Coloproctol 13(4):269–272

8. Albuszies G et al. (2005) Effect of increased cardiac output on hepatic and intestinal microcirculatory blood flow, oxygenation, and metabolism in hyperdynamic murine septic shock. Crit Care Med 33(10):2332–2338

9. Komen N et al. (2009) After-hours colorectal surgery: a risk factor for anastomotic leakage. Int J Colorectal Dis 24(7):789–795

10. Karliczek A et al. (2010) Intraoperative assessment of microperfusion with visible light spectroscopy for prediction of anastomotic leakage in colorectal anastomoses. Colorectal Dis 12:1018–1025

11. Posma LA et al. (2007) Transient profound mesenteric ischemia strongly affects the strength of intestinal anastomoses in the rat. Dis Colon Rectum 50(7):1070–1079

Part 3

Early diagnostics

Chapter 6

Anastomotic leakage, the search for a reliable biomarker. A review of the literature

N. Komen, R. W. F. de Bruin, G. J. Kleinrensink, J. Jeekel, J. F. Lange

Colorectal Dis. 2008 Feb;10(2):109-15

Abstract

Background

Colorectal anastomotic leakage (AL) is a severe complication leading to severe infection, sepsis and sometimes death. At present the diagnosis is made clinically, usually at 6–8 days after surgery. An objective biomarker reflecting the intra-abdominal milieu surrounding the anastomosis would be a useful additional diagnostic tool to make the diagnosis of AL before its clinical presentation. This review aims to assess the current status of the search for such a biomarker in peritoneal fluid.

Method

A literature search was carried out, using MEDLINE, PubMed and the Cochrane library, for all publications concerning human peritoneal fluid in relation to postoperative complications in general, and, more specific, anastomotic leakage after colorectal surgery.

Results

Analysis of several immune parameters, tissue repair parameters, parameters for ischaemia and microbiological composition of peritoneal fluid show that these can be determined reliably in the fluid, albeit with a large variance. Furthermore the data show that changes in concentration of these parameters precede AL and other postoperative complications by several days.

Conclusion

The results of the review demonstrate that it is possible to distinguish between patients with and without AL by measuring biomarkers in fluid from the peritoneal drain. Prospective studies with larger numbers of patients should, however, be performed and additional biomarkers should be studied to explore the full diagnostic potential of this approach.

Introduction

Leakage of a colorectal anastomosis is, by definition, a complication in which non-sterile intestinal content leaks into the abdominal or pelvic cavity through a defect in the anastomosis. Peritonitis may develop and lead to sepsis, multiple-organ failure, and ultimately death. The reported incidence varies between 10 and 13 % [1–3], with a mortality rate that can be as high as 33 % [4]. Current diagnostic methods include observation of clinical signs and symptoms while confirmation is obtained by imaging. These methods have several disadvantages. Neither clinical signs and symptoms nor systemic analysis of parameters such as C-reactive protein (CRP) and leucocytosis are specific. These can mimic less severe, postoperative infections including urinary tract infections and wound infection. Diagnostic tests for these presumed infections may delay the actual diagnosis of anastomotic leakage. Furthermore, when anastomotic leakage has progressed to a state of clinical manifestation, the patient is already ill and treatment needs to be initiated.

Imaging modalities, more specifically abdominal CT-scans and/with contrast enemas, are normally used to confirm a clinical diagnosis of anastomotic leakage, meaning the patient is already ill [5]. When these are used routinely, subclinical AL may be observed, not requiring any intervention [6]. Clinically relevant AL is usually diagnosed at about 6–8 days after surgery [1,7]. Some studies report an even longer interval, even up to 2 weeks [8]. Such long intervals are detrimental to the prognosis, increasing mortality rates [9]. An objective laboratory biomarker could help to decrease these intervals and make an early diagnosis.

The term biomarker is defined as an objectively measured characteristic, which is an indicator of a physiological or pathogenic process, or a pharmacological response to a therapeutic intervention [10]. A biomarker reflecting the intra-abdominal milieu surrounding the anastomosis could be a rapid and objective diagnostic tool in addition to current methods, allowing the diagnosis of AL before its clinical presentation. Such a biomarker could be found in the fluid retrieved from an intra-abdominal drain. It should, however, meet certain criteria, as defined in Table 1.

Criteria for a biomarker of AL in drain fluid
• Significant change in concentration in case of AL
• Stable in peritoneal environment and drain
• No significant influence of primary disease
• Maximum sensitivity and specificity
• Easy, fast, cheap and real-time testing

Table 1. Criteria for an objective biomarker for AL in drain fluid, composed by the authors

This fluid has to be retrieved by means of prophylactic drainage (PD), the aim of which is to prevent accumulation of blood in the pelvic or peritoneal cavity and to allow early detection of AL by faecal or purulent discharge from the drain. Despite the increasing use of 'fast track' surgery, PD is still often applied, mostly after low anterior resection as leakage rates are highest after this procedure [11,12].

Simply assessing the aspect of drainage fluid is not useful since in case of fecal discharge, severe leakage has already occurred. Imaging is a more accurate means of detection. Many studies have attempted to analyze the drain fluid in a more objective and sophisticated manner. The aim of this literature survey is to create an overview of studies in which such an analysis was performed and to see if a feasible biomarker for AL exists.

Method

A full search was performed of MEDLINE, PubMed and the Cochrane library for all papers concerning analysis of human peritoneal fluids, in relation to postoperative complications in general and, more specifically, AL after colorectal surgery. The terms 'drainage fluid' and 'AL' were used for the search. For each relevant article, all articles of the first author and all the related articles were identified. Potential biomarkers were compared to the criteria for a biomarker for AL in peritoneal drainage fluid as defined in Table 1.

Results

The search yielded ten studies which investigated a biomarker for postoperative complications. Of these studies, five aimed to find a biomarker specific for AL. All were prospective observational studies. The potential biomarkers described were divided into four groups, being immune parameters, tissue-repair parameters, parameters for ischemia, and microbiological parameters.

Immune parameters

Cytokines such as interleukin (IL)-1, 6, 10 and tumor necrosis factor-a (TNF-a), are polypeptides that mediate systemic changes associated with surgical trauma and infection such as fever, neutrophilia and increased hepatic acute phase protein synthesis [13]. Their major sources and effects are summarized in Table 2.

Cytokine	Major source	Major effects
Interleukin - 1	Macrophages	Stimulation of T cells and antigen-presenting cells. B-cell growth and antibody production. Promotes hematopoiesis (blood cell formation).
Interleukin - 6	Activated T cells, monocytes, endothelial cells	Stimulates B-cell differentiation, increases platelet production, stimulation of hepatocytes leading to increased production of CRP and fibrogen.
Interleukin - 10	Activated T cells, B cells and monocytes	Inhibition of cytokine production by macrophages and inhibition of the accessory functions of macrophages during T cell activation
TNF - α	Activated macrophages	Stimulates fibroblast growth and is a key mediary in the local inflammatory immune response

Table 2. Major source and effects of the reviewed cytokines (23-26)

During the first hours after abdominal surgery, levels of these cytokines are elevated in peritoneal fluid as part of the postoperative inflammatory response [14]. The systemic levels can be determined but compared with the peritoneal levels, they are secondary and are an inferior reflection of local events [14]. In the uncomplicated postoperative period, the cytokine levels in the peritoneal fluid will start to decrease within 24 h [14,15]. When complications such as AL occur, however, the response is different. Herwig et al. [16] found that a significant increase of IL-6 and TNF-a levels, as early as the first postoperative day, preceded clinical symptoms of peritonitis on account of leakage of a colorectal anastomosis by several days. Levels of IL-1 were significantly increased on the third postoperative day in patients who developed AL. Bertram et al. [17] found a sudden rise in TNF-a levels one day before the surgical diagnosis of AL. However it did not differ significantly from the control group. Levels of IL-6 were found to be the same in the two groups. These are the only two studies of AL after colorectal surgery, confirmed by imaging or laparotomy, as an outcome measure. In other studies, levels of peritoneal cytokines in relation to postoperative complications in general were analyzed. Van Bergen Henegouwen et al. [18] determined the levels of IL-6, IL-10 and TNF-a in peritoneal fluid of patients undergoing pancreaticoduodenectomy and gastric bypass. These levels were compared between patients with and without postoperative complications, which were defined as ‘any postoperative morbidity leading to medical or surgical intervention’. Decreasing cytokine levels were found in cases with an uncomplicated postoperative course. Additionally, they found a second rise of TNF-a in the peritoneal fluid to precede a postoperative complication by 1–3 days. Levels of IL-1 and 6 remained constant or decreased. Tsukada et al. found a significant correlation between IL-1b, IL-6, TNF-a and forms of surgical stress, such as longer operating time, more blood loss, and higher bacteria concentrations in drain fluid [19,20]. These findings were confirmed by Baker et al. [21] who found a correlation between IL-1b and IL-6 and postoperative complications. Unfortunately the studied populations were heterogeneous and postoperative complications were insufficiently defined.

The results of these studies suggested that monitoring of peritoneal cytokines could be predictive, or at least helpful in the early diagnosis of postoperative complications. However, with regard to the reported standard deviations, these studies included too few patients, and the definitions of postoperative complications were insufficient. Furthermore, patients underwent different surgical procedures in the same study. The results of these studies are therefore difficult to compare as it has been shown that the type of surgery influences the cytokine profile [22].

Tissue repair parameters

Matrix metalloproteinases (MMP) are a group of zinc-dependent endopeptidases that regulate the integrity and composition of the extracellular matrix (ECM) in both physiological and pathological processes. These are secreted as inactive pro-enzymes and need to be proteolytically activated in order to function. In peritoneal fluid, both the levels of the active form as well as the levels of the active form combined with the inactive form (Total MMP, T-MMP) could be determined. Wound repair and tissue regeneration depended on the balance between proteolysis by MMPs and prevention by their respective inhibitors like tissue inhibitors of metalloproteinase (TIMPs) on the one hand and by protein synthesis on the other [27]. Major activators and their effects are given in Table 3.

MMP	Enzyme	Activated by	Major effects
1	Collagenase-1	Plasmin, kallikrein, MMP-3, -10	Degradation of collagen, gelatin. Activator of MMP-2
2	Gelatinase A	MMP-3, -7, -13	Degradation of collagen, gelatin, elastin, fibronectin
9	Gelatinase B	MMP-2,-3,-13, Plasmin	Degradation of collagen, gelatin, elastin, fibronectin. Activator of MMP-9, -13
13	Collagenase-3	MMP-2, -3,-10, Plasmin	Degradation of collagen, gelatin. Activator of MMP-2, -9

Table 3. Activators and major effects of the reviewed MMP's (27)

Healing of colonic anastomoses in rats was enhanced by inhibiting MMP activity [28]. Compared with untreated controls, inhibition of MMP activity led to higher bursting pressures, a better preserved structural layer and an increased collagen network at the anastomotic site [28,29]. By analyzing the biopsies of macroscopically intact colon tissue, Stumpf et al. showed that patients with impaired anastomotic healing exhibited a significant higher expression of tissue MMP-1 and MMP-2 in the mucosal layers and of tissue MMP-2 and MMP-9 in the submucosal layers. In addition, the bowel wall of the leakage group contained a significantly lower collagen type I／III ratio compared to uncomplicated controls [30]. Type I and III collagen genes were normally overexpressed at the anastomotic site [31] and these collagen types were important targets of MMPs [27]. A correlation between intestinal tissue MMP-13 and AL was also found [32]. To gain more knowledge of the role of MMPs in the phase after oncological colorectal surgery, Baker et al. [33] determined daily profiles of MMPs and their inhibitors in peritoneal fluid. A positive correlation between peritoneal MMP-2, T-MMP-2, and T-MMP-9 and postoperative complications was observed. A negative correlation between TIMP-1 and -2 and postoperative complications was found. Unfortunately the studied population was heterogeneous and the types of postoperative complications were not specified in these publications. Although these studies suggested that determination of levels of MMPs in tissue or peritoneal fluid could serve as a biomarker for AL, the number of studies and numbers of patients used in these studies were small. In addition, the outcome measures used were not specific and did not permit definitive conclusions.

Parameters for ischaemia

Ischaemia of the intestine or, more locally, of the anastomosis is considered to be detrimental for healing and to increase the risk of AL [34,35]. Direct measurement of local ischaemia was shown to be predictive for AL [36]. However, postoperative in situ measurements are difficult and uncomfortable for the patient and therefore not ideal for routine surgical practice. When ischaemia occurs, the aerobic metabolism, present in healthy tissue, will convert to anaerobic metabolism. As a consequence, levels of lactate will increase and carbon dioxide will accumulate, lowering the pH. The decreased blood supply will lead to decreased levels of glucose. These processes will cause cell damage with breakdown of the membrane and release of the membrane’s phospholipids. These, in turn, will split up in free fatty acids and glycerol. These changes in metabolism were confirmed by measuring glucose, lactate and glycerol in peritoneal fluid by means of microdialysis in animal studies, in which hypoxic hypoxia was induced by adjusting ventilation [37] and in which regional occlusive ischaemia of the jejunum was induced [38]. Using microdialysis, peritoneal fluid could be evaluated indirectly by analyzing the dialysate. The dialysate will equilibrate with the peritoneal fluid surrounding the abdominal catheter, so that the levels of the dialysate will correspond to the levels of the local peritoneal fluid [39]. Indirect determination using microdialysis was also studied in patients after colorectal surgery [40,41]. In a pilot study including eight patients undergoing right hemicolectomy, Jansson et al. found the previously described changes in metabolic parameters to occur several hours before clinical signs of AL appeared [40]. The same group defined normal values for microdialysis in patients undergoing different gastro-intestinal operations [41].

Microbiological parameters

During colorectal resection, spillage of intestinal content may occur, leading to contamination of the abdominal cavity by microorganisms. Normally, this will be removed by the immune system and the postoperative course will be uncomplicated. When AL occurs, however, bacteria enter the abdominal or pelvic cavity in the postoperative phase. Qualitative and quantitative assessment of intraperitoneal bacterial load might therefore be a diagnostic tool for AL in an early phase. In this context the diagnostic value of lipopolysaccharides (LPS) in peritoneal fluid was studied. LPS forms part of the outer wall of gram-negative bacteria that are abundantly present in the gut as commensal flora. The diagnostic value of peritoneal LPS for peritonitis was reported by Beger et al. [42] and Muhammed et al. [43]. With the aim of identifying a biomarker for AL, Junger et al. [44] determined LPS levels in 22 patients having had a colorectal anastomosis. Three patients developed clinical signs of AL and they had significantly elevated LPS levels as early as day 3. The differences in LPS levels between the groups with and without anastomotic leakage were vast, however the standard deviation was great as well, rendering the number of patients too small.

Simmen et al. [45] studied indirect alterations of drainage fluid as a marker of bacterial infection. Analysis of the pH, pCO2 and pO2 of the drainage fluid was shown to allow early detection of infectious complications after abdominal surgery as early as the fourth postoperative day. These infectious complications contained only few cases of AL. Besides the small numbers in this study, all the patients underwent emergency laparotomy for a variety of indications rendering this population as too heterogeneous to allow clear conclusions to be drawn.

Discussion

At present AL is diagnosed by means of clinical symptoms and imaging. According to the literature, the interval between surgery and diagnosis of AL varies between 6 days and 2 weeks [7,8]. The longer the interval between onset and diagnosis, the higher the mortality will be [9]. Therefore AL should be diagnosed as early as possible to allow treatment to be instituted before the patient develops serious complications such as organ failure. This would decrease morbidity and mortality rates.

A reliable biomarker of AL would allow the diagnosis to be made earlier. Such a biomarker could be found in the surroundings of the anastomosis, i.e. drainage fluid. This should be present in the drainage fluid. Several studies measuring possible markers in drainage fluid have been reported with promising results. There are, however, several drawbacks. First, the position of the drain influences the composition of the drainage fluid [39], implying that the drain has to be located near the site of interest. Although the drain can be secured by attaching it to the skin, this will not guarantee its position at the anastomotic site. Second, even if the drain lies in the vicinity of the anastomosis, the surrounding peritoneal fluid can move with movements of the patient, like coughing. This could explain the variable results reported in the literature. Furthermore, this great variety renders the number of patients used in the reported studies, varying between 8 and 52 [21,40], too small. Third, the large variance in the measurements is often combined with great heterogeneity of the study populations [19,21,33]. For example, given the differences in anatomy and the complexity of the surgical procedure, it is not surprising that anterior resection and a right hemicolectomy show great differences. This is confirmed by Jansson et al. [39]. Fourth, there is a lack of detailed description of the criteria for patient inclusion in all but two of the reviewed studies [16,18]. The influence of selection bias is difficult to estimate. A fifth drawback that is encountered reviewing the literature concerning anastomotic leakage, is the vast variety in definitions of anastomotic leakage, as summarized by Bruce et al. [46]. In the articles discussed in this review the definition of anastomotic leakage varies from the occurrence of several undefined clinical signs and the presence of a fecal fistula or fecal fluid in the drain [44] to undefined clinical signs confirmed by imaging, microbiological investigation or diagnosis by laparotomy [16]. Concerning the criteria set out in Table 1, it can be said that the concentration of several potential biomarkers appears to change significantly in patients with AL, as shown in Table 4.

Potential biomarker	Significantly correlated to: AL	Postop compl.	Possible ischemia
Immune-parameters	(16)	(18, 21)	
Tissue repair parameters	(30, 32)	(33)	
Parameters of ischemia			(40)
Microbiological parameters	(44)	(45)	

Table 4. Representation of the studies relating a significant change of concentration of a potential biomarker (first column) to either AL, postoperative complications in general and visceral ischemia. Of the reviewed studies in only one no significant change was found(17).

The cytokines and MMPs are volatile, so that analysis of the peritoneal drainage fluid has to be immediate. In a routine ward this is very difficult to achieve. Moreover, the tests for the immune and tissue repair parameters are expensive in general, whereas analyses of the MMP-2 and -9 levels, pO2, pCO2 and pH may be more practical, because they are less costly [45]. The reviewed studies gave no data on sensitivity and specificity. To do so would be premature as no real cut-off levels of the studied biomarkers are defined. Furthermore, a larger number of patients are necessary and, in order to compare sensitivity and specificity between studies, a uniform definition of AL should be used.

Only a few studies correlate the potential biomarkers directly to AL. However, there are several studies finding a correlation with postoperative complications in general. Therefore these can be considered as potential biomarkers for AL as well (Table 4). At present, attempts to find a biomarker of AL in peritoneal fluid have focused on a narrow range of potential substances. Most studies have investigated immune- and tissue modulating factors, which are expensive to assay and have therefore limited the number of patients studied. For this reason other possible markers may be attractive. For example, C-reactive protein concentrations in drainage fluid should be studied to see whether it reflects inflammation faster than systemic measurement. Another possibility is to study the role of real-time PCR in detecting bacterial contamination of drainage fluid quantitatively. This technique has been reported to be fast and more sensitive than blood culture in detecting E. coli in the blood [47].

The detection of enzymes secreted by the exocrine pancreas, such as amylase and pancreas elastase-1, might also allow early diagnosis of AL. These enzymes are secreted into the intestinal lumen and, in the case of colorectal AL, detection in drainage fluid may be diagnostic, similar to the dehiscence of a pancreaticojejunostomy.

The only practical way of obtaining peritoneal fluid for analysis is by means of drainage. There is, however, controversy over the clinical value of a drain after colorectal surgery. Several meta-analyses have not shown any benefit for PD on the incidence of AL [48–50]. Adding to this controversy is the fact that with the current, primitive, diagnostic methods the drain is not a strong diagnostic tool. If, however, a biomarker can be found in peritoneal drainage fluid to diagnose AL before its clinical presentation, peritoneal drainage will become routine and no longer controversial. The reviewed studies indicate that analysis of drainage fluid has potential to detect AL in the early postoperative period.

Conclusion

Peritoneal fluid contains biomarkers indicating AL, potentially allowing early diagnosis. The analysis of peritoneal fluid might increase the understanding of anastomotic healing and the pathogenesis of AL. Analysis of the reviewed studies shows that it may be possible to distinguish between patients with and without AL by determining the presence and levels of potential biomarkers in drainage fluid. Studies with larger numbers of patients should be performed and additional biomarkers should be studied to explore the full diagnostic potential of this approach.

References

1. Kanellos I, Vasiliadis K, Angelopoulos S et al. Anastomotic leakage following anterior resection for rectal cancer. Tech Coloproctol 2004; 8 (Suppl. 1): s79–81.

2. Guenaga KF, Matos D, Castro AA, Atallah AN, Wille-Jorgensen P. Mechanical bowel preparation for elective colorectal surgery. Cochrane Database Syst Rev 2003; 2: CD001544.

3. Peeters KC, Tollenaar RA, Marijnen CA et al. Risk factors for anastomotic failure after total mesorectal excision of rectal cancer. Br J Surg 2005; 92: 211–6.

4. Docherty JG, McGregor JR, Akyol AM, Murray GD, Galloway DJ. Comparison of manually constructed and stapled anastomoses in colorectal surgery. West of Scotland and Highland Anastomosis Study Group. Ann Surg 1995; 221: 176–84.

5. Eckmann C, Kujath P, Schiedeck TH, Shekarriz H, Bruch HP. Anastomotic leakage following low anterior resection: results of a standardized diagnostic and therapeutic approach. Int J Colorectal Dis 2004; 19: 128–33.

6. Goligher JC, Lee PW, Simpkins KC, Lintott DJ. A controlled comparison one- and two-layer techniques of suture for high and low colorectal anastomoses. Br J Surg 1977; 64: 609–14.

7. Alves A, Panis Y, Pocard M, Regimbeau JM, Valleur P. Management of anastomotic leakage after nondiverted large bowel resection. J Am Coll Surg 1999; 189: 554–9.

8. Hyman N, Manchester TL, Osler T, Burns B, Cataldo PA. Anastomotic leaks after intestinal anastomosis: it's later than you think. Ann Surg 2007; 245: 254–8.

9. Macarthur DC, Nixon SJ, Aitken RJ. Avoidable deaths still occur after large bowel surgery. Scottish Audit of Surgical Mortality, Royal College of Surgeons of Edinburgh. Br J Surg 1998; 85: 80–3.

10. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 2001; 69: 89–95.

11. Rullier E, Laurent C, Garrelon JL, Michel P, Saric J, Parneix M. Risk factors for anastomotic leakage after resection of rectal cancer. Br J Surg 1998; 85: 355–8.

12. Yeh CY, Changchien CR, Wang JY et al. Pelvic drainage and other risk factors for leakage after elective anterior resection in rectal cancer patients: a prospective study of 978 patients. Ann Surg 2005; 241: 9–13.

13. Dinarello CA, Mier JW. Lymphokines. N Engl J Med 1987; 317: 940–5.

14. Wiik H, Karttunen R, Haukipuro K, Syrjala H. Maximal local and minimal systemic cytokine response to colorectal surgery: the influence of perioperative filgrastim. Cytokine 2001;

14: 188–92.

15. Jansson K, Redler B, Truedsson L et al. Intraperitoneal cytokine response after major surgery: higher postoperative intraperitoneal versus systemic cytokine levels suggest the gastrointestinal tract as the major source of the postoperative inflammatory reaction. Am J Surg 2004; 187: 372–7.

16. Herwig R, Glodny B, Kuhle C et al. Early identification of peritonitis by peritoneal cytokine measurement. Dis Colon Rectum 2002; 45: 514–21.

17. Bertram P, Junge K, Schachtrupp A, Gotze C, Kunz D, Schumpelick V. Peritoneal release of TNFalpha and IL-6 after elective colorectal surgery and anastomotic leakage. J Invest Surg 2003; 16: 65–9.

18. van Berge Henegouwen MI, van der Poll T, van Deventer SJ, Gouma DJ. Peritoneal cytokine release after elective gastrointestinal surgery and postoperative complications. Am J Surg 1998; 175: 311–6.

19. Tsukada K, Katoh H, Shiojima M, Suzuki T, Takenoshita S, Nagamachi Y. Concentrations of cytokines in peritoneal fluid after abdominal surgery. Eur J Surg 1993; 159: 475–9.

20. Tsukada K, Takenoshita S, Nagamachi Y. Peritoneal interleukin-6, interleukin-8 and granulocyte elastase activity after elective abdominal surgery. Apmis 1994; 102: 837–40.

21. Baker EA, Gaddal SE, Aitken DG, Leaper DJ. Growth factor profiles in intraperitoneal drainage fluid following colorectal surgery: relationship to wound healing and surgery. Wound Repair Regen 2003; 11: 261–7.

22. Baker EA, Leaper DJ. Proteinases, their inhibitors, and cytokine profiles in acute wound fluid. Wound Repair Regen 2000; 8: 392–8.

23. Tracey KJ, Cerami A. Tumor necrosis factor: a pleiotropic cytokine and therapeutic target. Annu Rev Med 1994; 45: 491–503.

24. http://gfme.free.fr/therap/interl.html (accessed August 2007).

25. http://www.web-books.com/MoBio/Free/Ch2G1.htm (accessed August 2007).

26. Abbas ALA, Pober J. (1994) Cellular and Molecular Immunology, 2nd edition. W.B. Saunders Company, Philadelphia, PA.

27. Chakraborti S, Mandal M, Das S, Mandal A, Chakraborti T. Regulation of matrix metalloproteinases: an overview. Mol Cell Biochem 2003; 253: 269–85.

28. Kiyama T, Onda M, Tokunaga A, Efron DT, Barbul A. Effect of matrix metalloproteinase inhibition on colonic anastomotic healing in rats. J Gastrointest Surg 2001; 5: 303–11.

29. de Hingh IH, Siemonsma MA, de Man BM, Lomme RM, Hendriks T. The matrix metalloproteinase inhibitor BB-94 improves the strength of intestinal anastomoses in the rat. Int J Colorectal Dis 2002; 17: 348–54.

30. 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.

31. Brasken P, Renvall S, Sandberg M. Fibronectin and collagen gene expression in healing experimental colonic anastomoses. Br J Surg 1991; 78: 1048–52.

32. Stumpf M, Cao W, Klinge U, Klosterhalfen B, Kasperk R, Schumpelick V. Collagen distribution and expression of matrix metalloproteinases 1 and 13 in patients with anastomotic leakage after large-bowel surgery. Langenbecks Arch Surg 2002; 386: 502–6.

33. Baker EA, Leaper DJ. Profiles of matrix metalloproteinases and their tissue inhibitors in intraperitoneal drainage fluid: relationship to wound healing. Wound Repair Regen 2003; 11: 268–74.

34. Thornton FJ, Barbul A. Healing in the gastrointestinal tract.Surg Clin North Am 1997; 77: 549–73.

35. Locke R, Hauser CJ, Shoemaker WC. The use of surface oximetry to assess bowel viability. Arch Surg 1984; 119: 1252–6.
36. Millan M, Garcia-Granero E, Flor B, Garcia-Botello S, Lledo S. Early prediction of anastomotic leak in colorectal cancer surgery by intramucosal pH. Dis Colon Rectum 2006; 49: 595–601.
37. Klaus S, Heringlake M, Gliemroth J, Bruch HP, Bahlmann L. Intraperitoneal microdialysis for detection of splanchnic metabolic disorders. Langenbecks Arch Surg 2002; 387: 276–80.
38. Sommer T, Larsen JF. Intraperitoneal and intraluminal microdialysis in the detection of experimental regional intestinal ischaemia. Br J Surg 2004; 91: 855–61.
39. Jansson K, Strand I, Redler B, Magnuson A, Ungerstedt U, Norgren L. Results of intraperitoneal microdialysis depend on the location of the catheter. Scand J Clin Lab Invest 2004; 64: 63–70.
40. Jansson K, Ungerstedt J, Jonsson T et al. Human intraperitoneal microdialysis: increased lactate ⁄ pyruvate ratio suggests early visceral ischaemia. A pilot study. Scand J Gastroenterol 2003; 38: 1007–11.
41. Jansson K, Jansson M, Andersson M, Magnuson A, Ungerstedt U, Norgren L. Normal values and differences between intraperitoneal and subcutaneous microdialysis in patients after non-complicated gastrointestinal surgery. Scand J Clin Lab Invest 2005; 65: 273–81.
42. Beger HG, Gogler H, Kraas E, Bittner R. [Endotoxins in bacterial peritonitis]. Chirurg 1981; 52: 81–8.
43. Muhammed KO, Ozener Q, Akoglu E. Diagnostic value of effluent endotoxin level in gram-negative peritonitis in CAPD patients. Perit Dial Int 2001; 21: 154–7.
44. Junger W, Junger WG, Miller K et al. Early detection of anastomotic leaks after colorectal surgery by measuring endotoxin in the drainage fluid. Hepatogastroenterology 1996; 43: 1523–9.
45. Simmen HP, Battaglia H, Giovanoli P, Blaser J. Analysis of pH, pO2 and pCO2 in drainage fluid allows for rapid detection of infectious complications during the follow-up period after abdominal surgery. Infection 1994; 22: 386–9.

46. Bruce J, Krukowski ZH, Al-Khairy G, Russell EM, Park KG. Systematic review of the definition and measurement of anastomotic leak after gastrointestinal surgery. Br J Surg 2001; 88: 1157–68.

47. Kane TD, Alexander JW, Johannigman JA. The detection of microbial DNA in the blood: a sensitive method for diagnosing bacteremia and ⁄ or bacterial translocation in surgical patients. Ann Surg 1998; 227: 1–9.

48. Merad F, Yahchouchi E, Hay JM, Fingerhut A, Laborde Y, Langlois-Zantain O. Prophylactic abdominal drainage after elective colonic resection and suprapromontory anastomosis: a multicenter study controlled by randomization. French Associations for Surgical Research. Arch Surg 1998; 133: 309–14.

49. Petrowsky H, Demartines N, Rousson V, Clavien PA. Evidence-based value of prophylactic drainage in gastrointestinal surgery: a systematic review and meta-analyses. Ann Surg 2004;240:1074–84; discussion 84–5.

50. Jesus EC, Karliczek A, Matos D, Castro AA, Atallah AN Prophylactic anastomotic drainage for colorectal surgery. Cochrane Database Syst Rev 2004; 4: CD002100.

Chapter 7

Detection of colon flora in peritoneal drain fluid after colorectal surgery: Can RT-PCR play a role in diagnosing anastomotic leakage?

The APPEAL-study: Analysis of Parameters Predictive for Evident Anastomotic Leakage.

N. Komen, M.C. Morsink, S. Beiboer, A. Miggelbrink, P. Willemsen, E. van der Harst, J.F. Lange, W.B. van Leeuwen

J Microbiol Methods. 2009 Oct;79(1):67-70

Abstract

A semi-quantitative Real-Time PCR strategy was developed to identify potential indicator organisms for anastomotic leakage in peritoneal drainage fluid, Escherichia coli and Enterococcus faecalis. The analytical performance of the amplification method was validated with 10 culture-positive and 7 culture-negative peritoneal drain fluid samples, obtained from 9 different patients with a colorectal anastomosis. Real-Time PCR results were fully concordant with the microbiological culture results. However, among the culture negative samples, four false-positive RT-PCR results were found. All false-positives originated from a single patient with a surgical site infection. This may indicate an elevated sensitivity of the RT-PCR method. The results showed that the semi-quantitative RT-PCR method has a clear potential to be useful as a powerful tool in early detection of anastomotic leakage.

Introduction

Anastomotic leakage is the most feared complication after colorectal surgery, with leakage rates varying between 2 and 24 % [1-4]. Anastomotic leakage (AL) is defined as intestinal content leaking into the peritoneal cavity through an anastomotic defect. Consequently colonic bacteria such as Escherichia coli and Enterococcus faecalis spread throughout the peritoneal cavity possibly leading to peritonitis. This, in turn, may lead to sepsis and mortality. Current diagnostic methods for AL include observation of clinical parameters such as symptoms of AL, wound aspect, change in vital signs and imaging. However, observation of clinical parameters is not specific and can mimic less severe surgical site infections.

Imaging modalities, such as CT-scan or conventional X-ray, are not routinely performed but only used for confirmation. A high index of suspicion remains necessary. With these diagnostic methods the interval between operation and the diagnosis of AL varies between 8 and 13 days [1,5,6]. By this time the patient will already be severely affected.

Prophylactic drainage is an accepted and widely used method to evacuate blood and wound fluid in the postoperative phase [7,8]. However, regarding AL, controversy remains on whether it has a beneficial effect on leakage rates [9,10]. In case of AL the bacterial load in the drainage fluid will increase over time. Detection of this trend could be diagnostic for anastomotic leakage and when detected in the early postoperative phase, morbidity and mortality rates may be reduced. To achieve this goal a fast and sensitive method like Real-Time polymerase chain reaction (RT-PCR) is required. Currently, no RT-PCR tests are available to detect colon flora in drainage fluid. Therefore we have proposed to develop a diagnostic test based on the detection of microorganisms indicative for AL in peritoneal drain fluid, using a quantitative Real-Time PCR approach. The indicative micro-organisms included E. coli and E. faecalis, which are both commonly present in the colon. The analytical performance of this strategy has been validated in a pilot study.

Materials and methods

Patient samples

Seventeen peritoneal drain fluid samples from a culture-positive (n=10) and a culture-negative pool (n=7) were obtained from nine patients enrolled in the pilot study preceding the APPEAL-study (Analysis of Predictive Parameters for Evident Anastomotic Leakage). This is a prospective observational multicenter study on the subject of potential biomarkers for AL in drainage fluid, registered in the Dutch Trial Register, study number NTR 1258 (http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=1258).

These selected patients underwent colorectal surgery with construction of an anastomosis and they received a closed, passive drainage system. Preoperatively they received prophylactic antibiotics intravenously, cefazoline 2 g and metronidazol 1.5 g. Postoperatively, the drains' reservoir was emptied twice daily, at 22.00 h and 9.00 h for 5 days after the intervention. Only morning collections were drained in a sterile Falcon tube, immediately transported to the laboratory and used for analysis. Cultures were performed directly by inoculating drainage fluid with a 10 μl inoculation loop on two plates, one Columbia Bloodagar and one MaC Conkeyagar. Afterwards, drainage fluid was centrifuged for 10 min, 2800×g and 4 °C. Supernatant was separated from the cellpellet and frozen in −80 °C until analysis. Drain fluid samples were indicated with a letter A through E, each referring to the consecutive postoperative day (A for the collection on postoperative day one, etc.) (Table 2).

DNA isolation

Prior to DNA isolation, 500 μl of drain fluid was spiked with 15 μl Phocine Herpes Virus (PhHV, supplied by the Department of Virology, Erasmus MC Rotterdam, The Netherlands) as an internal control. Drain fluid samples used for generating the standard curve were not spiked with PhHV. Subsequently, each sample was centrifuged at room temperature for 5 min at 100×g. Supernatant was diluted 10 times in a total volume of 250 μl and centrifuged at room temperature for 5 min at 8000×g. The resulting pellet was resuspended in 180 μl buffer containing 20 mMTris, 2 mMEDTA, 1 % Tween 80 and lysozyme (50 mg/ml) and incubated for 30 min at 37 °C on a shaking device at 600 rpm (Sanyo Orbital Shaker, München, Germany). DNA extraction was continued using a Macherey–Nagel NucleoSpin® Tissue Kit (Clontech Laboratories, Inc., Mountain View, CA, USA). First, 25 µl of protease was added to the sample, followed by incubation at 56 °C for 2 h at 700 rpm in a shaking device (Thermomixer Compact, Eppendorf, Hamburg, Germany). Protocol proceeded according to the manufacturer's instructions. Finally, template DNA was eluted in nuclease-free water in a total volume of 100 µl.

Semi-quantitative Real-Time PCR

All PCR reactions were performed in a total volume of 25 µl. The PCR-mix for detection of E. coli consisted of 12.5 µl 2× DyNAmo™ HS SYBR® Green mix (Finnzymes Oy, Espoo, Finland), 0.25 µl forward primer (50 pmol/µl), 0.25 µl reverse primer (50 pmol/µl), 0.25 µl 100 nM Fluorescein Calibration Dye (Bio-Rad, Hercules, CA, USA), 5 µl template DNA and 6.75 µl water. In order to detect E. faecalis, a PCR-mix containing 12.5 µl 2× DyNAmo™ HS SYBR® Green mix (Finnzymes Oy), 0.45 µl forward primer (50 pmol/µl), 0.15 µl reverse primer (50 pmol/µl), 0.25 µl 100 nM Fluorescein Calibration Dye (Bio-Rad), 5 µl template DNA and 6.65 µl water was used. Detection of the internal control PhHV was performed with a PCR-mix consisting of 12.5 µl 2× DyNAmo™ HS SYBR® Green mix (Finnzymes Oy), 0.2 µl forward primer (50 pmol/µl), 0.25 µl reverse primer (50 pmol/µl), 0.25 µl 100 nM Fluorescein Calibration Dye (Bio-Rad), 5 µl template DNA and 6.75 µl water. Following primers were used for Real-Time quantitative PCR: E. coli uidA gene forward primer 5′-GGC TTC TGT CAA CGC TGT TT-3′, E. coli uidA gene reverse primer 5′-CCC ATG GAA GAG AAA TGG AA-3′, E. faecalis 23S rRNA gene forward primer 5′-AGA AAT TCC AAA CGA ACT TG-3′, E. faecalis 23S rRNA gene reverse primer 5′-CAG TGC TCT ACC TCC ATC ATT-3′, PhHV forward primer 5′-GGG CGA ATC ACA GAT TGA ATC-3′, PhHV reverse primer 5′-GCG GTT CCA AAC GTA CCA A-3′. The Bio-Rad IQ5 ICycler (Bio-Rad, Veenendaal, The Netherlands) was used as Real-Time PCR platform and the PCR conditions for E. coli, E. faecalis and PhHV were as follows: a single predenaturation step of

15 min at 95 °C followed by 40 cycles of 15s at 95 °C and 1 min at 59 °C. Finally, the sample temperature was gradually increased to 95 °C in order to generate dissociation curves. These curves were used to assess the specificity of the PCR product. The dissociation temperature was 76.0 °C for the E. coli-specific PCR product and 77.0 °C for the E. faecalis-specific product. The PCR efficiency was calculated using the slope of the standard curve (efficiency=10−1/slope−1).

Standard curves

The semi-quantitative inoculum of indicator organisms potentially present in the peritoneal drain fluid at the time of anastomotic leakage, has been determined by using a reference dilution series of E. coli and E. faecalis inocula. Reference series were produced by spiking 500 µl of culture-negative drain fluid with a 10 log serial dilution (0, 10 through 105CFU) of both E. coli and E. faecalis. A standard curve was generated by comparing the Real-Time PCR results (threshold cycle or Ct-value) to the inoculum sizes. The approximate inoculum size of the query patient sample was determined after interpolation of its Ct-value within the standard curve. Patient samples were analyzed in duplicate.

Results

Characteristics of the patients of which drain fluid was retrieved are depicted in Table 1.

Patient	Age	Gender	Operation	Indication	Anastomosis	AL	Remarks
1	29	Female	Ileocecal resection	Crohn's disease	Side to side	No	
2	48	Female	Ileocecal resection	Crohn's disease	Side to side	No	
4	76	Male	Sigmoid resection	Adenocarcinoma	End to side	No	
5	71	Male	Ileocecal resection	Iatrogenic perforation by endoscopy	Side to side	No	Seropurulent peritonitis before operation
8	83	Female	Right hemicolectomy	Tubulovilleus adenoma	Side to side	No	
9	66	Male	Low anterior resection	Adenocarcinoma	End ot side	No	
10	71	Female	Sigmoid resection	Adenocarcinoma	End to side	No	
11	57	Male	Left hemicolectomy	Diverticular disease	Side to side	No	Wound dehiscence with infection
17	51	Male	Total mesorectal excision	Adenocarcinoma	Side to end	No	

Table 1. Patient characteristics of the studied population. All patients received antibiotic prophylaxis preoperatively consisting of cefazoline and metronidazol.

No patient suffered from clinical anastomotic leakage. The culture results and semi-quantitative RT-PCR data obtained from the drain fluid samples are summarized in Table 2.

Code	PO day	Culture	*E. coli* RT-PCR			*E. faecalis* RT-PCR			IC
			Ct-value	copy number	T melt	Ct-value	copy number	T melt	CT-value
1B	2	*Streptococcus oralis, Staphylococcus aureus*	33.8	10^4	76	36.4	10	77	33.5
1E	5	*Escherichia coli*	37.5	10^2	76	35.8	10	77	33.6
2D	4	*Escherichia coli*	30.5	10^5	76	37.2	-	74	30.7
4A	1	*Staphylococcus spp., Bacteroides melaninogenicus, non-identified anaerobic Grampos rod*	34.5	-	70-71	33.4	10^2	77	33.0
5A	1	*Escherichia coli*	32.9	10^4	76	32.0	10^2 -10^3	77	32.4
5C	3	*Escherichia coli*	32.3	10^4 -10^5	76	29.0	10^3 -10^4	77	31.2
8A	1	-	34.2	-	70-71	33.4	10^2	77	34.6
9A	1	-	36.4	10^3	76	N/A	-	-	33.1
9B	2	-	N/A	N/A	-	35.4	10	77	31.9
10A	1	Mixed skinflora	38.1	-	70	34.6	10^2	77	33.9
10E	5	-	34.1	-	72	35.7	-	72	33.1
11A	1	-	N/A	N/A	-	32.4	10^2 -10^3	77	31.0
11B	2	-	37.5	-	78	33.9	10^2	77	31.1
11C	3	-	38.3	-	80	31.4	10^3	77	30.4
11D	4	*Staphylococcus spp.*	37.1	-	70-80	33.1	10^2	77	30.9
11E	5	*E. faecalis*	36.3	-	70-74	28.1	10^4	77	31.1
17C	3	*Enterococcus faecalis, Escherichia coli, Candida albicans*	33.2	10^4	76	25.6	10^5	77	32.2

Table 2. Comparative analysis of microbiological culture and Real-Time PCR results for the colon flora indicator organisms *E. coli, E. faecalis* and the internal control (IC).
PO day, postoperative day and refers to the day of sampling; copy number, approximate number of bacteria after interpolation to the standard curve; T melt, melting temperature. Patient samples were analyzed in duplicate; indicated Ct-values, copy numbers and Tm-values are average data within 99.0% CI.

Results from both diagnostic techniques were fully concordant for patients 1, 2, 4, 5, 10 and 17. The first drain fluid sample after intervention obtained from patient 8 revealed E. faecalis-positive PCR and culture-negative result. A similar result was obtained from the first drain fluid sample from patient 9 of which E. coli-specific RT-PCR was positive, while no bacterial growth was observed in the microbiological culture. From patient 11 all consecutively sampled drain fluids were analyzed. E. faecalis could be cultured 5 days after surgical intervention. With semi-quantitative RT-PCR approximately 10^2–10^3 genome copies (Ct-value 32.4) could be detected in the first sample. The E. faecalis genome copy size remained stable for 4 days (Ct-values of 33.9, 31.4 and 33.1 for samples B, C and D respectively) and increased to 10^4 genome copies on day 5 (Ct-value 28.1 for sample E). All E. coli PCR results were negative for patient 11. The PCR product melting or dissociation temperature confirmed the product-specificity, which was 76 °C (+/−0.1 °C) for E. coli and 77 °C (+/−0.1 °C) for E. faecalis. Efficiencies of both E. coli-specific and E. faecalis-specific RT-PCR protocol were 97 %, which can be considered as very good. The aspecific Tm-values could all be explained by primer–dimer formation.

Discussion

In case of AL, the leaking intraluminal contents of the colon will affect the intraabdominal environment first, implying quantitative and qualitative microbiological analysis on drainage fluid could allow early diagnosis of AL [11]. E. coli and E. faecalis are facultative aerobic colon bacteria, ubiquitously present in faecal samples. Animal experiments suggest E. coli in blood detected by PCR, to be a useful tool in the diagnosis of AL [12]. E. coli and E. faecalis can be detected in drainage fluid after colorectal surgery by means of culture [13]. Therefore these bacteria are well suited to serve as indicator organisms for diagnosis of anastomotic leakage on peritoneal drainage fluid. In this article we have introduced a tool that allows semiquantitative, qualitative and fast detection of contamination of drainage fluid with these microorganisms. A Real-Time PCR protocol was used to semi-quantitatively detect and to identify species-specific target DNA in purified peritoneal drain fluid samples. The results obtained from the multiplex molecular-based detection of both E. coli and E. faecalis were concordant to the results obtained with conventional microbiological culture techniques that were considered to be the golden standard. This means that this technique allows reliable determination of contamination of peritoneal drain fluid with E. coli and E. faecalis. The RT-PCR showed several false positive results. E. coli was detected twice with RT-PCR, while it was not found in culture. E. faecalis was detected five times without culture confirmation. This discrepancy may be explained by the preoperative use of antibiotic prophylaxis, cefazoline and metronidazol. Due to the antibiotics the indicator organisms will not grow on culture media, however, the bacterial DNA can still be detected with RT-PCR. Another explanation could be the occurrence of false negative cultures, either due to the small bacterial load or due to the period between leaving the host and culture. The latter cannot be shortened since a certain volume of drainage fluid is needed to perform cultures. Despite the aforementioned arguments, our results indicate superior sensitivity of RT-PCR in detection of contaminated drain fluid compared to conventional cultures. In addition, RT-PCR can be performed more or less independent of differences in drain fluid transport conditions such as temperature and time. Therefore it allows flexibility in logistical matters, which is essential in a clinical setting.

The semi-quantitative aspect of this RT-PCR is important in the detection of anastomotic leakage. Initial positive samples could be positive for E. coli and E. faecalis due to intraoperative spill [13], however, in case of anastomotic leakage, the bacterial load will increase over time. Daily cultures can be semi-quantitative as well, however, RT-PCR is superior in terms of speed. Besides the clinically evident anastomotic leakage, subclinical anastomotic leakage can occur as well. This means leakage of intestinal content occurs but the patient is not affected by it. This condition does not require treatment and is often not even detected. Both intraoperative spill and subclinical AL may be responsible for positive test results on drainage fluid in this study, while no patient developed clinically manifest AL. However, it is expected that clinically relevant AL can be differentiated from subclinical AL and spill by the bacterial load which is probably vastly lower in the latter two conditions.

Drain fluid retrieval is prone to sample contamination through the person retrieving the fluid, which can be detected by culture as depicted in Table 2. However, E. coli and E. faecalis were selected as indicator bacteria since they are less likely to be brought into the drain fluid another way than through anastomotic leakage or intraoperative spill.

The specificity of species-specific DNA targets by Real-Time PCR amplification is guaranteed by the determination of the PCR product dissociation temperature, which is highly specific for each target DNA, with minor tolerance (+/−0.1 °C). We noticed that optimal results (highest sensitivity) could be retrieved by application of a short centrifugation step and subsequent DNA extraction from the supernatant of the drain fluid.

A RT-PCR test was developed that allows detection of E. coli and E. faecalis in peritoneal drain fluid equally sensitive as the golden standard, potentially more sensitive. This study does not allow to draw conclusions regarding the diagnostic potential of this test for AL since numbers are too small. In addition, none of the patients included in this study developed anastomotic leakage. However, this is currently being studied in the APPEAL-study.

References

1. Hyman, N., Manchester, T.L., Osler, T., Burns, B., Cataldo, P.A., 2007. Anastomotic leaks after intestinal anastomosis: it's later than you think. Ann. Surg. 245, 254–258.

2. Komen, N., Dijk, J.W., Lalmahomed, Z., Klop, K., Hop, W., Kleinrensink, G.J., Jeekel, H., Ruud Schouten, W., Lange, J.F., 2009. After-hours colorectal surgery: a risk factor for anastomotic leakage. Int. J. Colorectal Dis. 24, 789–795.

3. Matthiessen, P., Hallbook, O., Rutegard, J., Simert, G., Sjodahl, R., 2007. Defunctioning stoma reduces symptomatic anastomotic leakage after low anterior resection of the rectum for cancer: a randomized multicenter trial. Ann. Surg. 246, 207–214.

4. Sagar, P.M., Couse, N., Kerin, M., May, J., MacFie, J., 1993. Randomized trial of drainage of colorectal anastomosis. Br. J. Surg. 80, 769–771.

5. Alves, A., Panis, Y., Pocard, M., Regimbeau, J.M., Valleur, P., 1999. Management of anastomotic leakage after nondiverted large bowel resection. J. Am. Coll. Surg. 189, 554–559.

6. Makela, J.T., Kiviniemi, H., Laitinen, S., 2003. Risk factors for anastomotic leakage after left-sided colorectal resection with rectal anastomosis. Dis. Colon Rectum 46, 653–660.

7. Brown, S.R., Seow-Choen, F., Eu, K.W.,Heah, S.M.,Tang, C.L.,2001.Aprospective randomized study of drains in infra-peritoneal rectal anastomoses. Tech. Coloproctology 5, 89–92.

8. Lennox, M.S., 1984. Prophylactic drainage of colonic anastomoses. Br. J. Surg. 71, 10–11.

9. Karliczek, A., Jesus, E.C., Matos, D., Castro, A.A., Atallah, A.N., Wiggers, T., 2006. Drainage or nondrainage in elective colorectal anastomosis: a systematic review and metaanalysis. Colorectal Dis. 8, 259–265.

10. Peeters, K.C., Tollenaar, R.A., Marijnen, C.A., Klein Kranenbarg, E., Steup, W.H., Wiggers, T., Rutten, H.J., van de Velde, C.J., 2005. Risk factors for anastomotic failure after total mesorectal excision of rectal cancer. Br. J. Surg. 92, 211–216.

11. Komen, N., de Bruin, R.W., Kleinrensink, G.J., Jeekel, J., Lange, J.F., 2008. Anastomotic leakage, the search for a reliable biomarker. A review of the literature. Colorectal Dis. 10, 109–115 discussion 115–107.

12. Emet, T., Bilsel, Y., Tilki, M., Surmelioglu, A., User, Y., 2005. Early diagnosis of colorectal anastomotic leakages by detection of bacterial genome. Ulus. Travma Acil Cerrahi Derg. 11, 195–200.

13. Chylak, J., Michalska,W., Drews, M.,Marciniak, R., Lange, M., 2000. Comparison of bacterial flora found in the peritoneal cavity and drains after intraabdominal surgery.Med. Sci. Monit. 6, 285–290.

Chapter 8

RT-PCR of Enterococcus faecalis in drain fluid: the first screening test for symptomatic colorectal anastomotic leakage

The APPEAL-study: Analysis of Parameters Predictive for Evident Anastomotic Leakage.

N. Komen, J. Slieker, P. Willemsen, G. Mannaerts, P. Pattyn, T. Karsten, H. de Wilt, E. van der Harst, R. Bleker, W. van Leeuwen, C. Decaestecker, J. Jeekel, Johan F. Lange

Int J Colorectal Dis. 2013 Oct 11

Abstract

Purpose

With current diagnostic methods, the majority of patients with symptomatic colorectal anastomotic leakage (CAL) is identified approximately 1 week after operation. The aim of this study is to determine whether real-time polymerase chain reaction (RT-PCR) for detection of Escherichia coli and Enterococcus faecalis on drain fluid can serve as a screening test for CAL in the early postoperative phase.

Methods

All patients included in this multicenter prospective observational study underwent left-sided colorectal resection for both malignant and benign diseases with construction of an anastomosis. In all patients, an intra-abdominal drain was placed during operation. During the first five postoperative days, drain fluid was processed for RT-PCR. The quantitative results of the RT-PCR on days 2 to 5 were compared to the results of day 1 in order to detect concentration changes.

Results

In total, 243 patients, with both benign and malignant diseases, were included of whom 19 (7.8 %) developed symptomatic CAL. An increase in E. coli concentration was found in significantly more patients with CAL on day 4 and 5 [p =0.0004, diagnostic odds ratio (DOR) 7.9]. For E. faecalis, this result was found for days 2, 3, and 4 (p <0.003) with highest DOR on day 3 (31.6). Sensitivity and negative predictive values were 92.9 and 98.7 %, respectively, virtually ruling out CAL in case of negative test results on the third postoperative day.

Conclusion

Quantitative PCR for E. faecalis performed on drain fluid may be an objective, affordable and fast screening tool for symptomatic colorectal anastomotic leakage.

Introduction

Despite the vast body of evidence concerning colorectal anastomotic leakage (CAL), it remains a poorly understood complication of colorectal surgery. The reported incidence of CAL is estimated between 2.4 and 19 % [1–3] and mortality rates due to sepsis and multiple organ failure are around 15 % in patients who develop CAL [4]. With current screening and diagnostic methods, the interval between construction of the colorectal anastomosis and diagnosis of leakage varies between 6 and 13 days [5–7].

Several studies have suggested that delay of diagnosis of CAL is associated with higher mortality rates and that only early management improves clinical outcome [8–10]. Therefore, new screening methods allowing detection of CAL in the early postoperative phase are needed in addition to current methods.

Morbidity caused by CAL is due to the bacterial load leaking through an anastomotic defect. Gram-negative Escherichia coli and gram-positive Enterococcus faecalis belong to the most common species of the colon [11, 12]. When present in wound fluid obtained from the anastomotic site, it means there is contamination from the bowel. Increased concentrations of these bacteria are most likely reflecting an anastomotic defect. Therefore, these bacteria might be suited to screen for CAL [13, 14].

The golden standard for detection of bacterial contamination is culture. However, bacteria present in drain fluid in a collection bag outside the patient may not always be viable, which could render false negative results. In addition, it takes about 48 h of incubation before bacteria can be identified [14], which is an unacceptable delay. Real-time polymerase chain reaction (RT-PCR) is an alternative, molecular based technique that can be used to identify bacterial species. It is faster, more sensitive and less susceptible to contamination than culture and might therefore be a valuable screening tool for CAL. In addition, since virtually every clinical laboratory already has a RT-PCR machine, it is a cheap technique. The aim of this study is to study whether RT-PCR determination of E. coli and E. faecalis can serve as a screening test for CAL in the early postoperative phase after (left-sided) colorectal surgery.

Methods

Patients included in the APPEAL study received left-sided colorectal resection with construction of an anastomosis and were given an intra-abdominal drain. Seven medical centers in the Netherlands and Belgium participated in this study. The study, registered in the Dutch Trial Register (http://www.trialregister.nl, study number NTR 1258), was approved by the medical ethical committee of all the participating centers, in accordance with the ethical standards of the Helsinki Declaration of 1975 and all patients gave informed consent. Participating centers included patients consecutively between January 2007 and December 2009.

Inclusion and exclusion criteria

Subsequent patients undergoing left hemicolectomy, sigmoid resection, high anterior resection (HAR; with partial mesorectal excision (PME)), low anterior resection (LAR; with total mesorectal excision (TME)), and subtotal colectomy with ileorectal anastomosis were included. Oncologic resections as well as resections for inflammatory disease were included. Emergency operations were excluded due to the high probability of coexisting tissue damage and logistical difficulties. Reversals of colostomy were also excluded since the primary disease was already treated. Furthermore, patients under 18 years of age, patients who refused to participate, and patients who did not receive a drain were excluded.

Surgical procedure

The surgical procedure was left to the surgeon's discretion. All patients received preoperative antibiotic prophylaxis and an intra-abdominal drain. Guidelines concerning bowel preparation differed for each center and were respected. Patients were operated by laparotomy or laparoscopy, and the anastomosis was stapled or hand sutured. A diverting stoma was constructed according to the surgeon's preference. To obtain drain fluid, a drain was placed at the anastomotic site and was left in place during the first five postoperative days. The drains were all passive and closed drainage systems. The exact type of drain used was left to the surgeon's discretion.

Drain fluid

Drain fluid reservoirs were emptied two times a day with 12 h intervals, respecting rules of sterility. The evening collection was disposed of. The morning collection was centrifuged for 10 min at 2,800×g and 4 °C. The supernatant was brought into different cryotubes that were frozen at −80 °C to allow RT-PCR analysis in batch. Real-time polymerase chain reaction RT-PCR analysis of the drain fluids was performed in batch for efficiency purposes. The applied technique was described earlier [13].

DNA isolation

After thawing, each sample was centrifuged at room temperature for 5 min at 100×g. Supernatant was diluted 10 times in a total volume of 250 μl and centrifuged at room temperature for 5 min at 8,000×g. The resulting pellet was resuspended in 180 μl buffer containing 20mMTris, 2mMEDTA, 1 %Tween 80, and lysozyme (50 mg/ml) and incubated for 30 min at 37 °C on a shaking device at 600 rpm (Sanyo Orbital Shaker, München, Germany). DNA extraction was performed using a Macherey–Nagel NucleoSpin® Tissue Kit (Clontech Laboratories, Inc., Mountain View, CA, USA). First, 25 μl of protease was added to the sample, followed by incubation at 56 °C for 2 h at 700 rpm in a shaking device (Thermomixer Compact, Eppendorf, Hamburg, Germany). Protocol proceeded according to the manufacturer's instructions. Finally, template DNA was eluted in nuclease-free water in a total volume of 100 μl.

Semi-quantitative real-time polymerase chain reaction

All PCR reactions were performed in a total volume of 25 μl. The PCR mix for detection of E. coli consisted of 12.5 μl 2× DyNAmo™ HS SYBR® Green mix (Finnzymes Oy, Espoo, Finland), 0.25 μl forward primer (50 pmol/μl), 0.25 μl reverse primer (50 pmol/μl), 0.25 μl 100 nM fluorescein calibration dye (Bio-Rad, Hercules, CA, USA), 5 μl template DNA, and 6.75 μl water. In order to detect E. faecalis , a PCR mix containing 12.5 μl 2× DyNAmo™ HS SYBR® Green mix (Finnzymes Oy), 0.45 μl forward primer (50 pmol/μl), 0.15 μl reverse primer (50 pmol/μl), 0.25 μl 100 nM fluorescein calibration dye (Bio-Rad), 5 μl template DNA, and 6.65 μl water was used. As an extraction process control (internal control), phocine herpes virus (PhHV) was performed with a PCR mix consisting of 12.5 μl 2× DyNAmo™ HS SYBR® Green mix (Finnzymes Oy), 0.2 μl forward primer (50 pmol/ μl), 0.25 μl reverse primer (50 pmol/μl), 0.25 μl 100 nM fluorescein calibration dye (Bio-Rad), 5 μl template DNA, and 6.75 μl water. The following primers were used for realtime quantitative PCR:

E. coli uidA gene forward primer 5′-GGC TTC TGT CAA CGC TGT TT-3′, E. coli uidA gene reverse primer 5′-CCC ATG GAA GAG AAATGG AA-3′, E. faecalis 23S rRNA gene forward primer 5′-AGA AAT TCC AAA CGA ACT TG-3′, E. faecalis 23S rRNA gene reverse primer 5′-CAG TGC TCT ACC TCC ATC ATT-3′, PhHV forward primer 5′-GGG CGA ATC ACA GAT TGA ATC-3′, and PhHV reverse primer 5′-GCG GTT CCA AAC GTA CCA A-3′.

The Bio-Rad IQ5 ICycler (Bio-Rad, Veenendaal, The Netherlands) was used as real-time PCR platform, and the PCR conditions for E. coli, E. faecalis, and PhHV were as follows: a single predenaturation step of 15 min at 95 °C followed by 40 cycles of 15 s at 95 °C and 1 min at 59 °C. Finally, the sample temperature was gradually increased to 95 °C in order to generate dissociation curves. These curves were used to assess the specificity of the PCR product. The dissociation temperature was 76.0 °C for the E. coli –specific PCR product and 77.0 °C for the E. faecalis-specific product. The PCR efficiency was calculated using the slope of the standard curve (efficiency=10−1/slope−1).

Standard curves

The semi-quantitative inoculum of indicator organisms potentially present in the peritoneal drain fluid at the time of anastomotic leakage has been determined by using a reference dilution series of E. coli and E. faecalis inocula. Reference series were produced by spiking 500 μl of culture-negative drain fluid with a 10-log serial dilution of both E. coli and E.

faecalis. A standard curve was generated by comparing the real-time PCR results (threshold cycle or Ct value) to the inoculum sizes. The approximate inoculum size of the query patient sample was determined after interpolation of its Ct value within the standard curve. Patient samples were analyzed in duplicate.

Definitions

The endpoint of the APPEAL study was symptomatic colorectal anastomotic leakage. This was defined as a clinically manifest insufficiency of the anastomosis leading to a clinical state requiring intervention, confirmed by radiological studies, reoperation or fecal discharge from the drain.

Radiologic confirmation of CAL was defined as extravasation of endoluminally administrated water-soluble contrast and/or significant perianastomotic air on computed tomography or X-ray. Radiological studies were not routinely performed, only in case of clinical suspicion of CAL.

Interventions to treat CAL consisted of therapeutic drainage (prolonged stay of drain), use of therapeutic antibiotics, or a surgical intervention, i.e., construction of a diverting stoma, disconnection of the anastomosis and construction of a new anastomosis or a colostomy, or suturing of the leakage site.

All postoperative fistulas communicating with the surgical anastomosis were classified as a leak. Postoperative abscesses were classified as anastomotic leakage if there was extravasation of enteric contrast on radiological studies, if there was significant perianastomotic air or if communication with the anastomosis was noted after radiologic drainage.

The bacterial load of drain fluid was expected to rise in case of CAL, therefore, an increase detected by RT-PCR was scored positive and a decrease was scored negative.

Postoperative mortality was defined as patients that died within 30 days of operation in hospital and after discharge.

Data collection

Patients were followed from their preoperative admission on the ward until the first postoperative follow-up at the outpatient clinic. Demographic data of the patients, operative details, postoperative events and follow-up data were obtained through a standardized case record form and entered into a database. In case of CAL, the postoperative day of diagnosis was noted along with the manifestation of CAL, the diagnostic tool for detection of the leak, and the treatment.

Statistics

Categorical data are presented as numbers with percentages, numerical data are presented as means ± standard deviation (normally distributed), or medians with interquartile ranges (not normally distributed). Univariate analysis was performed using a chi-square test or Fisher exact test in case of categorical data and a Mann–Whitney U test in case of numerical data.

As test performance indicators sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic odds ratio (DOR) were calculated. Calculations were performed using SPSS 20 (SPSS Inc., Chicago, IL, USA).

Results

A total of 243 patients were included. The mean age was 64±12 years, 135 patients (56 %) were male and 108 (44 %) were female. Thirty-three patients (14 %) were treated for inflammatory diseases, 206 patients (84 %) were treated for malignancy, and four patients (2 %) had ischemic colitis. Fifty-six patients (23 %) underwent preoperative radiotherapy, and in 59 patients (25 %), a defunctioning stoma was constructed. A total of 92 (38 %) patients underwent a laparoscopic procedure, and 151 patients (62 %) were operated through laparotomy. Nineteen patients (7.8 %) developed clinical CAL. In nine patients it became manifest as sepsis, in seven patients as peritonitis, two patients developed a presacral abscess, and one patient developed an intra-abdominal abscess. In eight patients, the diagnosis was made by CT-scan, in seven patients by relaparotomy, and in four patients fecal discharge from the drain occurred. Median interval between operation and confirmation of CAL was 6 days (range 2–26 days). Two patients (0.8 %) developed an infection at the drain insertion site, both in the group without CAL. Average hospital stay of patients with CAL was significantly longer [28±22 days vs. 13± 13 days (p <0.0001)]. In the group of patients with CAL, three died (16 %), whereas six patients (3 %) died in the group without CAL (p = 0.002). Table 1 shows the baseline characteristics of the patients with and without CAL.

Variable	No CAL (n=224)	CAL (n=19)	p-value
Age	64.3 ± 12.0	65.3 ± 13.9	0.765
Gender			0.789
Male	125 (93 %)	10 (7 %)	
Female	99 (92 %)	9 (8 %)	
BMI (kg/m^2)			0.155
< 25	80 (91 %)	9 (9 %)	
25-30	109 (98 %)	5 (2 %)	
> 30	35 (88 %)	5 (12 %)	
ASA			0.532
1 - 2	173 (93 %)	13 (7 %)	
3 – 4	50 (89 %)	6 (11 %)	
Neoadjuvant radiotherapy			0.945
Yes	51 (91 %)	5 (9 %)	
No	173 (93 %)	14 (7 %)	
Type of resection			0.727
TME / LAR	76 (92 %)	7 (8 %)	
PME / HAR	55 (95 %)	3 (5 %)	
Left hemicolectomy	21 (96 %)	1 (4 %)	
Sigmoid resection	68 (90 %)	8 (10 %)	
Subtotal colectomy	4 (100 %)	0 (0 %)	
Height anastomosis			0.211
> 7cm	149 (94 %)	10 (6 %)	
< 7cm	74 (89 %)	9 (11 %)	
Construction anastomosis			0.735
Stapled	178 (93 %)	14 (7 %)	
Handsewn	45 (90 %)	5 (10 %)	
Configuration anastomosis			0.259
End-to-End	57 (86 %)	9 (14 %)	
End-to-Side	16 (94 %)	1 (6 %)	
Side-to-End	122 (94 %)	8 (6 %)	
Side-to-Side	23 (96 %)	1 (4 %)	
Protective ileostomy			0.933
Yes	55 (93 %)	4 (7 %)	
No	167 (92 %)	15 (8 %)	

Table 1. Univariate analysis of baseline characteristics of APPEAL-population.
CAL Colorectal Anastomotic Leakage; BMI Body Mass Index; ASA-score American Society of Anesthesiologists score; TME Total Mesorectal Excision; PME Partial Mesorectal Excision; HAR High Anterior Resection; LAR Low Anterior Resection

Sixty patients received a Penrose drain, all the other patients received a silicone tube drain. Drainage systems were all passive and closed. The production of drain fluid was not constant over time or between patients and varied greatly between 0 and 1,500 ml per day per patient (Table 2).

24 hour production	CAL	N	Mean	SD	p-value
Day 1	Yes	13	121.0	155.2	0.084
	No	196	179.8	172.1	
Day 2	Yes	14	79.3	85.0	0.551
	No	194	104.1	133.7	
Day 3	Yes	11	94.9	110.5	0.769
	No	177	124.1	182.2	
Day 4	Yes	11	129.7	159.3	0.737
	No	162	120.3	149.2	
Day 5	Yes	10	91.1	76.7	0.875
	No	134	119.4	151.3	

Table 2. Amount of drain fluid produced in ml per 24 hours versus colorectal anastomotic leakage (CAL)

The difference in production between patients with and without CAL was not significant. The quantitative results are depicted in Table 3.

RT-PCR	CAL	Day 1	Day 2	Day 3	Day 4	Day 5
E. coli (CFU/ml)	Yes	55	30	55	100000	1000000
	No	0	0	0	0	0
E. faecalis (CFU/ml)	Yes	300	7500	75000	75000	100000
	No	1000	1000	1000	0	1000

Table 3. Results of the semi-quantitative real-time PCR. The values are presented in colony forming units per milliliter (CFU/ml).

An increase of E. coli or E. faecalis as detected by RT-PCR was scored positive, whereas no change or a decrease was scored negative. An increase in E. coli concentration was found in significantly more patients with CAL on days 4 and 5 ($p = 0.0004$, DOR 7.9). For E. faecalis , this result was found for days 2, 3, and 4 ($p < 0.003$) with highest DOR on day 3 (31.6). Sensitivity and negative predictive values were 92.9 and 98.7 %, respectively. The results including sensitivity, specificity, PPV, NPV, and DOR for E. coli and E. faecalis are depicted in tables 4 and 5, respectively.

RT-PCR	Inter-val	Increase	CAL* Yes	CAL* No	p - value	Sens % 95 % CI	Spec % 95 % CI	PPV % 95 % CI	NPV % 95 % CI	DOR 95 % CI
E. coli	Day 1 → 2	Yes	3	11	0.185	-	-	-	-	-
		No	10	94						
	Day 1 → 3	Yes	6	23	0.102	-	-	-	-	-
		No	9	91						
	Day 1 → 4	Yes	9	16	0.0004	69.2 38.9 – 89.6	83.5 74.3 – 89.9	36.0 18.7 – 57.3	95.2 87.7 – 98.5	7.9 2.44 – 5.6
		No	4	81						
	Day 1 → 5	Yes	10	21	0.0004	66.7 41.7 – 84.8	79.8 71.1 – 86.4	32.3 17.3 – 51.5	94.3 86.6 – 97.9	7.9 2.4 – 25.6
		No	5	83						

Table 4. Quantitative increase of E. coli as determined by RT-PCR. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic odds ratio (DOR) are shown. All values are accompanied by their 95 % confidence interval (CI).
* Numbers of PCRs is less than number of included patients due to the APPEAL-study´s priority to biochemical analysis, lack of production, accidental drain removal by patient, early intervention for CAL

RT-PCR	Inter-val	Increase	CAL*		p - value	Sens % 95 % CI	Spec % 95 % CI	PPV % 95 % CI	NPV % 95 % CI	DOR 95 % CI
			Yes	No						
E. faecalis	Day 1 → 2	Yes	10	26	0.001	71.4 45.4 – 88.3	75.9 67.1 – 83.0	27.8 14.8 – 45.4	95.3 87.9 – 98.5	7.9 2.3 – 27.3
		No	4	82						
	Day 1 → 3	Yes	13	30	0.00001	92.9 68.5 – 98.7	70.9 61.5 – 78.8	30.2 17.7 – 46.3	98.7 92.7 – 99.8	31.6 4.0 – 252.7
		No	1	73						
	Day 1 → 4	Yes	9	26	0.003	75.0 46.8 – 91.1	72.6 62.9 – 80.6	25.7 12.5 – 43.2	95.8 88.3 – 99.1	7.9 2.0 – 31.8
		No	3	69						
	Day 1 → 5	Yes	7	38	0.388	-	-	-	-	-
		No	7	65						

Table 5. Quantitative increase of E. faecalis as determined by RT- PCR. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic odds ratio (DOR) are shown. All values are accompanied by their 95 % confidence interval (CI).
* Numbers of PCRs is less than number of included patients due to the APPEAL-study´s priority to biochemical analysis, lack of production, accidental drain removal by patient, early intervention for CAL

Discussion

Current screening methods for CAL consist of observation of clinical signs and symptoms and blood examination. These methods are not specific for CAL and may lead to various diagnostic procedures to exclude other less severe complications instead of ruling out CAL by means of highly specific imaging studies like CT-scan and/or water-soluble contrast radiography [9]. These additional diagnostics could lead to a delay in diagnosis of CAL [10]. Therefore, there is a need for a screening method that is objective and specific for CAL. This study shows that the number of patients with increased levels of E. faecalis between postoperative days 1 and 3 was significantly higher in case of CAL. This test has the highest DOR (31.6), reflecting the strong association between the test result and CAL. Considering high sensitivity (92.9 %) and NPV (98.7 %), a negative test result virtually rules out CAL at day 3 postoperatively. The false negative (1.3 %) rate is far lower than any other reported diagnostic test for CAL. However, since it does not equal zero, clinical observation remains important.

Specificity (70.9 %) and PPV (30.2 %) indicate a substantial number of false positive results. This is most likely due to subclinical anastomotic leakage, a long-known phenomenon with a reported incidence of 8 % [15, 16]. It could also be caused by intraoperative spill, however, the number of bacteria should have decreased at day 3. Regardless of the cause of the false positive results, positive test results should lead to additional imaging. Reported sensitivity and specificity of contrast radiography when performed in case of clinical suspicion are 68 and 94 %, respectively [17]. When performed routinely, reported sensitivity varies between 20 and 52 % and specificity is approximately 85 % [18, 19]. The reported sensitivity of CT-scan in the early postoperative period varies between 15 and 52 % [17, 20, 21]. The reported negative predictive value is 73 %, and the false negative rates vary between 35 and 53 % [17, 21]. Even though sensitivity of CT-scan is lower, it is preferable over contrast enema due to the additional information it provides.

As false positive RT-PCR results are most likely due to subclinical anastomotic leakage, CAL demonstrated on CT-scan might also remain subclinical and specific treatment may not be absolutely necessary. However, subclinical leakage is also associated with reduced quality of life and impaired bowel function [16], perhaps rendering treatment beneficial. The latter remains speculative and requires more research.

The number of PCRs performed, as depicted in the tables, does not add up to the number of included patients. This is due to insufficient production of drain fluid in most cases as illustrated by Table 2. The great variability is a drawback of this study that cannot easily be solved. A peritoneal lavage could be a solution; however, this may interfere with the quantitative PCR analysis. In addition, a few samples are missing due to accidental drain removal by patient, early intervention for CAL, and accidental loss of drain fluid either at the ward or at the processing laboratory.

Prophylactic drainage (PD), as performed on patients included in the APPEAL study, originally aimed to evacuate wound fluid and blood collections from the surgical site to prevent infectious complications and to detect AL by fecal or purulent discharge [22–24]. To date, the use of prophylactic drainage remains controversial. Several level 1 studies have shown that PD does not have a beneficial or a detrimental effect on the incidence of AL and on the morbidity afterwards [25–27]. A prospective study concerning pelvic anastomosis showed a higher leakage rate after routine irrigation–suction drainage in elective anterior resection [28]. A retrospective study showed drainage and the use of a defunctioning stoma to be beneficial in terms of reoperation rates as a result of anastomotic leakage after TME [29]. Despite this controversy surrounding the necessity to drain, prophylactic drainage remains common practice in many hospitals, particularly after rectal surgery [30]. In addition, the outcome measures used in these studies consist of leakage rates, hospital stay, radiological anastomotic leakage, infectious complications, and patient comfort. In this study, we have focused on and demonstrated the diagnostic capacity of the drain. Therefore, considering the low complication rate of PD, it should be placed routinely during surgery to allow collection of drain fluid for the first three postoperative days. In addition, it does not interfere with ERAS protocols since the results are known at day 3 and the drain can be removed [31].

Screening is defined by the World Health Organization as the systemic application of a test in an asymptomatic population in order to identify abnormalities that suggest presence of disease and refer these patients promptly for diagnosis and treatment [32]. The APPEAL study is the first study to define a promising screening tool for symptomatic CAL that is objective, fast, affordable and provides useful information concerning CAL as early as postoperative day 3.

Conclusion

RT-PCR for E. faecalis performed on drain fluid may be a useful screening tool for symptomatic colorectal anastomotic leakage in the early postoperative phase. Negative test results virtually rule out the presence of CAL. Positive results should lead to highly specific imaging studies for diagnosis of CAL.

Acknowledgments

The authors are grateful to all coworkers in the Departments of Surgery and Microbiology of the following hospitals:

ZNAMiddelheim, Antwerp, Belgium; Sint Franciscus Gasthuis, Rotterdam, The Netherlands; Erasmus MC, Rotterdam, The Netherlands; Reinier de Graaf Gasthuis, Delft, The Netherlands; University Hospital of Ghent, Ghent, Belgium; Daniel Den Hoed Kliniek, Erasmus MC, Rotterdam, The Netherlands; and Maasstad Ziekenhuis Rotterdam, Rotterdam, The Netherlands.

This research was made possible by grant from “Stichting Technische Wetenschappen”, Utrecht, The Netherlands.

References

1. Levack M, Berger D, Sylla P, Rattner D, Bordeianou L (2011) Laparoscopy decreases anastomotic leak rate in sigmoid colectomy for diverticulitis. Arch Surg 146(2):207–210

2. BoccolaMA, Buettner PG, RozenWM, Siu SK, Stevenson AR, Stitz R et al. (2011) Risk factors and outcomes for anastomotic leakage in colorectal surgery: a single-institution analysis of 1576 patients. World J Surg 35(1):186–195

3. Matthiessen P, Hallbook O, Rutegard J, Simert G, Sjodahl R (2007) Defunctioning stoma reduces symptomatic anastomotic leakage after low anterior resection of the rectum for cancer: a randomized multicenter trial. Ann Surg 246(2):207–214

4. Fraccalvieri D, Biondo S, Saez J, Millan M, Kreisler E, Golda T et al. (2012) Management of colorectal anastomotic leakage: differences between salvage and anastomotic takedown. Am J Surg 204(5):671–676

5. Buchs NC, Gervaz P, Secic M, Bucher P, Mugnier-Konrad B, Morel P (2008) Incidence, consequences, and risk factors for anastomotic dehiscence after colorectal surgery: a prospective monocentric study. Int J Colorectal Dis 23(3):265–270

6. Hyman N, Manchester TL, Osler T, Burns B, Cataldo PA (2007) Anastomotic leaks after intestinal anastomosis: it’s later than you think. Ann Surg 245(2):254–258

7. Bellows CF, Webber LS, Albo D, Awad S, Berger DH (2009) Early predictors of anastomotic leaks after colectomy. Tech Coloproctol 13(1):41–47

8. Macarthur DC, Nixon SJ, Aitken RJ (1998) Avoidable deaths still occur after large bowel surgery. Scottish audit of surgical mortality, royal college of surgeons of Edinburgh. Br J Surg 85(1):80–83

9. Alves A, Panis Y, Pocard M, Regimbeau JM, Valleur P (1999) Management of anastomotic leakage after nondiverted large bowel resection. J Am Coll Surg 189(6):554–559

10. den Dulk M, Noter SL, Hendriks ER, Brouwers MA, van der Vlies CH, Oostenbroek RJ et al. (2009) Improved diagnosis and treatment of anastomotic leakage after colorectal surgery. Eur J Surg Oncol 35(4):420–426

11. Marteau P, Pochart P, Dore J, Bera-Maillet C, Bernalier A, Corthier G (2001) Comparative study of bacterial groups within the human cecal and fecal microbiota. Appl Environ Microbiol 67(10):4939–4942

12. Balamurugan R, Rajendiran E, George S, Samuel GV, Ramakrishna BS (2008) Real-time polymerase chain reaction quantification of specific butyrate-producing bacteria, Desulfovibrio and Enterococcus faecalis in the feces of patients with colorectal cancer. J Gastroenterol Hepatol 23(8 Pt 1):1298–1303

13. Komen N, Morsink MC, Beiboer S, Miggelbrink A, Willemsen P, van der Harst E et al. (2009) Detection of colon flora in peritoneal drain fluid after colorectal surgery: can RT-PCR play a role in diagnosing anastomotic leakage? J Microbiol Methods 79(1):67–70

14. Fouda E, El Nakeeb A,Magdy A, Hammad EA, Othman G, Farid M (2011) Early detection of anastomotic leakage after elective low anterior resection. J Gastrointest Surg 15(1):137–144

15. Goligher JC, Lee PW, Simpkins KC, Lintott DJ (1977) A controlled comparison one- and two-layer techniques of suture for high and low colorectal anastomoses. Br J Surg 64(9):609–614

16. Lim M, Akhtar S, Sasapu K, Harris K, Burke D, Sagar P et al. (2006) Clinical and subclinical leaks after low colorectal anastomosis: a clinical and radiologic study. Dis Colon Rectum 49(10):1611–1619

17. Doeksen A, Tanis PJ, Wust AF, Vrouenraets BC, van Lanschot JJ, van TetsWF (2008) Radiological evaluation of colorectal anastomoses. Int J Colorectal Dis 23(9):863–868

18. Hoffmann J, Jensen RH, Shokouh-Amiri MH, Damm P (1988) Clinical value of water-soluble contrast enema in assessing the integrity of left colonic anastomoses. J R Coll Surg Edinb 33(1):23–24

19. Akyol AM, McGregor JR, Galloway DJ, George WD (1992) Early postoperative contrast radiology in the assessment of colorectal anastomotic integrity. Int J Colorectal Dis 7(3):141–143

20. Nicksa GA, Dring RV, Johnson KH, SardellaWV, Vignati PV, Cohen JL (2007) Anastomotic leaks: what is the best diagnostic imaging study? Dis Colon Rectum 50(2):197–203

21. Khoury W, Ben-Yehuda A, Ben-Haim M, Klausner JM, Szold O (2009) Abdominal computed tomography for diagnosing postoperative lower gastrointestinal tract leaks. JGastrointest Surg 13(8):1454–1458

22. Averbach AM, Sugarbaker PH (1995) The use of drains in elective surgery for colorectal cancer: always, never or selectively? Tumori 81(3 Suppl):89–97

23. Brown SR, Seow-Choen F, Eu KW, Heah SM, Tang CL (2001) A prospective randomised study of drains in infra-peritoneal rectal anastomoses. Tech Coloproctol 5(2):89–92

24. LennoxMS (1984) Prophylactic drainage of colonic anastomoses. Br J Surg 71(1):10–11

25. Merad F, Yahchouchi E, Hay JM, Fingerhut A, Laborde Y, Langlois-Zantain O (1998) Prophylactic abdominal drainage after elective colonic resection and suprapromontory anastomosis: a multicenter study controlled by randomization. French Associations for Surgical Research. Arch Surg 133(3):309–314

26. Jesus EC, Karliczek A, Matos D, Castro AA, Atallah AN (2004) Prophylactic anastomotic drainage for colore ctal surgery. Cochrane Database Syst Rev 4, CD002100

27. Petrowsky H, Demartines N, Rousson V, Clavien PA (2004) Evidence-based value of prophylactic drainage in gastrointestinal surgery: a systematic review and meta-analyses. Ann Surg 240(6): 1074–1084, discussion 84–5

28. Yeh CY, Changchien CR, Wang JY, Chen JS, Chen HH, Chiang JM et al. (2005) Pelvic drainage and other risk factors for leakage after elective anterior resection in rectal cancer patients: a prospective study of 978 patients. Ann Surg 241(1):9–13

29. Peeters KC, Tollenaar RA, Marijnen CA, Klein Kranenbarg E, Steup WH, Wiggers T et al. (2005) Risk factors for anastomotic failure after totalmesorectal excision of rectal cancer. Br J Surg 92(2): 211–216

30. Karliczek A, Jesus EC, Matos D, Castro AA, Atallah AN,Wiggers T (2006) Drainage or nondrainage in elective colorectal anastomosis: a systematic review and meta-analysis. Colorectal Dis 8(4):259–265

31. ScatizziM, KroningKC, Boddi V, De Prizio M, Feroci F (2010) Fasttrack surgery after laparoscopic colorectal surgery: is it feasible in a general surgery unit? Surgery 147(2):219–226

32. WHO. World Health Organization: Cancer 2011

Chapter 9

Acute phase proteins in drain fluid: a new screening tool for colorectal anastomotic leakage?

APPEAL-study: Analysis of Parameters Predictive for Evident Anastomotic Leakage.

N. Komen, J. Slieker, P. Willemsen, G. Mannaerts, P. Pattyn, T. Karsten, H. de Wilt, E. van der Harst, M. Murawska, J. Jeekel, Y.B. de Rijke, J.F. Lange

Accepted for publication in Am J Surg 2014

Abstract

Background

We aim to determine if C-reactive protein (CRP), Lipopolysaccharide Binding Protein (LBP) and Procalcitonin (PCT) in drain fluid can serve as screening tools for CAL.

Methods

Patients included in this multicenter prospective observational study underwent left hemicolectomy, sigmoid resection, high anterior resection, low anterior resection or subtotal colectomy. During the first five postoperative days CRP, LBP and PCT were determined on drain fluid.

Results

In total 243 patients were included of whom 19 (8 %) developed CAL. CRP levels were higher in patients with leakage on day 3 and day 5, levels of LBP were higher on day two, three and four and PCT levels were higher on day 5. Multivariate analysis showed LBP to be significantly related to CAL. An increase from the average initial value at the first postoperative day with 1 standard deviation, increased the risk of leakage 1.6 times.

Conclusion

Increased concentrations of LBP in drain fluid might a screening tool for CAL

Introduction

Despite the vast body of evidence, colorectal anastomotic leakage (CAL) remains a poorly understood complication of colorectal surgery. The reported incidence of CAL is estimated between 2.4 % and 19 % [1-3] and mortality rates due to sepsis and multi organ failure are around 15 % [4]. With current screening and diagnostic methods the interval between construction of the colorectal anastomosis and diagnosis of leakage varies between 6 and 13 days [5-7]. Several studies have suggested that delay of diagnosis of CAL is associated with higher mortality rates and that only early management improves clinical outcome [8-10]. Therefore, new screening methods allowing detection of CAL in the early postoperative phase are needed. A biomarker reflecting the local inflammation, i.e. around the anastomosis, could be an objective screening tool for CAL in an early phase.

C - reactive protein (CRP) is an acute-phase protein displaying rapid and pronounced rise of its serum concentration in response to infection or inflammation. It is mainly produced by hepatocytes, however Kupffer cells, blood monocytes and alveolar macrophages have been shown to produce CRP as well [11].

Lipopolysacharide Binding Protein (LBP) is a glycoprotein produced mainly in the liver, but also in skin, lung, intestine and at local sites of injury and infection. Particularly LBP produced at local sites of infection is considered to contribute to bacterial clearing [12].

Procalcitonin (PCT) is a potential marker of acute inflammation that is released from different parenchymal tissues and differentiated cell types throughout the body, mostly from the liver and adipose tissue, in response to increased interleukins levels in bacterial infections [13-16].

The aim of this study is to determine if CRP, LBP and PCT in drain fluid can serve as non-invasive screening tools for CAL in the early postoperative phase after colorectal surgery.

Methods

Patients included in the "APPEAL" study received left-sided colorectal resection with construction of an anastomosis and were given an intra-abdominal drain. Seven medical centers in the Netherlands and Belgium participated in this study. The study, registered in the

Dutch Trial Register (http://www.trialregister.nl, study number NTR 1258), was approved by the medical ethical committee of all the participating centers, in accordance with the ethical standards of the Helsinki Declaration of 1975, and all patients gave informed consent. Participating centers included patients consecutively between January 2007 and December 2009.

Inclusion and exclusion criteria

Patients undergoing left hemicolectomy, sigmoid resection, high anterior resection (HAR; with partial mesorectal excision: PME), low anterior resection (LAR; with total mesorectal excision: TME), and subtotal colectomy with ileorectal anastomosis were included. Oncologic resections as well as resections for benign disease were included. Emergency operations were excluded due to the high probability of coexisting tissue damage and logistical difficulties. Reversals of colostomy were also excluded since the primary disease was already treated. Furthermore, patients under 18 years of age, patients who refused to participate and patients who did not receive a drain were excluded.

Surgical procedure

The surgical procedure was left to the surgeon's discretion. All patients received preoperative antibiotic prophylaxis and an intra-abdominal drain. Guidelines concerning bowel preparation in the participating centers were respected. Patients were operated by laparotomy or laparoscopy and the anastomosis was stapled or hand sutured. A diverting stoma was constructed according to the surgeon's preference. To obtain drain fluid, a drain was placed at the anastomotic site and was left in place during the first 5 postoperative days. The type of drain used was left to the surgeon's discretion.

Determination of CRP, LBP and PCT

Drain fluid reservoirs were emptied two times a day with 12 hour intervals, respecting rules of sterility. The evening collection was disposed of. The morning collection was centrifuged for

10 minutes at 2800xg and 4 °C. The supernatant was brought into different cryotubes that were frozen at -80°C to allow analysis in batch.

LBP was determined by a two-sited chemiluminescent immunometric enzyme assay (Immulite 1000, Siemens, Los Angeles, USA). For CRP determination the Tina-quant assay on the Hitachi 912 (Roche Diagnostics, Penzberg, Germany) was used. PCT was determined with an immunoluminometric assay (LUMI-Test PCT, BRAHMS Diagnostika, Berlin, Germany).

Definitions

The endpoint of the APPEAL-study was symptomatic colorectal anastomotic leakage. This was defined as a clinically manifest insufficiency of the anastomosis leading to a clinical state requiring intervention, confirmed by radiological studies, reoperation or faecal discharge from the drain.

Radiologic confirmation of CAL was defined as extravasation of endoluminally administrated water-soluble contrast and/or significant perianastomotic air on computed tomography or X-ray. Radiological studies were not routinely performed, only in case of clinical suspicion of CAL.

Interventions to treat CAL consisted of therapeutic drainage (prolonged stay of drain), use of therapeutic antibiotics or a surgical intervention, i.e. construction of a diverting stoma, disconnection of the anastomosis and construction of a new anastomosis or a colostomy.

All postoperative fistulas communicating with the surgical anastomosis were classified as a leak. Postoperative abscesses were classified as anastomotic leakage if there was extravasation of enteric contrast on radiological studies, if there was significant perianastomotic air, or if communication with the anastomosis was noted after radiologic drainage.

Postoperative mortality was defined as patients that died within 30 days of operation, in hospital and after discharge.

Data collection

Patients were followed from their pre-operative admission on the ward until the first postoperative follow-up at the outpatient clinic. Demographic data of the patients, operative details, postoperative events, and follow-up data were obtained through a standardized case record form and entered into a database. In case of CAL the postoperative day of diagnosis was noted along with the manifestation of CAL, the diagnostic tool for detection of the leak, and the treatment.

Statistics

Categorical data are presented as numbers with percentages, numerical data are presented as means ± standard deviations (normally distributed), or medians with interquartile ranges (not normally distributed). Univariate analysis was performed using a chi-square test in case of categorical data and an unpaired t-test or one way ANOVA (normally distributed) and Mann-Whitney U test (not normally distributed) in case of numerical data. To determine whether the individual CRP, PCT, LBP fluctuations in time were related to the risk of CAL we fitted mixed effects model with random cubic splines for the normalized responses of CRP, LBP and PCT to account for the nonlinear character of the responses. Each model contained following fixed effects: age, ASA-classification, gender, approach (laparoscopic or open), use of corticosteroids, time effects of the order up to 3 or less (depending on the response) and the interaction terms between time effects and factors ASA-classification, gender, approach (laparoscopic or open), use of corticosteroids. Each model contained as random effects a random intercept and random effects of time.

From each univariate model random effects were estimated using empirical Bayes estimates and plugged into the logistic model for CAL and the survival model for time to AL. To correct for uncertainty of the plug-in covariates we sampled random effects from the fitted polynomial mixed regression models and fitted the logistic/survival models for each sample. As the final estimate we took an average of the obtained estimates. Final standard errors were calculated taking into account within and between samples variability.

Results

A total of 243 patients were included. The mean age was 64 ± 12 years, 135 patients (56 %) were male and 108 (44 %) were female. Thirty-three patients (14 %) were treated for inflammatory diseases, 206 patients (84 %) were treated for malignancy, and four patients (2 %) had ischemic colitis. Fifty-six patients (23 %) underwent pre-operative radiotherapy, and in 59 patients (25 %) a defunctioning stoma was constructed. A total of 92 (38 %) patients underwent a laparoscopic procedure and 151 patients (62 %) were operated through laparotomy.

Nineteen patients (8 %) developed clinical CAL. In 9 patients it became manifest as sepsis, in 7 patients as peritonitis, 2 patients developed a pre-sacral abscess and one patient developed an intra-abdominal abscess. In 8 patients the diagnosis was made by computed tomography, in 7 patients by relaparotomy and in 4 patients faecal discharge from the drain occurred. Median interval between operation and confirmation of CAL was 6 days (range 2 – 26 days). Two patients (0,8 %) developed an infection at the drain insertion site, both in the group without CAL. Average hospital stay of patients with CAL was significantly longer (28 ± 22 days vs. 13 ± 13 days ($p<0{,}0001$)). In the group of patients with CAL three died (16 %), whereas 6 patients (3 %) died in the group without CAL ($p=0.002$).

Table I shows the baseline characteristics of the patients with CAL compared to the patients without CAL. The incidence of pulmonary comorbidity and the number of patients using corticosteroids was significantly higher in the leakage group.

Drain fluid production was compared between the group with and without leakage. No significant difference between the two groups was found.

Variable		No CAL (n=224)	CAL (n=19)	p-value
Age (years)		64 ± 12	65 ± 13	0.765
Gender	Male	125 (93 %)	10 (7 %)	0.789
	Female	99 (92 %)	9 (8 %)	
BMI (kg/m^2)	< 25	80 (91 %)	9 (9 %)	0.155
	25-30	109 (98 %)	5 (2 %)	
	> 30	35 (88 %)	5 (12 %)	
Smoking	Yes	50 (94 %)	3 (6 %)	0.750
	No	168 (92 %)	15 (8 %)	
Steroids	Yes (chronic)	2 (40 %)	3 (60 %)	0.000
	Yes (perioperatively)	16 (80 %)	4 (20 %)	
	No	205 (94 %)	12 (6 %)	
ASA-score *	1 – 2	173 (93 %)	13 (7 %)	0.532
	3 – 4	50 (89 %)	6 (11 %)	
Neoadjuvant radiotherapy	Yes	51 (91 %)	5 (9 %)	0.945
	No	173 (93 %)	14 (7 %)	
Type of resection #	TME / LAR	76 (92 %)	7 (8 %)	0.727
	PME / HAR	55 (95 %)	3 (5 %)	
	Left hemicolectomy	21 (96 %)	1 (4 %)	
	Sigmoid resection	68 (90 %)	8 (10 %)	
	Subtotal colectomy	4 (100 %)	0 (0 %)	
Height anastomosis	> 7cm	149 (94 %)	10 (6 %)	0.211
	< 7cm	74 (89 %)	9 (11 %)	
Construction anastomosis	Stapled	178 (93 %)	14 (7 %)	0.735
	Handsewn	45 (90 %)	5 (10 %)	
Configuration anastomosis	End-to-End	57 (86 %)	9 (14 %)	0.259
	End-to-Side	16 (94 %)	1 (6 %)	
	Side-to-End	122 (94 %)	8 (6 %)	
	Side-to-Side	23 (96 %)	1 (4 %)	
Protective ileostomy	Yes	55 (93 %)	4 (7 %)	0.933
	No	167 (92 %)	15 (8 %)	

Table **I.** Univariate analysis of baseline characteristics of the total APPEAL-population: patients without colorectal anastomotic leakage (CAL) versus the patients with CAL.
* ASA: American Society of Anesthesiologists score
# TME Total Mesorectal excision; LAR Low Anterior Resection; PME Partial Mesorectal Excision; HAR High Anterior Resection

Univariate analysis shows CRP levels are significantly higher in patients with leakage on day 3 and day 5 postoperatively (figure 1). Levels of LBP are significantly higher in patients with leakage on day two, three and four after the operation (figure 2).

The PCT concentration is significantly higher on day 5 in case of leakage (figure 3).

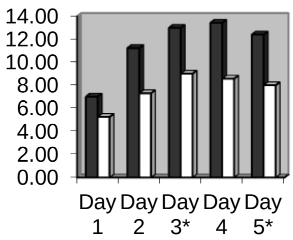

Figure 1. Differences in CRP concentrations (mg/l) between patients with and without AL.
* Postoperative day on which differences are significant (day 3 p=0.003; day 5 p=0.013)

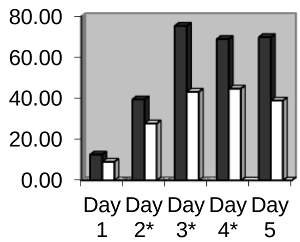

Figure 2. Differences in LBP concentrations (μg/ml) between patients with and without AL
* Postoperative day on which differences are significant (day 2 p=0.040; day 3 p=0.039; day 4 p=0.003)

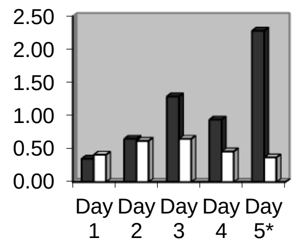

Figure 3. Differences in PCT concentrations (ng/ml) between patients with and without AL
* Postoperative day on which differences are significant (day 5 p=0.013)

In the multivariate analysis for LBP we found a significant effect of the random intercept after correcting by sampling method (table II).

Coefficient	log HR	Standard Error	p-value
Intercept	-2.63	0.47	1.8e-08
b.s [, 3]	0.30	0.19	0.12
int.s LBP	0.50	0.22	0.024
Use of steroids at home (Yes vs. No)	4.14	1.32	0.002
Use of steroids intraoperatively (Yes vs.No)	1.56	0.71	0.027
Approach (Open vs. Laparoscopy)	-0.83	0.57	0.14

Table II. Final logistic regression model for CAL after correcting for standard error using sampling method. The intercept estimate reflects the reference value of the log hazard ratio (HR) for the average person in a population. B.s [,3] stands for b-spline basis function of order 3. Int.s LBP stands for random intercept for LBP.

This suggests that the higher the initial value of LBP the higher the risk of leakage. Both LBP and the random effects were normalized. Therefore, this means that an increase from the average initial value of LBP at the first postoperative day with 1 standard deviation, increases the risk of leakage exp(0.5) = 1.6 times. In addition we found use of steroids to be independently associated with CAL as well. No significant results were obtained in the multivariate analysis of PCT and CRP.

Univariate analysis of the APP data on each postoperative day was also performed with outcome variables other than CAL. Comparison of means between patients undergoing laparoscopic versus open surgery resulted in significant higher concentration in the open surgery group of CRP on day 1 (p=0.014) and 2 (p=0.018) and of PCT on day 2 (p=0.026). No significantly different concentrations for LBP were found. Similar analysis comparing patients with a diverting stoma to patients without a stoma showed no significant results for neither of the three analyzed APPs (p>0.120). Indication for operation (malignancy, inflammatory, other) as outcome variable also did not reveal significant results (p>0.087).

Discussion

Acute Phase Proteins (APPs) are produced in the liver, extrahepatically and at the site of injury in case of LBP. Therefore levels at the local milieu could be a more specific indicator for local infection, like CAL.

CRP plays a role in recognizing pathogens and activating the complement system and phagocytic cells. Furthermore, it contributes to restoration of normal structure and function of injured tissues [17]. CRP in plasma has been used for a long time to objectify disease activity in infective or non-infective inflammatory states [18]. For CRP in drain fluid, univariate analysis shows a significant difference between patients with CAL and patients without CAL on days 3 and 5 postoperatively. Multivariate analysis renders no significant results. The differences between laparoscopic versus open surgery may be explained by the greater tissue damage resulting from open surgery, leading to a greater inflammatory reaction.

LBP enhances the inflammatory response to gram-negative bacteria by transferring LPS (LipoPolySaccharide) to a host membrane protein (m)CD14, causing the release of proinflammatory cytokines that recruit neutrophils as an early, innate immune response [19]. High serum LBP levels have been shown to correlate to the onset of bacteremia and sepsis [20]. Since gram negative bacteria are abundantly present in the colon, significantly higher LBP concentrations in patients with CAL on day 2, 3 and 4 postoperatively could be expected. Moreover, multivariate analysis has shown that an increase of LBP levels in drain fluids is associated with a significantly higher risk for CAL.

In a non-septic condition, PCT is produced primarily in neuroendocrine C cells of the thyroid [15]. However, bacterial infection has been shown to induce a release of PCT from different parenchymal tissues and differentiated cell types throughout the body [37]. The serum level of PCT has been suggested to be a sensitive indicator of ongoing abdominal sepsis and could help deciding whether to perform a relaparotomy [21]. In drain fluid, a significant difference between patients with CAL and patients without CAL was found only on day 5 postoperatively. However, multivariate analysis renders no significant results. Levels of PCT were significantly higher in the open surgery group on day 2. Perhaps this could be due to a larger intraoperative spill in the open group. However, on day one the difference is not significant contradicting this hypothesis.

This is the first study concerning acute phase proteins in drain fluid and was conducted to study if APPs have diagnostic value when determined in the perianastomotic environment.

Several studies in search of a biomarker for CAL in drain fluid have been performed. The majority of studies concern cytokines like IL-1b and TNF-α [22]. Unfortunately, no thresholds could be defined due to great variability in results rendering this parameters not yet suited for clinical use [23]. The same problem is encountered with LBP and, in analogy to cytokine data, consequently one can only conclude a correlation exists between LBP levels in drain fluid and CAL. Probably, combining several biomarkers more specifically diagnoses CAL and further studies should aim to find a biomarker profile diagnostic for CAL.

The number of samples used for each analysis does not add up to the number of included patients. This is due to variable and insufficient production of drain fluid in most cases. Peritoneal lavage could be a solution, however, this may interfere with the concentrations of the APPs. Few samples are missing due to accidental removal of the drain by the patient, early intervention for CAL and accidental loss of drain fluid either at the ward or at the processing laboratory.

Corticosteroids are known to impair wound healing however, their influence on the healing of colorectal anastomoses is unclear and studies have reported conflicting results [24-26]. This study shows use of corticosteroids to be correlated to CAL. It is a confirmation of the results published by Slieker et al., who used the same database [27].

To date, the use of prophylactic drainage remains controversial. Several level 1 studies have shown that PD does not have a beneficial or a detrimental effect on the incidence of AL and on the morbidity afterwards [28-30]. A prospective study concerning pelvic anastomosis showed a higher leakage rate after routine irrigation-suction drainage in elective anterior resection [31]. A retrospective study showed drainage and the use of a defunctioning stoma to be beneficial in terms of reoperation rates as a result of anastomotic leakage after TME [32]. Despite this controversy surrounding the necessity to drain, prophylactic drainage remains common practice in many hospitals, particularly after rectal surgery [33]. In addition, the outcome measures used in these studies consist of leakage rates, hospital stay, radiological anastomotic leakage, infectious complications and patient comfort. In this study we have shown diagnostic potential of the drain. Therefore, considering the low complication rate of

PD, it should be placed routinely during surgery to allow collection of drain fluid for the first 3 postoperative days.

The statistical methods used in this article allow analysis of longitudinal data on an individual level, i.e. analysis of individual profiles of the acute phase proteins. We found that an increase of LBP levels with one standard deviation leads to a 1.6 times increased risk of CAL in the individual patient. This means LBP and CAL are related. However, since it is not possible to calculate a standard deviation on one measurement this finding by itself cannot be used in clinical practice. In addition, due to the vast variability of the obtained values between patients and between samples of the same patient it is not possible to define general cut-off values. A personalized, "tailor made" approach is mandatory. In analogy with cardiovascular research concerning risk scores (Framingham score), a more personalized approach can be achieved through the development of a prognostic model, determining whether repeated measurements of a specific biomarker, in this study LBP, can ultimately provide a better understanding of disease progression. The most common are dynamic predictions of survival probabilities using the recorded longitudinal information, namely landmarking [34] and joint modeling [35]. Because the subject-specific longitudinal trajectories can be quite complex (e.g. nonlinear, plateaus) different features of these trajectories may be more predictive for the event of interest. In our study we do not encounter strictly joint-modeling setting, meaning that the recorded longitudinal information influences the survival submodel but not the other way around. Therefore using the two-stage model is sufficient. Based upon that model we can perform a dynamic prediction of the patient-specific hazard for a new patient by updating the patient-specific random effect related to LBP intercept as more longitudinal data for this patient are available. For this purpose we can apply the similar procedure as in the typical joint modeling situation by using Metropolis-Hastings sampling [36]. To simplify matters, a statistical black box should be visualized that allows calculation of the chance of CAL after putting in a diagnostic factor like the concentration of LBP on postoperative day 1 and 3 for example. Other postoperative signs and symptoms like fever, serum CRP and white blood cell count could be factored in as well.

Conclusion

Increased concentrations of Lipopolysacharide Binding Protein in drain fluid are significantly associated to a higher chance of CAL and could contribute in a future prognostic model for CAL.

Acknowledgements

The authors are grateful to all co-workers in the departments of surgery and microbiology of the following hospitals:

ZNA Middelheim, Antwerp, Belgium

Sint Franciscus Gasthuis, Rotterdam, The Netherlands

Erasmus MC, Rotterdam, The Netherlands

Reinier de Graaf Gasthuis, Delft, The Netherlands

University Hospital of Ghent, Ghent, Belgium

Daniel Den Hoed Kliniek, Erasmus MC, Rotterdam, The Netherlands

Maasstad Ziekenhuis Rotterdam, Rotterdam, The Netherlands

The authors are also grateful to the laboratory technicians Mrs. G.P. Koelewijn-van Vliet and Mrs. M.R. Blokland-de Wit for their assistance with laboratory analyses.

This research was made possible by grant from “Stichting Technische Wetenschappen”, Utrecht, The Netherlands.

References

1. Levack M, Berger D, Sylla P, Rattner D, Bordeianou L. Laparoscopy decreases anastomotic leak rate in sigmoid colectomy for diverticulitis. Arch Surg;146:207-10.

2. 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;35:186-95.

3. Matthiessen P, Hallbook O, Rutegard J, Simert G, Sjodahl R. Defunctioning stoma reduces symptomatic anastomotic leakage after low anterior resection of the rectum for cancer: a randomized multicenter trial. Ann Surg 2007;246:207-14.

4. Fraccalvieri D, Biondo S, Saez J, et al. Management of colorectal anastomotic leakage: differences between salvage and anastomotic takedown. Am J Surg.

5. Buchs NC, Gervaz P, Secic M, Bucher P, Mugnier-Konrad B, Morel P. Incidence, consequences, and risk factors for anastomotic dehiscence after colorectal surgery: a prospective monocentric study. Int J Colorectal Dis 2008;23:265-70.

6. Hyman N, Manchester TL, Osler T, Burns B, Cataldo PA. Anastomotic leaks after intestinal anastomosis: it's later than you think. Ann Surg 2007;245:254-8.

7. Bellows CF, Webber LS, Albo D, Awad S, Berger DH. Early predictors of anastomotic leaks after colectomy. Tech Coloproctol 2009;13:41-7.

8. Macarthur DC, Nixon SJ, Aitken RJ. Avoidable deaths still occur after large bowel surgery. Scottish Audit of Surgical Mortality, Royal College of Surgeons of Edinburgh. Br J Surg 1998;85:80-3.

9. Alves A, Panis Y, Pocard M, Regimbeau JM, Valleur P. Management of anastomotic leakage after nondiverted large bowel resection. J Am Coll Surg 1999;189:554-9.

10. den Dulk M, Noter SL, Hendriks ER, et al. Improved diagnosis and treatment of anastomotic leakage after colorectal surgery. Eur J Surg Oncol 2009;35:420-6.

11. Dong Q, Wright JR. Expression of C-reactive protein by alveolar macrophages. J Immunol 1996;156:4815-20.

12. Su GL, Freeswick PD, Geller DA, et al. Molecular cloning, characterization, and tissue distribution of rat lipopolysaccharide binding protein. Evidence for extrahepatic expression. J Immunol 1994;153:743-52.

13. Rau B, Kruger CM, Schilling MK. Procalcitonin: improved biochemical severity stratification and postoperative monitoring in severe abdominal inflammation and sepsis. Langenbecks Arch Surg 2004;389:134-44.

14. Assicot M, Gendrel D, Carsin H, Raymond J, Guilbaud J, Bohuon C. High serum procalcitonin concentrations in patients with sepsis and infection. Lancet 1993;341:515-8.

15. Linscheid P, Seboek D, Nylen ES, et al. In vitro and in vivo calcitonin I gene expression in parenchymal cells: a novel product of human adipose tissue. Endocrinology 2003;144:5578-84.

16. Becker KL, Nylen ES, White JC, Muller B, Snider RH, Jr. Clinical review 167: Procalcitonin and the calcitonin gene family of peptides in inflammation, infection, and sepsis: a journey from calcitonin back to its precursors. J Clin Endocrinol Metab 2004;89:1512-25.

17. Volanakis JE. Human C-reactive protein: expression, structure, and function. Mol Immunol 2001;38:189-97.

18. Pepys MB. C-reactive protein fifty years on. Lancet 1981;1:653-7.

19. Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. Science 1990;249:1431-3.

20. Froon AH, Dentener MA, Greve JW, Ramsay G, Buurman WA. Lipopolysaccharide toxicity-regulating proteins in bacteremia. J Infect Dis 1995;171:1250-7.

21. Novotny AR, Emmanuel K, Hueser N, et al. Procalcitonin ratio indicates successful surgical treatment of abdominal sepsis. Surgery 2009;145:20-6.

22. Komen N, de Bruin RW, Kleinrensink GJ, Jeekel J, Lange JF. Anastomotic leakage, the search for a reliable biomarker. A review of the literature. Colorectal Dis 2008;10:109-15; discussion 15-7.

23. Cini C, Wolthuis A, D'Hoore A. Peritoneal fluid cytokines and matrix-metalloproteinases as early markers of anastomotic leakage in colorectal anastomosis. A literature review and meta-analysis. Colorectal Dis.

24. Konishi T, Watanabe T, Kishimoto J, Nagawa H. Risk factors for anastomotic leakage after surgery for colorectal cancer: results of prospective surveillance. J Am Coll Surg 2006;202:439-44.

25. Tresallet C, Royer B, Godiris-Petit G, Menegaux F. Effect of systemic corticosteroids on elective left-sided colorectal resection with colorectal anastomosis. Am J Surg 2008;195:447-51.

26. Post S, Betzler M, von Ditfurth B, Schurmann G, Kuppers P, Herfarth C. Risks of intestinal anastomoses in Crohn's disease. Ann Surg 1991;213:37-42.

27. Slieker JC, Komen NA, Mannaerts GH, et al. Long-term and Perioperative Corticosteroids in Anastomotic Leakage: A Prospective Study of 259 Left-Sided Colorectal Anastomoses. Arch Surg.

28. Merad F, Yahchouchi E, Hay JM, Fingerhut A, Laborde Y, Langlois-Zantain O. Prophylactic abdominal drainage after elective colonic resection and suprapromontory anastomosis: a multicenter study controlled by randomization. French Associations for Surgical Research. Arch Surg 1998;133:309-14.

29. Jesus EC, Karliczek A, Matos D, Castro AA, Atallah AN. Prophylactic anastomotic drainage for colorectal surgery. Cochrane Database Syst Rev 2004:CD002100.

30. Petrowsky H, Demartines N, Rousson V, Clavien PA. Evidence-based value of prophylactic drainage in gastrointestinal surgery: a systematic review and meta-analyses. Ann Surg 2004;240:1074-84; discussion 84-5.

31. Yeh CY, Changchien CR, Wang JY, et al. Pelvic drainage and other risk factors for leakage after elective anterior resection in rectal cancer patients: a prospective study of 978 patients. Ann Surg 2005;241:9-13.

32. Peeters KC, Tollenaar RA, Marijnen CA, et al. Risk factors for anastomotic failure after total mesorectal excision of rectal cancer. Br J Surg 2005;92:211-6.

33. Karliczek A, Jesus EC, Matos D, Castro AA, Atallah AN, Wiggers T. Drainage or nondrainage in elective colorectal anastomosis: a systematic review and meta-analysis. Colorectal Dis 2006;8:259-65.

34. van Houwelingen H, Putter, H. Dynamic Prediction in Clinical Survival Analysis: Taylor & Francis Inc; 2011.

35. Rizopoulos D. Dynamic predictions and prospective accuracy in joint models for longitudinal and time-to-event data. Biometrics;67:819-29.

36. Murawska M, Rizopoulos D, Lesaffre E. A Two-Stage Joint Model for Nonlinear Longitudinal Response and a Time-to-Event with Application in Transplantation Studies. Journal of Probability and Statistics 2011;2012.

37. Müller B, White JC, Nylen ES, Snider RH, Becker KL, Habener JF. Ubiquitous expression of the calcitonin-i gene in multiple tissues in response to sepsis. J Clin Endocrinol Metab 2001;86:396-404.

Part 4

Potential prevention strategies

Chapter 10

Colorectal Anastomotic Leakage: A New Experimental Model

N. Komen, H.C. van der Wal, M. Ditzel, G.J. Kleinrensink, H. Jeekel, J. F. Lange

J Surg Res. 2009 Jul;155(1):7-12

Background.

Anastomotic leakage is the major complication after colorectal surgery. To date, animal experiments concerning colorectal anastomosis focus on anastomotic healing instead of anastomotic leakage. This study aims to develop a new experimental model for colorectal anastomotic leakage.

Methods.

A control group, receiving an anastomosis with 12 interrupted sutures, was compared to a group receiving an anastomosis with 6 interrupted sutures. When the leakage rate was observed to be too low, the number of sutures was decreased stepwise, to 5 or less. Each group contained 9 ‘‘C57Bl6–mice’’. After 7 d the Anastomotic Bursting Pressure (ABP) was determined.

Results.

In the first experiment, one mouse (11.1 %) in the case group and none in the control group developed leakage. Average ABP was 152,2 mm Hg in the control group and 138,8 mm Hg in the case group (p = 0,111). In the second experiment, case group receiving an anastomosis with 5 sutures, 4 mice (44.4 %) in the case group developed leakage. This experiment was repeated twice resulting in leakage rates of 33.3 % and 44.4 %. The average overall ABP in the case group was 142,7 mmHg vs. 179,9 mmHg (p = 0,022) in the control group. The mice without leakage showed a stabilization of average weight loss around day 2 and 3 and a decrease afterwards. The mice with leakage showed a decrease only after day 5. The difference in wellness-scores between the groups with- and without leakage was 2 points, increasing during follow-up.

Conclusions.

The model of anastomotic leakage caused by creating an anastomosis with 5 interrupted sutures is feasible. Weight loss and wellness-scores are good predictors of leakage.

Introduction

Anastomotic leakage is the most important complication after colorectal surgery. The incidence varies between 2 % and 24 % [1–4] and mortality rates can be as high as 33 % [5]. Attempting to solve this problem, a great number of animal experimental studies have been performed. However, despite the great number of studies [6–10], knowledge of this complication is still limited. Contributing to these limitations may be the experimental rat model that has mostly been used for these experiments. This model consists of construction of an anastomosis after which the studied intervention is performed [11–13]. After approximately a week, anastomotic bursting pressures (ABP) and hydroxyproline concentrations are determined as primary outcome measures. Although these outcome measures provide useful information on the healing of the anastomosis, it does not provide information on anastomotic leakage. Leakage rates of these studies are reported but vary greatly between 0 % [14] and 40 % [15], occur after different interventions, and reproducibility has never been tested. This could explain why very few animal experimental results find their way into the clinical setting. An animal model for anastomotic leakage more interesting for the surgeon would be a model in which leakage has a similar cause, manifestation, and incidence as in the human situation. In the human situation, even though multiple factors contribute to the occurrence, technical insufficiency is considered to be one of the causes of anastomotic leakage [16–18]. Van der Ham et al. have constructed technically insufficient anastomoses by using only 4 sutures [12], however no clear leakage was observed. The authors suggested this to be caused by the consistency of feces. In addition, rats are known to be resistant to infection. Therefore, chances of causing anastomotic leakage by technical insufficiency, manifesting itself as fecal peritonitis or abscess formation, are most likely higher in animals less resistant to infection. This study aimed to evaluate the feasibility of an experimental model of anastomotic leakage caused by standardized technical failure in the mouse.

Methods

Animals

Since anastomotic leakage becomes manifest as either abscess formation or fecal peritonitis, the animal used for the model should be sensitive to infection. Therefore, these experiments were conducted with the C57Bl6-mice, since they are considered to be less resistant to infection than the rat. The weight of the mice varied between 23 and 33 g. Standard mouse chow (Tecnilab-BMI, Someren, The Netherlands) and water were supplied ad libitum.

Anesthesia and Operation

The procedure consisted of anesthetizing the mouse (nose mask, FiO2 60 %, isoflurane 2 %), shaving, and disinfecting the abdomen. Afterwards the abdomen was entered under aseptic conditions through a 3-cm midline incision. One centimeter aborally to the caecum, the mesentery was cleaved without damaging the vessels, after which the colon was transected. An end-to-end anastomosis was constructed with Dafilon 8-0 (B. Braun). The colon was repositioned and the abdominal wall was closed in two layers with Safil 5-0 (B. Braun). All intra-abdominal manipulations were done with microscope (Konan Camera K-880, Tokyo, Japan) to ensure optimal vision.

Study Design

Each experiment in this series contained a control group in which a sufficient anastomosis was constructed with 12 interrupted sutures, and an intervention group, in which a potentially insufficient anastomosis was constructed by means of too few sutures. In the first experiment, a potentially insufficient anastomosis was constructed with six sutures.

The number of sutures was to be decreased when the experiment was not successful, according to Table 1. The experiment was considered to be a success when a leakage rate between 20 % and 50 % was achieved. Lower rates would not be cost-effective since too many animals would be needed for a difference to reach significance. Higher rates were considered to be incomparable to the human situation. The successful experiment was repeated twice to test reproducibility, and when the results appeared to be reproducible, the experiments would be stopped in accordance with the committee on Animal Research.

After the operation, twice a day during 1 wk, the weight of the mice was noted, and the mice were observed. During observation, the mice were scored for parameters of wellness, as depicted in Table 2. This score consists of parameters selected from a previously published wellness score [19] in combination with parameters that were considered to represent illness of mice by the committee on Animal Research. Primary outcome measure was anastomotic leakage manifesting itself by either fecal peritonitis or abscess formation around the anastomosis. Secondary outcome measure was ABP, measured in vivo in the anesthetized mice. After one wk the experiment was ended. The mice were anesthetized and re-laparotomy was performed. The abdomen was checked for signs of fecal peritonitis or abscess formation. The anastomosis than was exposed and the ABP determined. When mice were considered to be too ill during the observation period, they were removed from the experiment and the aforementioned protocol was executed. These experiments were approved by the committee on Animal Research of the University Medical Center Rotterdam, Erasmus MC, Rotterdam, The Netherlands. Statistical analysis was performed with SPSS, version 11.0 (SPSS Inc., Chicago, IL).

Experiment number	Number of sutures Intervention group	Number of sutures control group
1	6	12
2	5	12
3	4	12

Table 1. Number of sutures in the different experimental groups.

Parameters	Grading	Score
Activity	Normal / Medium / Low	2 / 1 / 0
Fur	Smooth / Fluffy / Erect	2 / 1 / 0
Eyes	Clean & Open / Clean & Closed / Dirty & Closed	2 / 1 / 0
Able to stand strait	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 2. Scored parameters for wellness. The best grade was awarded the highest score. Minimum score was 0, maximum score was 12. In case of a score of 4 or lower the mouse was removed from the experiment.

Results

Experiment 1

Nine mice received an anastomosis with 12 sutures and nine with six sutures. No animal was removed from the experiment, however, during the sacrificing of the mice one (11.1 %) of the intervention group mice appeared to have an abscess on the anastomotic site. The average anastomotic bursting pressures (ABP), wellness scores per day, and the average weight loss per day are depicted in Tables 3, 4, and Fig. 1.

Anastomotic Bursting Pressure compared between groups			
Experiment	**6**	**12**	**p-value**
1	138,8 ± 21,8	152,2 ± 22,5	0,111

Table 3. Mean ABPs compared between groups

Score vs. AL							
	Score Day 1	Score Day 2	Score Day 3	Score Day 4	Score Day 5	Score Day 6	Score Day 7
AL	8 ± -	7 ± -	6 ± -	6 ± -	5 ± -	7 ± -	6 ± -
No AL	10,5 ± 1,0	9,1 ± 2,4	9,2± 2,5	9,5 ± 2,0	9,4 ± 2,7	9,4 ± 2,3	9,6 ± 1,8

Table 4. Average wellness scores per day and standard deviation versus the presence of anastomotic leakage. No standard deviations could be calculated for the AL group since only one mouse developed AL.

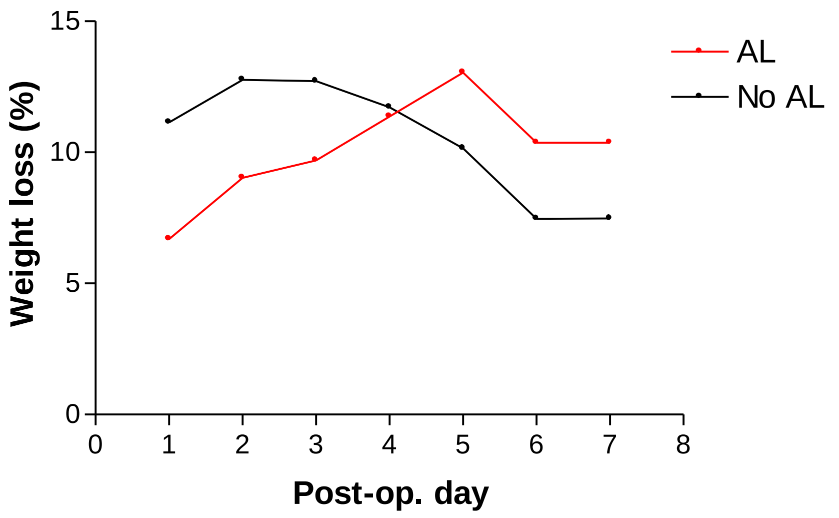

Figure 1. Visual representation of average weight loss per day. The group without leakage shows a stabilization of average weight loss around day 2 and 3 and a decrease afterwards. The group with leakage shows a decrease of average weight loss only after day 5

Experiment 2

Nine mice received an anastomosis with 12 sutures and nine with five sutures. None of the control mice developed anastomotic leakage, whereas 4 (44.4 %) mice of the intervention group developed anastomotic leakage. One mouse (wellness score 2) was removed from the experiment within 24 h after construction of the anastomosis, two mice (wellness score 2 and 1) within 36 h, and one (wellness score 1) within approximately 48 h. They all had developed fecal peritonitis because of anastomotic leakage. This experiment appeared to be successful and was therefore repeated (Experiment 2b). Again, nine mice received an anastomosis with 12 sutures and nine with five sutures. None of the control mice developed anastomotic leakage, whereas 3 (33.3 %) mice of the case group developed anastomotic leakage. One mouse (wellness score 1) was removed from the experiment within 48 h after construction of the anastomosis, one mouse (wellness score 4) on the fifth d postoperatively, and one appeared to have a large abscess at the anastomotic site at 7 d postoperatively, during sacrificing procedures. This experiment appeared to be successful as well and was therefore repeated (Experiment 2c). Again, nine mice received an anastomosis with 12 sutures and nine with five sutures. One of the control mice developed an abscess, which was observed during sacrificing procedures. Four (44.4 %) mice of the case group developed anastomotic leakage. One mouse (wellness score 3) was removed from the experiment within 24 h after construction of the anastomosis, one mouse (wellness score 3) on the fifth d postoperatively, and two appeared to have large abscesses at the anastomotic site at 7 d postoperatively, during the sacrificing procedure. The average ABP, wellness scores per day, and the average weight loss per day for experiments 2a, 2b, and 2c are depicted in Tables 5, 6, and Figs. 2 and 3. The state of the anastomosis directly after construction, after 7 d with- and without abscess formation are shown in Figs. 4, 5, and 6. Since these experiments were successful, in concordance with the committee on Animal Research, no experiments with lower number of sutures were performed.

Anastomotic Bursting Pressure compared between groups			
Experiment	**5**	**12**	**P-value**
2a	134,3 ± 41,4	178,0 ± 29,8	0,039
2b	131,8 ± 32,2	160,2 ± 35,9	0,201
2c	124,1 ± 73,7	204,1 ± 147,5	0,613
Total	142,7 ± 33,4	179,9 ± 84,3	0,022

Table 5. Mean ABPs compared between groups. The ABPs of all mice that were removed from the experiment before day 7 were not used for calculation.

			Score vs. AL						
Exp.	**AL / No AL**	**N**	**Score Day 1**	**Score Day 2**	**Score Day 3**	**Score Day 4**	**Score Day 5**	**Score Day 6**	**Score Day 7**
2a	**AL**	4	1,5 ± 0,6	0,5 ± 1,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0
	No AL	14	10,9 ± 0,7	11,5 ± 0,7	11,6 ± 0,5	11,6 ± 1,1	11,6 ± 0,8	11,7 ± 0,8	11,9 ± 0,5
2b	**AL**	3	9,0 ± 3,5	7,0 ± 5,2	3,7 ± 3,2	4,3 ± 4,5	2,0 ± 3,5	3,3 ± 5,8	3,3 ± 5,8
	No AL	15	10,7 ± 0,6	11,2 ± 1,4	10,9 ± 1,8	11,2 ± 1,4	11,5 ± 0,9	12,0 ± 0,0	11,9 ± 0,3
2c	**AL**	5	8,4 ± 5,1	8,4 ± 5,4	7,6 ± 5,0	6,4 ± 5,4	6,2 ± 5,8	6,0 ± 6,0	6,2 ± 6,0
	No AL	13	10,6 ± 1,2	10,1 ± 2,4	10,0 ± 3,2	9,9 ± 3,2	10,4 ± 2,2	9,8 ± 3,7	9,8 ± 3,6
Total	**AL**	12	6,3 ± 4,9	5,4 ± 5,4	4,1 ± 4,8	3,8 ± 4,8	3,1 ± 4,8	3,3 ± 5,1	3,4 ± 5,2
	No AL	42	10,7 ± 0,8	11,0 ± 1,7	10,9 ± 2,1	10,9 ± 2,1	11,2 ± 1,5	11,2 ± 2,3	11,3 ± 2,2

Table 6. Average wellness scores per day and standard deviation versus the presence of anastomotic leakage.

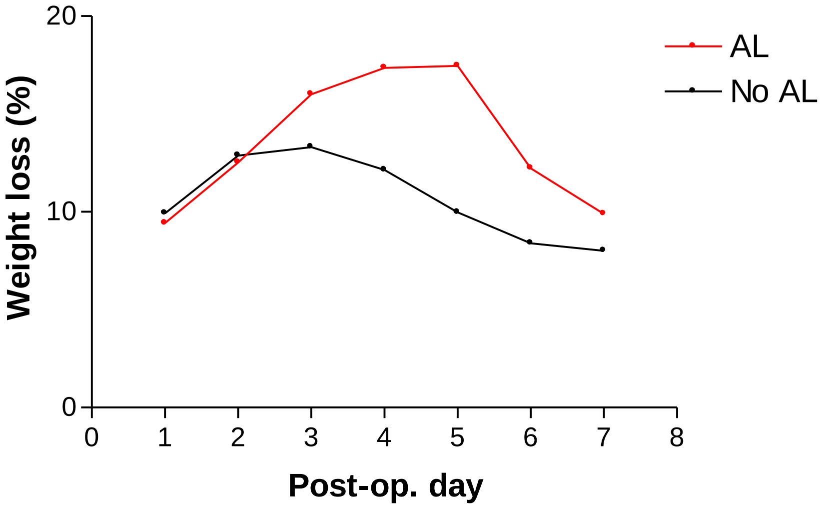

Figure 2. Visual representation of average weight loss per day. The group without leakage shows a stabilization of average weight loss around day 2 and 3 and a decrease afterwards. The group with leakage shows a decrease of average weight loss only after day 5

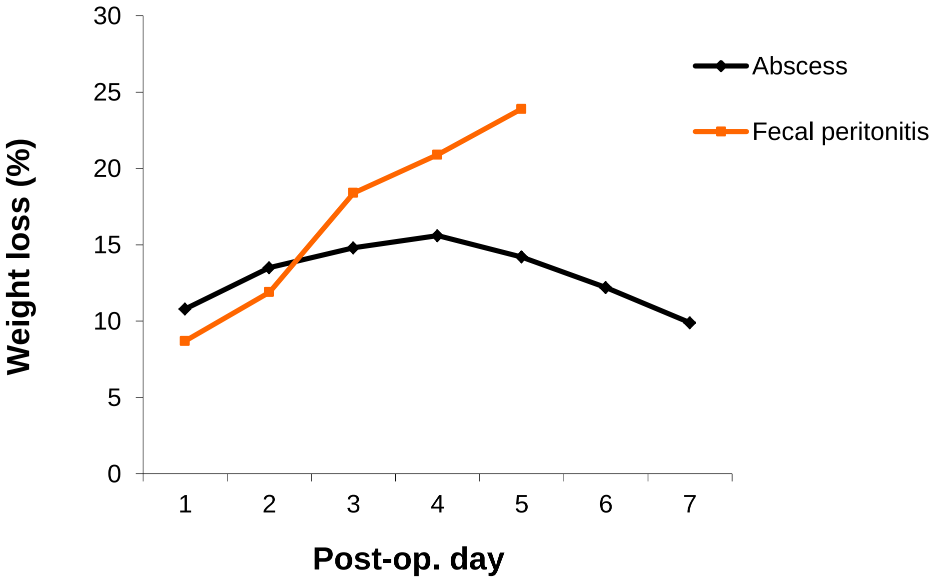

Figure 3. Visual representation of relative weight loss in mice with anastomotic leakage, comparing mice with fecal peritonitis to mice with abscess formation.

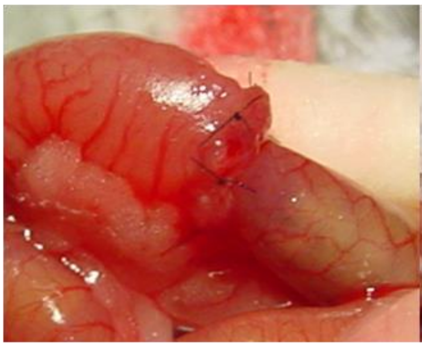

Figure 4. Anastomosis with 5 sutures directly after construction.

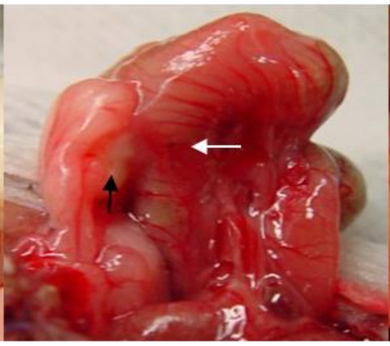

Figure 5. Anastomosis after 7 days with abscess formation. White arrow indicates a suture, black arrow indicates an abscess.

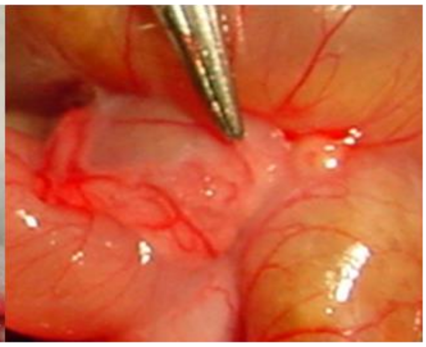

Figure 6. Anastomosis after 7 days. This figure shows the vast formation of adhesions.

Discussion

To date, no animal model has been described in which a spontaneously leaking anastomosis can be reproducibly constructed. Van der Ham et al. [12] tested a model of insufficient anastomotic suturing in rats. No fecal peritonitis or abscess formation occurred, which meant no anastomotic leakage occurred. The authors explained this to be caused by the high consistency of the feces. In addition, the rats' great resistance to infection could explain it as well. Considering these data and the fact that mice are more sensitive to infection than rats, a comparable model was tested on mice. These series of experiments have shown that anastomotic leakage can be induced by constructing an anastomosis with five sutures. The incidence varied between 33 % and 44 % in three similar experiments, which means leakage can be reproducibly induced in this model. The incidence is higher than in the human situation, however, since the majority of mice heal well, this model can be compared to the human situation.

The construction of the anastomosis in this model is insufficient. The colonic mucosa is everting (Fig. 4), which is an exaggeration of the technical failure that can occur in the human situation. Due to this exaggeration, the number of animals needed to reach significance remains limited. In addition, due to the same reason the model is very well suited to test potential protective barriers against anastomotic leakage such as tissue glues and meshes. In order to evaluate anastomotic healing in this model ABP, being the primary outcome measure in the rat model of anastomotic healing was determined as well. These experiments show that ABP is lower in case of a decreased number of sutures (Tables 3 and 5), which means the anastomosis is less strong in case of insufficient suturing. This effect is probably even stronger than represented in Tables 3 and 5 since the ABPs of the mice that were removed from the experiment before day 7 because of leakage were not included in this calculation. In addition, only 52 % of the anastomosis of the control group actually ruptured on the anastomosis, while this is 100 % and 79 % for the anastomosis constructed with six and five sutures respectively.

In order to compare the general state of the mice per day, a wellness score is needed. To our knowledge, only one, not very extensive, scoring method is published [19]. In this study, the items mentioned in Table 2 are the observed wellness features. Tables 4 and 6 show that the wellness score of the mice developing anastomotic leakage will decrease rapidly after the operation and that the scores do not exceed 8, whereas the scores of the healthy mice vary around 10. In addition to the wellness scores, the weight was observed as well. Figures 1 and 2 show the average weight loss for each postoperative day, comparing the group with leakage to the group without. In both experiments, the weight loss of the mice without leakage appeared to stabilize around postoperative day 2 and day 3 and to decrease afterwards, meaning a gain in weight. In the mice with leakage, stabilization of the weight loss occurred later, at postoperative day 5. Afterwards, the weight loss decreased, indicating that the mice with leakage gained weight. This was most likely due to the fact that all mice with fecal peritonitis were removed from the experiment, leaving only the mice with abscess formation (Fig. 3). The latter group showed a slight recovery.

Regarding the wellness score and weight loss, it can be stated that a wellness score lower than 8 and an increasing weight loss after postoperative day 2 are indicators for the presence of anastomotic leakage.

To our knowledge, only one other model of anastomotic leakage has been published. This model, described by Nordentoft et al. [20] concerns a model in pigs in which an anastomosis is created around a tube. A leakage rate of 100 % was realized. It is an interesting model, however, there are several disadvantages. First, the cause of leakage in this model does not exist in the human situation. Second, since the anastomosis is intact and leakage occurs merely through the created defect, this model may be considered as a model for colon perforation rather than anastomotic leakage.

Third, since pigs are large and costly animals, this model is not practical.

Conclusion

The mouse model of anastomotic leakage caused by constructing an anastomosis with only five sutures induces a reproducible incidence of anastomotic leakage and is therefore a feasible model.

References

1. Hagmuller E, Lorenz D, Werthmann K, et al. [Uses and risks of drainage following elective colon resection. A prospective, randomized and controlled clinical study.] Nutzen und Risiken einer Drainage nach elektiven Colonresektionen. Eine prospektive, randomisierte und kontrollierte klinische Studie. Chirurg 1990;61:266.

2. Hyman N, Manchester TL, Osler T, et al. Anastomotic leaks after intestinal anastomosis: It's later than you think. Ann Surg 2007;245:254.

3. Sagar PM, Couse N, Kerin M, et al. Randomized trial of drainage of colorectal anastomosis. Br J Surg 1993;80:769.

4. Matthiessen P, Hallbook O, Rutegard J, et al. Defunctioning stoma reduces symptomatic anastomotic leakage after low anterior resection of the rectum for cancer:A randomized multicenter trial. Ann Surg 2007;246:207.

5. Suturing or stapling in gastrointestinal surgery: A prospective randomized study. West of Scotland and Highland Anastomosis Study Group. Br J Surg 1991;78:337.

6. 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.

7. Kanellos I, Mantzoros I, Demetriades H, et al. Sutureless colonic anastomosis in the rat: A randomized controlled study. Tech Coloproctol 2002;6:143.

8. Egger B, Tolmos J, Friess H, et al. [Keratinocyte growth factor significantly improves healing of left-sided colon anastomoses.] Keratinozyten-Wachstumsfaktor verbessert signifikant die Heilung von linksseitigen Kolonanastomosen. Langenbecks Arch Chir Kongressbd 1998;115(Suppl 1):95.

9. Shikata J, Shida T. Effects of tension on local blood flow in experimental intestinal anastomoses. J Surg Res 1986;40:105.

10. Waninger J, Kauffmann GW, Shah IA, et al. Influence of the distance between interrupted sutures and the tension of sutures on the healing of experimental colonic anastomoses. Am J Surg 1992;163:319.

11. Kanellos D, Blouhos K, Pramateftakis MG, et al. Effect of 5-fluorouracil plus interferon on the integrity of colonic anastomoses covering with fibrin glue. World J Surg 2007;31:186.

12. 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.

13. Uzunkoy A, Akinci OF, Coskun A, et al. Effects of anti-adhesive agents on the healing of intestinal anastomosis. Dis Colon Rectum 2000;43:370.

14. Akgun A, Kuru S, Uraldi C, et al. Early effects of fibrin sealant on colonic anastomosis in rats: An experimental and case-control study. Tech Coloproctol 2006;10:208.

15. 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.

16. Borowski DW, Kelly SB, Bradburn DM, et al. Impact of surgeon volume and specialization on short-term outcomes in colorectal cancer surgery. Br J Surg 2007;94:880.

17. Ishii Y, Hasegawa H, Nishibori H, et al. The application of a new stapling device for open surgery (contour curved cutter stapler) in the laparoscopic resection of rectal cancer. Surg Endosc 2006;20:1329.

18. Beard JD, Nicholson ML, Sayers RD, et al. Intraoperative air testing of colorectal anastomoses: A prospective, randomized trial. Br J Surg 1990;77:1095.

19. te Velde EA, Voest EE, van Gorp JM, et al. Adverse effects of the anti-angiogenic agent angiostatin on the healing of experimental colonic anastomoses. Ann Surg Oncol 2002;9:303.

20. Nordentoft T, Sorensen M. Leakage of colon anastomoses: Development of an experimental model in pigs. Eur Surg Res 2007; 39:14.

.

Part 5

General discussion, summary and future perspectives

As has been mentioned several times throughout this manuscript colorectal anastomotic leakage (CAL) is the major complication of colorectal surgery. We found that CAL occurred in 8.7 % of the 739 patients operated in our hospital. Mortality rate amongst these patients was 14.1 %, significantly higher than in the group of patients that did not develop CAL. The mean hospital stay after operation and the stay at the ICU of patients with CAL were 47 days and 7 days, respectively, whereas patients without CAL stayed in the hospital 14 days and 1 day at the ICU, respectively [1]. These findings correspond to the reported figures in literature [2-5], as depicted in the introduction, indicating the problem of CAL is widespread. Considering that in the Netherlands, annually, approximately 10.000 people will undergo colorectal resection, it can be stated that CAL is a severe complication [6].

Preoperative phase

In the preoperative phase risk factors for CAL can be assessed. This means checking the presence of factors like diverticular disease, rectal resection, urgent operation, smoking, body mass index (BMI), gender, use of steroids, radio- and/or chemotherapy, American Society of Anesthesiologists (ASA) score, a history of cardiac and vascular disease, operating time and use of non-steroidal anti-inflammatory drugs (NSAID) as mentioned in the introduction. These factors are often studied in literature with as many confirming as contradicting results. Subsequently, assuming not all risk factors are known, several new potential risk factors are explored in this thesis. In our retrospective series (Chapter 1) we found obesity (BMI > 30 kg/m2) to be an independent risk factor for CAL, confirming earlier reports [7].This may be due to a defective tissue structure and healing, increased intra-abdominal pressure or the technically more demanding construction of an anastomosis because of thick mesenteries and epiploic appendices. A possible solution might be to construct a diverting stoma in this population. However, since construction of a stoma is difficult in patients with high BMI, awareness and standardized postoperative monitoring, shown to decrease delay until diagnosis of CAL [8], is a more logical consequence of this finding.

In addition, we found that patients operated upon after-hours had more than a twofold increased risk of anastomotic leakage, a finding that has not been reported before. Many potential confounders have been accounted for and are not significant in multivariate analysis. Therefore a possible explanation could be that a decreased technical performance of the operating team has contributed to a higher leakage rate after hours. Medical errors tend to occur more often at night [9, 10] and physicians appear to be less proficient at night, which leads to more errors than during daytime [11-13]. Decreased non-technical skills of the operating team at night, like teamwork- and management skills and situational awareness, could have contributed to higher leakage rates as well. Situational awareness (SA), defined as the ability of each team member to observe, understand and predict events in the operating room (OR), appears to be closely related to technical error rates [14]. The introduction of the surgical safety checklist has been shown to reduce morbidity and mortality after surgery and may contribute to the reduction of CAL rates, specifically in patients operated upon after hours [15].

Healing of an anastomosis leads to a state of higher metabolic activity and higher oxygen demand, requiring an increased blood inflow [16]. Atherosclerosis is a known cause of tissue ischemia [17, 18] and is suggested to have a detrimental effect on anastomotic healing [19]. However it has never been proven. To date, it is possible to determine the atherosclerotic load, represented by the calcium score, mass and volume, of a patient on CT-scan by means of special software (Chapter 2). It was found that atherosclerotic macroangiopathy, i.e. calcifications in the left and right common iliac arteries, is an independent risk factor for colorectal anastomotic leakage. More specifically, an increase of 1 U of calcium score, mass, or volume leads to an increased risk for AL of 0.1 %, 0.3 % and 0.2 %, respectively. Obviously, anastomotic blood supply does not come directly from the common iliac arteries. However, atherosclerosis is a systemic disease and the atherosclerotic load of the common iliac arteries may represent the atherosclerotic load of the colonic and rectal arteries and perhaps also of the colonic microvasculature. Since abdominal CT-scan is often performed for preoperative staging of colorectal tumors, this tool can easily be used for preoperative risk assessment for CAL, allowing tailor made decisions on the construction of an anastomosis and/or a protective or definite stoma. The study presented in Chapter 2 shows the relation between atherosclerosis and CAL. It should be followed by a prospective study aiming to define cut off values in order to become a more concrete risk assessment tool for CAL. Another potential, albeit controversial, risk factor for CAL is use of corticosteroids. Corticosteroids are known to impair wound healing, however their influence on the healing of colorectal anastomosis is unclear [20-23]. This may be because most studies were retrospective, had broad inclusion criteria, and focused on patients with Crohn´s disease. In addition, outcome measures often consisted of postoperative complications in general and CAL was not specifically addressed. Chapter 3 describes the first prospective study demonstrating a significant relationship between patients receiving perioperative corticosteroid treatment for the reduction of postoperative pulmonary complications and clinical AL. The data also show that these patients did not receive a diverting stoma more often than patients who did not take corticosteroids, concluding that, in practice, the risk of CAL due to perioperative use of steroids is not considered to outweigh the risk of morbidity due to a diverting stoma. Even though the group of patients using corticosteroids as a whole was small (27 patients, 11 %), a substantial proportion of patients with CAL took corticosteroids perioperatively (4 patients, 21 %). Only 17 patients (7 %) in the group without CAL used corticosteroids perioperatively. The difference is even more outspoken when the long term users are included (37 % vs. 8 %). Therefore, in this patient category a diverting stoma is recommended.

Intraoperative phase

Adequate blood supply is considered essential for proper anastomotic healing. Therefore, intraoperative identification of the colon´s vasculature during operation is important. Riolan´s arch, considered to be a connection between the superior (SMA) and inferior mesenteric (IMA) arteries, is often referred to during this process. However, as explained in Chapter 4, this eponym leads to confusion due to conflicting results of anatomical studies, the large interindividual variety and due to lack of publications on this matter by Jean Riolan the Younger himself. Therefore, it is proposed to abandon this eponym and describe the left colon´s vasculature in regular terms as mentioned in the Terminologia Anatomica, i.e. marginal artery (MA), left colic artery (LCA) and its ascending branch (ALCA) [24]. Besides this potentially hazardous confusion, the vascular anatomy holds another risk factor. The MA might sometimes be incomplete at the level of the splenic flexure and/or descending colon, rendering perfusion of the proximal anastomotic loop insufficient. Therefore, respecting ALCA's terminal bifurcation, bridging the MA at the splenic flexure (Griffiths' critical point), is of primordial importance under those circumstances. Unfortunately, it is not possible to determine patency of MA at the splenic flexure intraoperatively. Therefore, respecting ALCA and its terminal bifurcation may be beneficial for anastomotic healing and CAL rates.

In colorectal surgery two predominant approaches to vascular ligature exist, as illustrated in Chapter 5. High tie (HT) ligation, i.e. at the origin of the IMA, allows anastomotic perfusion only through MA. After low tie (LT) ligation, i.e. after the origin of LCA, anastomotic perfusion is allowed not only through MA, but through LCA and ALCA as well. As previous studies have shown that after HT ligation anastomotic perfusion is decreased [25, 26], no evidence existed on whether LT is better in terms of perfusion. Chapter 5 describes a study comparing the two techniques by means of Laser Doppler Flowmetry (LDF). Anastomotic perfusion was found to decrease only slightly after HT ligation, whereas after LT perfusion had increased. These results differ from previous findings which may be explained by the longer time interval between arterial ligation and measurement in our study. During this interval recruitment of colonic arteries occurs, allowing recovery of blood flow, which is more outspoken after LT. The increased perfusion after LT as compared to the initial values might be due to reactive hyperemia following manipulation of the bowel [27]. The results of this study favor LT in terms of anastomotic perfusion. In addition, knowing that intestinal blood flow recovers after ligation should lead to restraint and patience of the surgeon concerning resection of a bowel segment that does not seem to be well perfused.

Postoperative phase

With current screening and diagnostic methods the interval between construction of a colorectal anastomosis and diagnosis of leakage varies between 6 and 13 days [28-30]. Several studies have suggested that delay of diagnosis of CAL is associated with higher mortality rates and that only early management will improve clinical outcome [8,31,32]. Therefore, new screening methods allowing detection of CAL in the early postoperative phase, i.e. before the patient manifestly becomes ill, are needed. As discussed in Chapter 6 several studies have been reported aiming to identify a reliable biomarker in drain fluid, originating from the perianastomotic environment. Concentrations of several cytokines have been shown to increase in case of CAL. In addition, studies on several parameters for tissue repair, ischemia and microbiology have shown that it is possible to distinguish between patients with and without CAL. Unfortunately, the number of patients in these studies is rather small and the variability in the obtained values large. Moreover, the main focus is on volatile, costly parameters like IL-1 (interleukin – 1), IL-6, IL-10, TNF-α, MMP-1, MMP-2 and MMP-9. However, for a biomarker to have a clinical role, it should be stable in drain fluid and relatively cheap to determine. In addition, it should not be affected by the primary disease and have optimal sensitivity and specificity.

We hypothesized that in case of CAL the bacterial load in the peri-anastomotic region and consequently in drain fluid will increase over time. Moreover, colonic flora is abundantly present in the colon, so probably easy to detect. In order to detect bacterial contamination fast, sensitive and relatively cheap a real-time PCR test was developed as described in Chapter 7. E. coli and E. faecalis were selected since these bacteria are abundantly present in fecal content and have been detected in drain fluid by culture before [33]. In addition, they are facultatively aerobic and can therefore be cultured in the routine fashion. With cultures serving as the golden standard, RT-PCR results for both E. coli and E. faecalis were generally concordant to culture results, except for several false positive RT-PCRs. The majority of the false positive results occurred on samples taken on day 1 after the operation. At that moment prophylactic antibiotics may have killed colonic bacteria present in drain fluid, explaining negative cultures. With RT-PCR DNA of bacteria is detected whether they are alive or not, rendering positive results.

After validation of these RT-PCR tests to cultures, the potential for screening for CAL was studied in the APPEAL-study as described in Chapter 8. Quantitative PCR for E. faecalis was found to have a high diagnostic odds ratio (DOR = 31.6), high sensitivity (92.9 %) and low false negative rates (1.3 %) and was therefore considered most suited to serve as screening test for CAL. The drawback of this test is the high rate of false positives, which could be due to the aforementioned, intrinsic aspect of PCR, i.e. detection of DNA. However, when the bacteria are not alive when they are detected, it is less likely that their detected concentrations increase. Consequently it is more likely that the false positives are due to subclinical colorectal anastomotic leakage, a long-known phenomenon with a reported incidence of 8 % [34, 35]. Even though it is considered to be asymptomatic it is associated with reduced quality of life and impaired bowel function [35]. Therefore these patients could benefit from treatment after early detection by RT-PCR. The latter remains speculative and requires more research.

The high number of false positives demands additional highly specific testing by means of contrast radiography or CT-scan with rectal contrast testing before intervention. This may lead to overshooting, however, since PCR screening is a cheap technique, the benefits of early detection of CAL by means of PCR screening followed by contrast CT will surely outweigh the cost of a patient with CAL. Whether this screening tool leads to reduced morbidity and mortality remains to be studied.

In Chapter 9 acute phase proteins (APP) levels in drain fluid were evaluated as a possible screening tool for CAL. When determined in serum, CRP, PCT and LBP have been shown to be indicators for inflammatory processes, however, not specific. We hypothesized that these APPs determined in drain fluid would be indicators specifically for CAL. Only increased levels of LBP were found to be associated with a higher risk of CAL. When comparing the means, a clear difference in levels of CRP and PCT could be found between patients with and without CAL. However, the large standard deviation rendered the differences as not significant. The variability may be due to varying amounts of drain fluid produced and limited freshness of samples, which are issues not easy to solve. However, a more personalized approach can be achieved through the development of a prognostic model, determining whether repeated measurements of a specific biomarker, in this study LBP, can ultimately provide a better understanding of disease progression. Development of this prognostic model with the factors to be included should be subject for future research.

Prophylactic drainage (PD), as performed on patients included in the APPEAL-study, aims to evacuate wound fluid and blood collections from the surgical site to prevent infectious complications and to detect AL by fecal or purulent discharge [36-38]. To date, the use of prophylactic drainage remains controversial. Several level 1 studies have shown that PD does not have a beneficial or a detrimental effect on the incidence of CAL and on the morbidity afterwards [39-41]. A prospective study concerning pelvic anastomosis showed a higher leakage rate after routine irrigation-suction drainage in elective anterior resection [42]. A retrospective study however showed PD and the use of a defunctioning stoma to be beneficial in terms of reoperation rates as a result of anastomotic leakage after TME [43]. Despite this controversy surrounding the necessity to drain, PD remains common practice, particularly after rectal surgery due to higher leakage rates [44]. In addition, the outcome measures used in these studies consist of leakage rates, hospital stay, radiological anastomotic leakage, infectious complications and patient comfort. In the APPEAL-study however, we focused on the diagnostic capacity of the drain. Since our results suggest drain fluid analysis has diagnostic value for CAL, the drain´s diagnostic importance has increased tremendously. Therefore, considering the low complication rate of PD, it should be placed routinely during surgery with colorectal anastomosis to allow collection of drain fluid.

Potential Prevention Strategies

To date, no ways of preventing CAL exist. However, a vast body of evidence exists concerning the evaluation of potential prevention strategies in animal experimental setting. In the majority of studies potential sealants are studied, mostly in a rat model [45, 46]. This model allows determination of anastomotic bursting pressures (ABP) and hydroxyproline concentrations as primary outcome measures. Although these outcome measures provide useful information on the healing of the anastomosis, it does not provide information on anastomotic leakage. Leakage rates of these studies are reported but vary greatly between 0 % [47] and 40 % [48], occur after different interventions, and reproducibility has never been tested. This could explain why very few animal experimental results find their way into the clinical setting. Therefore a mouse model of anastomotic leakage caused by constructing an insufficient anastomosis with only five sutures was developed. Anastomotic leakage can be induced with a reproducible incidence of approximately 40 % and is suited for testing of potential anastomotic sealants. The only drawback is the size of the mice, i.e. around 30 grams, which requires technical skill and a microscope. The model is demonstrated in a video available through following link: http://www.youtube.com/watch?v=BOKTIungr0A.

So far six sealants have been tested in this model (Evicel, Omnex, VascuSeal, PleuraSeal, BioGlue, Colle Chirurgicale Cardial) [56]. Leakage rates were around 40 % - 50 % and were not significantly smaller in the group treated with a sealant. More standardized research in this area is unequivocal.

Future perspectives

When restoring continuity after colorectal surgery the most important question is whether to protect the anastomosis by constructing a deviating stoma or not. To do so routinely is not desirable due to the psychological impact on the patient and the morbidity that comes with closure [49-51]. Consequently we have to be able to identify high risk patients so a diverting stoma can be selectively constructed. Therefore we need to know all risk factors for CAL. We have shown in this thesis that not all risk factors for CAL are known. The newly found risk factors “after hours surgery” and atherosclerosis should be confirmed in future studies, but also other potential risk factors like renal insufficiency and the amount of intraabdominal fat should be studied. The latter could be a better predictor for CAL than BMI and can be accurately determined on CT-scan [52,53]. In addition, since CAL is a multifactorial problem, future studies should focus on combinations of risk factors to see whether high risk patients can be identified better by means of combinations of risk factors instead of just an individual factor. Furthermore, operative technique and its impact on anastomotic blood perfusion and viability needs to be studied extensively. We have shown that anastomotic blood flow recovers better after low ligation of IMA, however our series was too small to allow analysis of the impact on CAL. Future studies on this matter should not only include more patients but should also aim to study anastomotic viability postoperatively. A wireless perianastomotic oxygen sensor would allow detection of ischemia and potentially CAL in real time and bed-side. First studies towards the development of such a device have been performed [54, 55].

As mentioned in this thesis we have developed and validated a screenings method for CAL, being a RT-PCR for E. faecalis. This technique is objective, affordable and easy to implement in hospitals. Future studies should aim to confirm these data and to determine sensitivity and specificity of additional CT-scans and/or contrast enemas performed in case of positive testing. An additional challenge might be the identification of subclinical leaks since they are most likely responsible for the substantial number of false positives with RT-PCR screening. Since LBP is a mediator in the inflammatory response to viable gram-negative bacteria, it could be of value in determining which of the RT-PCR positive patients is in fact false positive.

Animal experimental research remains important in CAL research, particularly in the search for an anastomotic sealant and a means to improve anastomotic healing. The model described in this thesis is suited to study potential sealants. Several have been tested in this model already, however, none of them was shown to be a feasible sealant [56].

References

1. Komen N, Dijk JW, Lalmahomed Z, Klop K, Hop W, Kleinrensink GJ, et al. After-hours colorectal surgery: a risk factor for anastomotic leakage. Int J Colorectal Dis. 2009 Jul;24(7):789-95.

2. Matthiessen P, Hallbook O, Rutegard J, Simert G, Sjodahl R. Defunctioning stoma reduces symptomatic anastomotic leakage after low anterior resection of the rectum for cancer: a randomized multicenter trial. Ann Surg. 2007 Aug;246(2):207-14.

3. Mann B, Kleinschmidt S, Stremmel W. Prospective study of hand-sutured anastomosis after colorectal resection. Br J Surg. 1996 Jan;83(1):29-31.

4. Alves A, Panis Y, Trancart D, Regimbeau JM, Pocard M, Valleur P. Factors associated with clinically significant anastomotic leakage after large bowel resection: multivariate analysis of 707 patients. World J Surg. 2002 Apr;26(4):499-502.

5. Pakkastie TE, Luukkonen PE, Jarvinen HJ. Anterior resection controls cancer of the rectum as well as abdominoperineal excision. Eur J Surg. 1995 Nov;161(11):833-9.

6. DCCG. Dutch Surgical Colorectal Audit. 2013 [updated 2013; cited 2013]; Available from: http://www.dccg.nl/colorectalaudit.

7. Benoist S, Panis Y, Alves A, Valleur P. Impact of obesity on surgical outcomes after colorectal resection. Am J Surg. 2000 Apr;179(4):275-81.

8. den Dulk M, Noter SL, Hendriks ER, Brouwers MA, van der Vlies CH, Oostenbroek RJ, et al. Improved diagnosis and treatment of anastomotic leakage after colorectal surgery. Eur J Surg Oncol. 2009 Apr;35(4):420-6.

9. Peberdy MA, Ornato JP, Larkin GL, Braithwaite RS, Kashner TM, Carey SM, et al. Survival from in-hospital cardiac arrest during nights and weekends. Jama. 2008 Feb 20;299(7):785-92.

10. Neuberger JS, Harris JA, Kundin WD, Bischone A, Chin TD. Incidence of needlestick injuries in hospital personnel: implications for prevention. Am J Infect Control. 1984 Jun;12(3):171-6.

11. Smith-Coggins R, Rosekind MR, Hurd S, Buccino KR. Relationship of day versus night sleep to physician performance and mood. Ann Emerg Med. 1994 Nov;24(5):928-34.

12. Rollinson DC, Rathlev NK, Moss M, Killiany R, Sassower KC, Auerbach S, et al. The effects of consecutive night shifts on neuropsychological performance of interns in the emergency department: a pilot study. Ann Emerg Med. 2003 Mar;41(3):400-6.

13. Catchpole K, Mishra A, Handa A, McCulloch P. Teamwork and error in the operating room: analysis of skills and roles. Ann Surg. 2008 Apr;247(4):699-706.

14. Mishra A, Catchpole K, Dale T, McCulloch P. The influence of non-technical performance on technical outcome in laparoscopic cholecystectomy. Surg Endosc. 2008 Jan;22(1):68-73.

15. Haynes AB, Weiser TG, Berry WR, Lipsitz SR, Breizat AH, Dellinger EP, et al. A surgical safety checklist to reduce morbidity and mortality in a global population. N Engl J Med. 2009 Jan 29;360(5):491-9.

16. Chang N, Goodson WH, 3rd, Gottrup F, Hunt TK. Direct measurement of wound and tissue oxygen tension in postoperative patients. Ann Surg. 1983 Apr;197(4):470-8.

17. Greenwald DA, Brandt LJ, Reinus JF. Ischemic bowel disease in the elderly. Gastroenterol Clin North Am. 2001 Jun;30(2):445-73.

18. Scharff JR, Longo WE, Vartanian SM, Jacobs DL, Bahadursingh AN, Kaminski DL. Ischemic colitis: spectrum of disease and outcome. Surgery. 2003 Oct;134(4):624-9; discussion 9-30.

19. Foster ME, Brennan SS, Morgan A, Leaper DJ. Colonic ischaemia and anastomotic healing. Eur Surg Res. 1985;17(3):133-9.

20. Konishi T, Watanabe T, Kishimoto J, Nagawa H. Risk factors for anastomotic leakage after surgery for colorectal cancer: results of prospective surveillance. J Am Coll Surg. 2006 Mar;202(3):439-44.

21. Post S, Betzler M, von Ditfurth B, Schurmann G, Kuppers P, Herfarth C. Risks of intestinal anastomoses in Crohn's disease. Ann Surg. 1991 Jan;213(1):37-42.

22. Tresallet C, Royer B, Godiris-Petit G, Menegaux F. Effect of systemic corticosteroids on elective left-sided colorectal resection with colorectal anastomosis. Am J Surg. 2008 Apr;195(4):447-51.

23. Jex RK, van Heerden JA, Wolff BG, Ready RL, Ilstrup DM. Gastrointestinal anastomoses. Factors affecting early complications. Ann Surg. 1987 Aug;206(2):138-41.

24. (IFAA). FCoATFatIFoAoA. Terminologia anatomica: international anatomical terminology Stuttgart: Thieme; 1998.

25. Seike K, Koda K, Saito N, Oda K, Kosugi C, Shimizu K, et al. Laser Doppler assessment of the influence of division at the root of the inferior mesenteric artery on anastomotic blood flow in rectosigmoid cancer surgery. Int J Colorectal Dis. 2007 Jun;22(6):689-97.

26. Dworkin MJ, Allen-Mersh TG. Effect of inferior mesenteric artery ligation on blood flow in the marginal artery-dependent sigmoid colon. J Am Coll Surg. 1996 Oct;183(4):357-60.

27. Karliczek A, Benaron DA, Baas PC, Zeebregts CJ, Wiggers T, van Dam GM. Intraoperative assessment of microperfusion with visible light spectroscopy for prediction of anastomotic leakage in colorectal anastomoses. Colorectal Dis. 2009 Jul 21.

28. Buchs NC, Gervaz P, Secic M, Bucher P, Mugnier-Konrad B, Morel P. Incidence, consequences, and risk factors for anastomotic dehiscence after colorectal surgery: a prospective monocentric study. Int J Colorectal Dis. 2008 Mar;23(3):265-70.

29. Hyman N, Manchester TL, Osler T, Burns B, Cataldo PA. Anastomotic leaks after intestinal anastomosis: it's later than you think. Ann Surg. 2007 Feb;245(2):254-8.

30. Bellows CF, Webber LS, Albo D, Awad S, Berger DH. Early predictors of anastomotic leaks after colectomy. Tech Coloproctol. 2009 Mar;13(1):41-7.

31. Macarthur DC, Nixon SJ, Aitken RJ. Avoidable deaths still occur after large bowel surgery. Scottish Audit of Surgical Mortality, Royal College of Surgeons of Edinburgh. Br J Surg. 1998 Jan;85(1):80-3.

32. Alves A, Panis Y, Pocard M, Regimbeau JM, Valleur P. Management of anastomotic leakage after nondiverted large bowel resection. J Am Coll Surg. 1999 Dec;189(6):554-9.

33. Chylak J, Michalska W, Drews M, Marciniak R, Lange M. Comparison of bacterial flora found in the peritoneal cavity and drains after intraabdominal surgery. Med Sci Monit. 2000 Mar-Apr;6(2):285-90.

34. Goligher JC, Lee PW, Simpkins KC, Lintott DJ. A controlled comparison one- and two-layer techniques of suture for high and low colorectal anastomoses. Br J Surg. 1977 Sep;64(9):609-14.

35. Lim M, Akhtar S, Sasapu K, Harris K, Burke D, Sagar P, et al. Clinical and subclinical leaks after low colorectal anastomosis: a clinical and radiologic study. Dis Colon Rectum. 2006 Oct;49(10):1611-9.

36. Averbach AM, Sugarbaker PH. The use of drains in elective surgery for colorectal cancer: always, never or selectively? Tumori. 1995 May-Jun;81(3 Suppl):89-97.

37. Brown SR, Seow-Choen F, Eu KW, Heah SM, Tang CL. A prospective randomised study of drains in infra-peritoneal rectal anastomoses. Tech Coloproctol. 2001 Aug;5(2):89-92.

38. Lennox MS. Prophylactic drainage of colonic anastomoses. Br J Surg. 1984 Jan;71(1):10-1.

39. Merad F, Yahchouchi E, Hay JM, Fingerhut A, Laborde Y, Langlois-Zantain O. Prophylactic abdominal drainage after elective colonic resection and suprapromontory anastomosis: a multicenter study controlled by randomization. French Associations for Surgical Research. Arch Surg. 1998 Mar;133(3):309-14.

40. Petrowsky H, Demartines N, Rousson V, Clavien PA. Evidence-based value of prophylactic drainage in gastrointestinal surgery: a systematic review and meta-analyses. Ann Surg. 2004 Dec;240(6):1074-84; discussion 84-5.

41. Jesus EC, Karliczek A, Matos D, Castro AA, Atallah AN. Prophylactic anastomotic drainage for colorectal surgery. Cochrane Database Syst Rev. 2004(4):CD002100.

42. Yeh CY, Changchien CR, Wang JY, Chen JS, Chen HH, Chiang JM, et al. Pelvic drainage and other risk factors for leakage after elective anterior resection in rectal cancer patients: a prospective study of 978 patients. Ann Surg. 2005 Jan;241(1):9-13.

43. Peeters KC, Tollenaar RA, Marijnen CA, Klein Kranenbarg E, Steup WH, Wiggers T, et al. Risk factors for anastomotic failure after total mesorectal excision of rectal cancer. Br J Surg. 2005 Feb;92(2):211-6.

44. Karliczek A, Jesus EC, Matos D, Castro AA, Atallah AN, Wiggers T. Drainage or nondrainage in elective colorectal anastomosis: a systematic review and meta-analysis. Colorectal Dis. 2006 May;8(4):259-65.

45. Vakalopoulos KA, Daams F, Wu Z, Timmermans L, Jeekel JJ, Kleinrensink GJ, et al. Tissue adhesives in gastrointestinal anastomosis: a systematic review. J Surg Res. Apr;180(2):290-300.

46. Pommergaard HC, Achiam MP, Rosenberg J. External coating of colonic anastomoses: a systematic review. Int J Colorectal Dis. Oct;27(10):1247-58.

47. Akgun A, Kuru S, Uraldi C, Tekin O, Karip B, Tug T, et al. Early effects of fibrin sealant on colonic anastomosis in rats: an experimental and case-control study. Tech Coloproctol. 2006 Oct;10(3):208-14.

48. Kanellos I, Christoforidis E, Kanellos D, Pramateftakis MG, Sakkas L, Betsis D. The healing of colon anastomosis covered with fibrin glue after early postoperative intraperitoneal chemotherapy. Tech Coloproctol. 2006 Jul;10(2):115-20.

49. Tsunoda A, Tsunoda Y, Narita K, Watanabe M, Nakao K, Kusano M. Quality of life after low anterior resection and temporary loop ileostomy. Dis Colon Rectum 2008;51:218-22.

50. den Dulk M, Smit M, Peeters KC, et al. A multivariate analysis of limiting factors for stoma reversal in patients with rectal cancer entered into the total mesorectal excision (TME) trial: a retrospective study. Lancet Oncol 2007;8:297-303.

51. Chow A, Tilney HS, Paraskeva P, Jeyarajah S, Zacharakis E, Purkayastha S. The morbidity surrounding reversal of defunctioning ileostomies: a systematic review of 48 studies including 6,107 cases. Int J Colorectal Dis 2009;24:711-23.

52. Sottier D, Petit JM, Guiu S, et al. Quantification of the visceral and subcutaneous fat by computed tomography: Interobserver correlation of a single slice technique. Diagn Interv Imaging.

53. Ballian N, Lubner MG, Munoz A, et al. Visceral obesity is associated with outcomes of total mesorectal excision for rectal adenocarcinoma. J Surg Oncol;105:365-70.

54. Tanase D, Komen N, Kleinrensink GJ, Jeekel J, Lange J.F., Draaijer A, French PJ. Oxygen-tension measurements - the first step towards prevention and early detection of anastomotic leakage. Sensors, 2007 IEEE 2007:68 - 71

55. Tanase D, Komen N, Draaijer A, Lange JF, Kleinrensink GJ, Jeekel J, French PJ. Animal studies using an oxygen-tension sensor for tissue viability monitoring. BIODEVICES 2008 2008;1:50-5.

56. Slieker JC, Vakalopoulos KA, Komen N, Jeekel J, Lange JF. Prevention of leakage by sealing colon anastomosis: experimental study in a mouse model. J Surg Res. 2013 Oct;184(2):819-24

Samenvatting

Naadlekkage (NL) van de anastomose is de belangrijkste complicatie na colorectale chirurgie. Zoals beschreven in hoofdstuk 1 treedt deze complicatie op in 8.7 % van de patienten die colorectale chirurgie ondergaan. De mortaliteit bedraagt 14 %, significant hoger dan de patienten die geen NL ontwikkelen. Daarnaast is de opnameduur langer: 47 in plaats van 14 dagen. Gezien jaarlijks in Nederland ongeveer 10000 mensen colorectale chirurgie ondergaan, moet NL dus als een belangrijk probleem beschouwd worden waarmee rekening gehouden moet houden in de preoperatieve, peroperatieve en postoperatieve fase.

Preoperatieve fase

In de preoperatieve fase kan het risico op NL ingeschat worden aan de hand de aan- of afwezigheid van risicifactoren. Tot de potentiële risicofactoren behoren diverticulose/-itis, resectie van het rectum, urgente ingrepen, roken, body mass index (BMI), geslacht, gebruik van corticosteroiden, radio- en/of chemotherapie, American Society of Anesthesiologists (ASA) score, voorgeschiedenis van cardiale en/of vasculaire ziekte, verwachte operatieduur gebruik van non-steroidal anti-inflammatory drugs (NSAID). Naast studies die de risicofactoren bevestigen zijn er ook een groot aantal studies zijn die juist het tegendeel aantonen. Om deze reden werden een aantal nieuwe potentiële risicofactoren bestudeerd in deze thesis.

In Hoofdstuk 1 wordt een retrospectieve studie beschreven van de patiënten die colorectale chirurgie in een groot academisch ziekenhuis (Erasmus MC, Rotterdam) ondergingen. Een BMI groter dan 30 kg/m2 en een ingreep, verricht tijdens diensturen, bleken onafhankelijke risicofactoren te zijn voor NL.

Genezing van een anastomose gaat gepaard met een verhoogde metabole activiteit en verhoogde lokale zuurstofnood. Atheroslerose is een bekende risicofactor voor ischemie en in de literatuur is reeds gesuggereerd dat het ook een risicofactor voor NL kan zijn. In Hoofdstuk 2 wordt de mate van atherosclerose gekwantificeerd met een nieuwe techniek. Met behulp van de “Siemens Calcium Score” – software werd de mate van atherosclerose bepaald als calcium score, calcium volume en calcium massa. Het bleek dat macroangiopathie, i.e. de calcium score, massa en volume in de beide aa. iliacae communes een onafhankelijke risicofactor is voor NL.

Zoals reeds eerder vermeld wordt het gebruik van corticosteroiden ook beschreven als mogelijke risicofactor, maar de gepubliceerde resultaten zijn tegenstrijdig. Dit komt wellicht doordat meeste studies retrospectief waren met ruime inclusie criteria en voornamelijk gericht waren op patienten met de ziekte van Crohn. Daarnaast werd als uitkomstmaat meestal algemene postoperatieve complicaties gebruikt en werd niet specifiek naar NL gekeken. In hoofdstuk 3 wordt de eerste prospectieve studie beschreven waaruit blijkt dat perioperatief gebruik van corticosteroiden gepaard gaat met een verhoogd risico op NL.

Peroperatieve fase

Een goede bloedvoorziening van de anastomose is essentiëel voor een goede genezing. Daarom is het belangrijk tijdens de operatie de vaten van het colon te identificeren zodat bloedvoorziening van de anastomose zeker gespaard blijft. Hierbij wordt vaak de arcade van Riolan, een verbinding tussen de a. mesenterica superior (AMS) en a. mesenterica inferior (AMI), genoemd. In Hoofdstuk 4 wordt een literatuurstudie naar dit eponym beschreven. Het blijkt een verwarrende term door de tegenstrijdige resultaten van anastomische studies, de grote interindividuele variabiliteit en het gebrek aan publicaties hieromtrent door Jean Riolan zelf. Daarom wordt in dit hoofdstuk voorgesteld het eponym te verlaten en de vasculatuur van het linker colon te beschrijven alleen aan de hand van termen gebruikt in de *Terminologia Anatomica*, t.w. de a. marginalis (AM), a. colica sinistra (ACS) en zijn opstijgende tak. Meestal volgt de AM het colon, maar soms is de arterie incompleet ter hoogte van de flexura lienalis waardoor de bloedvoorziening van de proximale lis van de anastomose onvoldoende is. Daarom is het belangrijk de terminale bifurcatie van de opstijgende tak van de ACS te respecteren, zodat een mogelijks insufficiënte AM ter hoogte van de flexura lienalis (Griffiths' critical point) overbrugd wordt.

In de colorectale carcinoomchirurgie zijn er momenteel twee geaccepteerde benaderingen ten aanzien van het niveau van de vasculaire ligatuur. Bij een zogenaamde "High Tie" (HT)

wodrt de AMI ter hoogte van de origo geligeerd waardoor de anastomose alleen door de AM wordt bevloeid. Bij een Low Tie (LT), waarbij de arterie wordt na aftakking van de ACS wordt geligeerd, wordt de anastomose bevloeid zowel door de AM als door de ACS en zijn opstijggende tak. Er zijn meerdere publicaties waaruit blijkt dat na HT de bloedvoorziening van de anastomose verminderd is, maar of dat ook geldt voor LT is onbekend.
In Hoofdstuk 5 wordt de studie beschreven waarbij de twee technieken met elkaar worden vergeleken, waaruit blijkt dat de bloedvoorziening van de anastomose na LT beter is dan na HT. De bloedvoorziening na LT blijkt zelfs beter dan voor LT, wat reactieve hyperaemie suggereert.

Postoperatieve fase

Laattijdige diagnose van NL gaat gepaard met hogere mortaliteit waardoor een vroegtijdige diagnose essentieel is. Tegenwoordig wordt voornamelijk nog gelet op de kliniek en het bloedonderzoek. Dit beleid is subjectief en aspecifiek. Om de diagnose vroegtijdig te stellen is er behoefte aan een objectieve screeningsmethode. Drainvocht, afkomstig uit een peroperatief geplaatste drain zou voor screening kunnen dienen. Hoofdstuk 6 beschrijft een literatuur studie naar alle reeds gepubliceerde potentieële biomarkers voor NL in drainvocht. Eiwitten zoals IL-1 (interleukin – 1), IL-6, IL-10, TNF-α, MMP-1, MMP-2 and MMP-9 worden in verhoogde concentratie gedetecteerd in geval van NL. Deze potentieële biomarkers zijn echter vluchtig, duur en de variabiliteit in de resultaten is groot. Daarom is er gezocht naar een sneller, sensitiever en goedkoper alternatief in de vorm van “Real-Time Polymerase Chain Reaction” (RT-PCR) screening. Er werd daarbij aangenomen dat in geval van NL het aantal bacteriën in het drainvocht toeneemt. In Hoofdstuk 7 wordt de ontwikkeling beschreven van de RT-PCR test voor de detectie van E. coli en E. faecalis alsook de validatie met behulp van kweken, i.e. de gouden standaard. Vervolgens is onderzocht of RT-PCR voor E. coli en/of E. faecalis kan dienen als screeningsmethode voor NL (Hoofdstuk 8). Hieruit blijkt RT-PCR voor E. faecalis het meest geschikt als screeningsmethode.

In Hoofdstuk 9 wordt de studie naar acute fase eiwitten als potentieële biomarker voor NL beschreven. De concentraties van C-reatief proteine (CRP), Lipopolysacharide Bindend Proteine (LBP) en Procalcitonine (PCT) werden in drainvocht bepaald en vergeleken tussen patienten met en zonder NL. Een toenemende concentratie LBP ging gepaard met een verhoogde kans op NL. De grote variabiliteit in de bepaalde concentraties liet geen eenvoudige bepaling van grenswaarden toe. Derhalve wordt voorgesteld een prognostisch model te ontwikkelen waar de factor LBP in betrokken moet worden.

Dierexperimentele studie

De beste behandeling voor NL is preventie. Veel dierexperimentele studies zijn reeds verricht waarin de preventieve capaciteit van verschillende “sealants” werd getest. Helaas worden de bevindingen niet naar de kliniek geëxtrapoleerd. Een van de oorzaken hiervoor is gelegen in het gebruikte model, dat een model is voor onderzoek van naadgenezing en niet van naadlekkage. Daarom werd een nieuw dierexperimenteel model ontwikkeld zoals beschreven in Hoofdstuk 10.

Publications

N. Komen, J.C. Slieker, P. Willemsen, G. Mannaerts, P. Pattyn, T. Karsten, H. de Wilt, E. van der Harst, Y.B. de Rijke, M. Murawska, J. Jeekel, J.F. Lange. *Acute phase proteins in drain fluid: a new screening tool for colorectal anastomotic leakage ? APPEAL-study: Analysis of Parameters Predictive for Evident Anastomotic Leakage.* Accepted for publication American Journal of Surgery 2014

N. Komen, J. Slieker, P. Willemsen, G. Mannaerts, P. Pattyn, T. Karsten, H. de Wilt, E. van der Harst, W. van Leeuwen, C. Decaestecker, H. Jeekel, J.F. Lange. *Polymerase chain reaction for Enterococcus faecalis in drain fluid: the first screening test for symptomatic colorectal anastomotic leakage. The Appeal-study: Analysis of Parameters Predictive for Evident Anastomotic Leakage.* Int J Colorectal Dis. 2013 Oct 11

J.C. Slieker, K.A. Vakalopoulos, **N. Komen**, J. Jeekel, J.F. Lange. *Prevention of leakage by sealing colon anastomosis: experimental study in a mouse model.* J Surg Res. 2013 Oct;184(2):819-24

M. Ditzel, E.B. Deerenberg, **N. Komen**, I.M. Mulder, H. Jeekel, J.F. Lange. *Postoperative adhesion prevention with a new barrier: an experimental study.* Eur Surg Res. 2012;48(4):187-93. Epub 2012 Jun 5

J.C. Slieker, **N. Komen**, G.H. Mannaerts, T.M. Karsten, P. Willemsen, M. Murawska, H. Jeekel, J.F. Lange. *Long-term and perioperative corticosteroids in anastomotic leakage: a prospective study of 259 left-sided colorectal anastomoses.* Arch Surg. 2012 May;147(5):447-52

N. Komen, S. Vercauteren, D. de Roover. *Aortic hammer syndrome.*J Endovasc Ther. 2011 Aug;18(4):497-500

N. Komen, P. Klitsie, J.J. Hermans, W.J. Niessen, GJ. Kleinrensink, H. Jeekel, J.F. Lange. *Calcium scoring in unenhanced and enhanced CT data of the aorta-iliacal arteries: impact of image acquisition, reconstruction, and analysis parameter settings.* Acta Radiol. 2011 Nov 1;52(9):943-50. Epub 2011 Oct 3

N. Komen, P. Klitsie, J.W. Dijk, J.C. Slieker, J.J. Hermans, K. Havenga, M. Oudkerk, J. Weyler, GJ. Kleinrensink, J.F. Lange. *Calcium score: a new risk factor for colorectal anastomotic leakage.* Am J Surg. 2011 Jun;201(6):759-65. Epub 2010 Sep 27

N.C. Duchateau, **N. Komen**, S.D. Colpaert. *Smoke evacuation during electrosurgery.* Acta Chir Belg. 2011 Jan-Feb;111(1):55-6

N. Komen, J.C. Slieker, P. de Kort, J.H. de Wilt, E. van der Harst, P.P. Coene, M.P. Gosselink, G. Tetteroo, E. de Graaf, T. van Beek, R. den Toom, W. van Bockel, C. Verhoef, J.F. Lange. *High tie versus low tie in rectal surgery: comparison of anastomotic perfusion*. Int J Colorectal Dis. 2011 Aug;26(8):1075-8. Epub 2011 Mar 29

N. Komen, M.C. Morsink, S. Beiboer, A. Miggelbrink, P. Willemsen, E. van der Harst, J.F. Lange, W.B. van Leeuwen. *Detection of colon flora in peritoneal drain fluid after colorectal surgery: Can RT-PCR play a role in diagnosing anastomotic leakage?* J Microbiol Methods 2009 Aug 14

N. Komen, H.C. van der Wal, M. Ditzel, G.J. Kleinrensink, H. Jeekel, J.F. Lange. *Colorectal anastomotic leakage: a new experimental model.* J Surg Res. 2009 Jul; 155(1):7-12 2008 Sep 16

N. Komen, J.W. Dijk, Z. Lalmahomed, K. Klop, W. Hop, G.J. Kleinrensink, H. Jeekel, W.R. Schouten, J.F. Lange. *After hours colorectal surgery: a risk factor for anastomotic leakage.* Int J Colorectal Dis. 2009 Jul; 24 (7):789-95 2009 Mar 21

N. Komen, J. Jeekel. *Chapter 147, Meckel's Diverticulum*, in "General Surgery: Principles and international practice." KI Bland, MW Büchler, A Csendes, OJ Garden, MG Sarr, J Wong, eds, 2007

N. Komen, R.W.F. de Bruin, GJ. Kleinrensink, J. Jeekel, J.F. Lange. *Anastomotic leakage, the search for a biomarker*, Colorectal Dis. 2008 Feb;10(2):109-15; discussion 115-7

N. Komen, M.J.O.E. Bertleff, L.J. van Doorn, J.F. Lange, P.W. de Graaf. *Helicobacter Genotyping and Detection in Peroperative Lavage Fluid in Patients with Perforated Peptic Ulcer*, J Gastrointest Surg. 2008 Mar;12(3):555-60

J.F. Lange, **N. Komen**, G. Akkerman, E. Nout, H. Horstmanshoff, F. Schlesinger, J. Bonjer, GJ. Kleinrensink. *Riolan's arch: confusing, misnomer, and obsolete. A literature survey of the connection(s) between the superior and inferior mesenteric arteries*, Am J Surg, 2007

D.Tanase, **N.Komen**, A.Draaijer, GJ. Kleinrensink, J. Jeekel, Johan F. Lange, Paddy J. French. *Tissue-viability monitoring using an oxygen-tensionsensor.* Communications in Computer and Information Science, 2008

Dankwoord

Professor Lange, de open, creatieve sfeer en de grote vrijheid die ik van u kreeg maakten het onderzoeken voor u en met u fantastisch. Veel dank voor alle mogelijkheden die u heeft gegeven binnen de REPAIR-groep.

Professor Jeekel, uw benadering van chirurgie en chirurgisch onderzoek, met een open vizier en altijd kritisch, is inspirerend. Veel dank voor de lessen, inzichten en mogelijkheden die ik van u gekregen heb.

Professor Kleinrensink, bedankt voor uw motivatie, optimisme en ondersteuning, met name ook op onze jaarlijkse besprekingen in Utrecht.

Professor de Wilt, dank voor de hulp met de verschillende studies, de nuttige tips en het grondige nazicht van de artikelen.

Professor Pattyn, dank voor uw inspanningen om de APPEAL-studie tot een goed einde te brengen. Daarnaast dank voor uw bereidheid deel uit te maken van mijn promotiecommissie.

Professor Verhoef, Professor van Eijck, Professor Bruno, Professor Tilanus, bedankt voor uw bereidheid plaats te nemen in mijn promotiecommissie.

Dr. van Leeuwen, beste Willem, zonder jouw hulp geen RT-PCR. Heel veel dank voor je inspanningen om van een idee een bruikbaar instrument te maken.

Dr. de Rijke, beste Yolanda, “zou je het LBP ook niet bepalen”, bedankt voor je uitleg en ideeën.

Dr. Hermans, beste John, bedankt voor de hulp bij de ontrafeling van de calcium score en de regelmatige discussies die vaak meer vragen opleverden dan antwoorden.

Beste Ron, beste Henk, bedankt voor jullie hulp en adviezen op het lab.

Beste REPAIR – ders: Jens, Arthur, Hans-Christiaan, Mark, Ruben, Jeroen, Juliette, Joris, Max, en medeonderzoekers: Marna, Jill, Zarina, Martin, Dustin, Mariëlle, Tessa, Stefan en Martijn, bedankt voor jullie hulp en gezelligheid.

Dank ook aan de studenten geneeskunde betrokken bij de verschillende onderzoeken: Pieter Klitsie, Jan- Willem Dijk, Peter de Kort, Sander Schouten, Karel Klop, Wouter van Bockel.

Beste Mark, λιπον, jouw blik op het vacature bord in het EMC heeft uiteindelijk geleid tot dit proefschrift. Laten we “de koers” weer oppakken.

Gabriëlle, beste paranimf, eerlijk, betrouwbaar, correct en direct, ik hoop dat we nog vaak de afstand kunnen overbruggen.

Kees, beste paranimf, vriend, van samen een biertje en voetballen tot promoveren, ik ben blij dat je naast me staat.

Aan mijn familie, met name mijn ouders, mijn zus Antoinet en Kris, bedankt voor jullie onvoorwaardelijke en eindeloze steun, begrip en motivatie, zowel bewust als onbewust.

Marie, sterk, mooi, lief, intelligent, fantastische vrouw, merci….

Nola, door jou weet ik wat écht belangrijk is.

PhD Portfolio

Name PhD student	N.A.P. Komen
Erasmus MC Department	Surgery
PhD period	2005 - 2013
Promotor	Prof. dr. J.F. Lange
Copromotor	Prof. dr. J. Jeekel

PhD training	*Year*	*ECTS*
Good Clinical Practice	2007	3
Article 9, certificate required for animal experimental work	2005	3
National Conferences		
Dutch Taskforce on Anastomotic Leakage (oral presentation)	2006	1
Nederlandse Vereniging voor Heelkunde (5 oral + 1 poster presentations)	2007-2013	5
SEOHS (poster presentation)	2007	1
International Conferences		
Congress of ISDS (oral presentation)	2006	1
European Society for Surgical Research (4 oral presentations)	2007-2008	4
Belgian Surgical Week (4 oral presentations)	2009-2010	4
European Society for Coloproctology (oral presentation)	2013	1
Belgian Section of Abdominal Wall Surgery (oral presentation)	2013	1
Teaching		
Surgical teaching of OR – nurses and anaesthesiology nurses	2005-2008	
Coordinator surgical teaching OR – nurses and anaesthesiology nurses	2007-2008	
Teaching pathology in a thanatopraxie course, a preserving embalmment technique	2007-2008	
Supervising 6 Master theses	2006-2008	6

Curriculum vitae

Nicolaas (Niels) Antonius Petrus Komen was born 19th of August of 1979 in Oisterwijk, the Netherlands. After graduation at the Jeroen Bosch College, ´s-Hertogenbosch, he started his medical studies in 1997 at the University of Antwerp. In 2003 he did his clinical rotations that brought him for three months to Greece and two months to the Republic of South Africa. After graduating in 2004 he worked at the department of neurosurgery of the Erasmus MC, Rotterdam, for one year (Prof. C. Avezaat). In 2005 he started to work as a researcher for the REPAIR-group, lead by Prof. J.F. Lange and Prof. J. Jeekel, which resulted in this thesis. In 2008 he started his surgical training at the ZNA Middelheim Hospital Antwerp, Belgium (Dr. M. Vanderveken). After his third year of training at University Hospital of Antwerp, Belgium (Prof. dr. W. Vaneerdeweg) he continued his training at the Sint–Augustinus Hospital in Wilrijk, Belgium (Dr. M. Huyghe). In 2014 he will finish his surgical training at the University Hospital of Antwerp, Belgium (Prof. dr. G. Hubens). Niels is living in Antwerp with Marie and their daughter Nola.