Gut Microbiota and Colorectal Surgery: Impact on Postoperative Complications

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Abstract

Keywords

- ▶ anastomotic leak
- ▶ postoperative complications
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- ▶ colorectal

Colorectal anastomotic leakage is a dreaded complication after colorectal surgery and causes high morbidity and mortality. The pathophysiology of anastomotic healing remains unclear despite numerous studies. In this article, our aim is to provide different perspectives on what is known about the role of the gastrointestinal tract microbiome and its relation to anastomotic integrity.

Colorectal anastomotic leak is a devastating complication of colorectal surgery. Despite advances in surgical technology, anastomotic leak rates after colon and rectal surgery continue to occur. The clinical manifestations of anastomotic leak range from fever and pain requiring antibiotics to its most extreme form with uncontrolled extravasation, septic shock with multisystem organ failure, and even death.¹ This leads to severe consequences such as pelvic sepsis, risk for permanent stoma, all-cause mortality, and in cancer patients, increased recurrence.² After almost a century of investigation in patient risk factors and surgical technique, we have yet to fully understand the pathogenesis of intestinal leakage. The overall incidence of colorectal anastomotic leak varies widely in the literature and ranges from 1 to 24%.³ This considerable variation is due in part to the lack of a uniform definition and diagnostic parameters. An attempt at standardization is depicted in ▶ **Tables 1** and **2**.⁴ It is also well recognized and accepted that leak rates increase as the distance from the anal verge decreases.^{5,6} Proposed explanations such as technical challenges in the pelvis resulting in tissue trauma, increased tension, or decreased blood supply seem plausible. However, there remains no universally accepted explanation. Most colorectal surgeons can anecdotally describe anastomotic leaks which occurred during seemingly technically sound procedures with careful consideration and preservation of intraoperative technical factors in an effort to prevent potentially catastrophic anastomotic failures. Unfortunately, it has

been shown that the surgeon's intraoperative judgment in predicting anastomotic leak based on objective evaluation of anastomotic integrity has extremely low sensitivity and specificity.⁷ Thus, despite advances in surgical technique and patient selection, leak rates have largely remained stagnant. It has thus been proposed that factors unrelated to the technical characteristics of the anastomosis may contribute to the development of anastomotic leak.

Microbiome

We have only recently begun to acknowledge the importance of microbial organisms and our symbiotic relationship which allows for our survival and health maintenance. The human microbiome is defined as the collective set of genomes of the microbes associated with the human body.⁸ This includes species of bacteria, and several viruses that can infect humans directly or that can infect other microbes. The involvement of the human microbiome is thought to play a key role in the pathogenesis of obesity, gastrointestinal (GI) malignancies, and Crohn disease.⁹ The human body consists of around 10 trillion cells, whereas 100 trillion bacteria colonize our surfaces and intestinal tract which directly interact with the host and participate in health homeostasis.⁹ This coinhabitation in the GI tract is both beneficial and essential to the human host by providing digestion of foods, nutrient processing, and immune functions.¹⁰ In the GI tract, which

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Table 1 Definition and severity grading of anastomotic leakage after anterior resection of the rectum⁴

Definition	Defect of the intestinal wall integrity at the colorectal or coloanal anastomotic site (including suture and staple lines of neorectal reservoirs) leading to a communication between the intra- and extraluminal compartments. A pelvic abscess close to the anastomosis is also considered as anastomotic leakage	
Grade	A	Anastomotic leakage requiring no active therapeutic intervention
	B	Anastomotic leakage requiring active therapeutic intervention but manageable without relaparotomy
	C	Anastomotic leakage requiring relaparotomy

harbors the majority of microbes associated with the human body, it has been estimated that only between 10 and 50% of organisms can be successfully grown in clinical microbiology laboratories, and their role in anastomotic healing is the topic of renewed interest.

Involvement of Bacteria in the Pathogenesis of Anastomotic Leakage

The role of bacteria in the development of anastomotic complications has been the topic of study for some time. Antibiotic protection of colon anastomoses was first described by Cohn and Rives during a landmark study which proposed that the gut and specifically colon microbiome had a significant role in protection of devascularized intestinal anastomoses.¹¹ Blain and Kennedy also demonstrated that parenteral penicillin partially protected ischemic bowel from necrosis,¹² and a broad spectrum antibiotic was even more effective.¹³ Cohn and Rives went on to find that intraluminal use of antibiotics was able to protect a devascularized segment of colon, specifically at an anastomotic site.¹¹

Recently, Shogan et al have been proposing a significant role for bacteria in the pathophysiology of anastomotic leakage.¹⁴ They propose an interaction between intraluminal content and the layers of the bowel wall which may play a role in the physiology of anastomotic healing. These additional interactions at a cellular level has turned our study of intestinal anastomosis away from that of solely technical considerations to one that likely harbors cellular and microscopic interactions which may allow us to manipulate it in such a way to reduce its incidence and possibly complications related to its failure.

Host-Pathogen Interactions

The restoration of the epithelial barrier represents one of the most proximal events in anastomotic healing.¹⁵ Impairment of restitution of the epithelial barrier may lead to excessive exposure of the underlying tissue layers to detrimental factors in the intestinal lumen (such as bacteria) and can represent a potential early point of compromise of anastomotic healing.¹⁵ The contribution of this process in the

Table 2 Typical clinical characteristics of patients with different severity grades of anastomotic leakage after anterior rectal resection⁴

	Grade A	Grade B	Grade C
Clinical condition	Good	Mild/moderate discomfort	Severely impaired
Clinical symptoms	No	Yes Abdominal/pelvic pain May have fever Purulent/fecal vaginal discharge (rectovaginal fistula) Turbid/purulent rectal discharge	Yes Peritonitis Septicemia/sepsis
Contents from the drain (if present)	Serous fluid May have turbid or feculent contents from the drain	Turbid/purulent (fecal) content	Fecal (purulent) content
Laboratory tests	Normal	Leukocytosis C-reactive protein elevation	Leukocytosis C-reactive protein elevation May have changes owing to sepsis (e.g., leukopenia)
Radiologic evaluation	Small, contained anastomotic leakage	Anastomotic leakage May have local complications (e.g., pelvic abscess)	Anastomotic leakage May have generalized complications (i.e., peritonitis)
Specific treatment	No	Yes Antibiotics Interventional drainage Transanal lavage/drainage	Yes Relaparotomy with control of septic focus

pathogenesis of anastomotic leak is substantiated by the growing recognition that host-pathogen interactions (HPI) significantly influence gut biology. In vitro and in vivo studies have demonstrated that environmental host cues can lead to activation of virulent bacteria and resultant tissue damage.^{16,17} This may effectively explain how alterations in the gut microbiome potentiates and fosters healing impairment of GI anastomoses. The resultant HPI at the site of GI anastomoses may have significant deleterious effects on the reestablishment of an effective epithelial barrier, which may demonstrate how pathogenic organisms contribute to anastomotic leak.¹⁵ However, most anastomoses heal successfully in the face of bacterial interactions with the epithelium suggesting the requirement of an additional insult to precipitate disruptions in anastomotic healing. This hypothesis was investigated by Stern et al which demonstrated that simulated radiation effects may alter the phenotypic virulence of specific bacteria. Specifically, host factors induced by neoadjuvant radiation and subsequently present at anastomotic sites can transform *Pseudomonas aeruginosa* to an anastomotic-disrupting phenotype (P2) which is associated with anastomotic leak in a rat model. This additional tissue disruption may be the requisite component to produce clinical anastomotic failure by virtue of tight junction disruption followed by epithelial cell death and complete monolayer destruction.¹⁸ Although HPI almost certainly play a part in anastomotic healing, further experimental studies looked further at the results of these interactions to attempt to elucidate specific actions of these pathogenic bacteria and their methods of destruction.

Matrix Metalloproteinases

It appears that the actions of certain bacteria seem to put intestinal anastomoses at increased risk. Shogan et al hypothesized that the capacity of intestinal bacteria to degrade collagen may be an important mechanism underlying anastomotic leak. Their laboratory has previously shown that virulent bacteria with high collagenase activity may contribute to the development of anastomotic leakage.¹ This was investigated with a rat model that found *Enterococcus faecalis* contributes to anastomotic leak through its collagenolytic and matrix metalloproteinase (MMP)9-activating functions, suggesting that there may exist a leak phenotype among intestinal microbes that colonize anastomotic tissues based on a given bacterial strain's collagen-degrading activity and its ability to cleave host intestinal MMP9 to its active extracellular matrix-degrading form.¹ Parenteral cefoxitin did not kill *E. faecalis* in the rat model, nor did it prevent anastomotic leakage. A preliminary survey of 64 bacterial strains isolated from human anastomotic tissues demonstrated that only *P. aeruginosa* and *E. faecalis* expressed the collagen-degrading/MMP9 phenotype. The importance of MMPs and their role in anastomotic integrity is further characterized by case series which investigated the strength of intestinal and specifically colonic anastomoses after administration of MMP inhibitors, to include the antibiotic doxycycline.^{19,20} These studies found that addition of these MMP inhibitors improved the tissue

strength and tissue integrity of anastomoses postoperatively, and furthermore that the tissue surrounding a colonic anastomosis seems to harbor heavy infiltration of MMPs, and thus further collagenolysis, which may lead to loss of tissue integrity.²¹ Thus, the actions of certain MMPs and bacteria which seem to preferentially secrete them may provide a target for further study in an effort to prevent their deleterious effects and reduce anastomotic complications.

Butyrate

Bacteria in the lumen of the colon ferment soluble fiber into short-chain fatty acids (SCFA) and other metabolites with potentially beneficial properties. Butyrate is an abundant SCFA that is transported into the colonic epithelium. It accounts for $\geq 70\%$ of the energy used by normal colonocytes.²² Their absence can lead to mucosal atrophy.²³ In a rat model, the administration of butyrate enemas postoperatively after left-sided colonic resection and anastomosis enhanced anastomotic bursting strength which can be explained by increased collagen synthesis and maturation.²⁴ This was corroborated by another group which similarly found strengthened bursting wall tension in those subjects receiving butyrate enemas postoperatively after right and left colon resection.²⁵ To look at this pathophysiology further, Levison demonstrated that SCFAs inhibited in vitro growth of *P. aeruginosa*²⁶ exactly the pathogen that was later identified as being able to transform into tissue destroying phenotype with high collagenase activity, as previously discussed.¹⁸ Taken together, it tends to reason that the gut microbiome may additionally influence anastomotic healing by production of SCFAs, namely butyrate, which have beneficial effects on anastomotic integrity, and possibly limiting the growth of deleterious bacterial pathogens.

Butyrate and its parent compound fiber may also influence the gut microbiota by virtue of its mechanical properties in that insoluble fiber bulks luminal contents and may speed colonic transit to minimize the exposure of the colonic epithelium to ingested carcinogens such as nitrosamines from charred meat.²² The concept of stool consistency and flow rate have also been studied with its relation to the gut microbiome and anastomotic healing.

Stool Consistency

Flow rate of material through the gut also seems to influence microbial structure as microbial content is most likely influenced by the growth rate and ability to adhere to the gut mucosal layer.²⁷

Recent evidence in mice has shown that the microbial community undergoes significant shifts in structure with changes in the timing of food delivery; therefore, "flow rate" of material through the gut will also influence microbial structure.²⁸ A direct correlate would be the recent evidence that mechanical bowel preparation (MBP) with the administration of antibiotics reduces both surgical site infections and anastomotic leaks.

Mechanical Bowel Preparation

One of our most salient and controversial topics, and its relation to the gut microbiota, is the use of bowel cleansing before colon resection. A mass of microflora resides in the lower GI tract.

MBP is performed before elective colorectal surgery to reduce massive bowel contents, which is a source of colorectal leakage and infectious pathogens, thereby minimizing the chance for surgical site infection (SSI).²⁹ The use of MBP has declined in recent years due to the fact that MBP has consistently failed in numerous randomized clinical trials to demonstrate an independent protective effect against postoperative surgical site infection or anastomotic leakage.^{30–33} This lack of any identifiable benefit, combined with the discomfort that mechanical cleansing can cause patients, has led some to conclude that MBP should no longer be routinely performed. However, these trials failed to include analysis with the coadministration of oral antibiotic preparations.

Other investigators have postulated that the primary reason for mechanical cleansing is to reduce fecal bulk and thereby increase the delivery of oral antibiotics to the colonic mucosa.^{34,35} Recently, nearly 5,000 patients from the 2012 Colectomy-Targeted ACS NSQIP (American College of Surgeons National Surgery Quality Improvement Program) database found that combined bowel preparation with mechanical cleansing and oral antibiotics results in a significantly lower incidence of incisional surgical site infection, anastomotic leakage, and hospital readmission when compared with no bowel preparation.³⁶

In addition, a recent meta-analysis by Chen et al consisted of 7 randomized controlled trials and 1,769 cases. The authors demonstrated that both total SSI and incisional SSI were significantly reduced in patients who received oral systemic antibiotics and MBP compared with patients who received systemic antibiotics alone and MBP (total SSI: 7.2 vs. 16.0%, $p < 0.00001$; incisional SSI: 4.6 vs. 12.1%, $p < 0.00001$).³⁷

Thus, recent data do suggest that a combination of bowel cleansing and intraluminal delivery of antibiotics appear to limit both superficial surgical site infections and anastomotic complications, further supporting an integral role of the gut microbiota in relation to anastomotic integrity.

Conclusion

In summary, despite extensive observational and experimental research in animal models and in humans, the incidence of intestinal anastomotic leakage has largely remained unchanged over time. We believe that this is largely because there lies various yet fully undescribed causal factors leading to colorectal anastomotic leaks that are only now being recognized. Certainly, anastomotic leaks have a complex multifactorial pathophysiology, wherein various intraoperative technical factors, patient comorbidities and characteristics, anastomotic ischemia, inflammation, and bacteria within the gut are all involved. Given the available data, the gut microbiome and its complex interplay within the anastomotic microenvironment appears to be intimately involved in

various aspects of anastomotic healing, which we are only beginning to understand. However, definitive clinical implications for these findings are lacking. Certainly, more research is required to completely elucidate this role and its significance with regard to the ability to manipulate the microbiome in a way which may be able to reduce anastomotic complications and ultimately improve patient outcomes.

References

- Shogan BD, Belogortseva N, Luong PM, et al. Collagen degradation and MMP9 activation by *Enterococcus faecalis* contribute to intestinal anastomotic leak. *Sci Transl Med* 2015;7(286):286ra68
- Shogan BD, Carlisle EM, Alverdy JC, Umanskiy K. Do we really know why colorectal anastomoses leak? *J Gastrointest Surg* 2013;17(9):1698–1707
- Paun BC, Cassie S, MacLean AR, Dixon E, Buie WD. Postoperative complications following surgery for rectal cancer. *Ann Surg* 2010;251(5):807–818
- Karanjia 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* 2010;147(3):339–351
- 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
- Karanjia ND, Corder AP, Bearn P, Heald RJ. Leakage from stapled low anastomosis after total mesorectal excision for carcinoma of the rectum. *Br J Surg* 1994;81(8):1224–1226
- Karliczek A, Harlaar NJ, Zeebregts CJ, Wiggers T, Baas PC, van Dam GM. Surgeons lack predictive accuracy for anastomotic leakage in gastrointestinal surgery. *Int J Colorectal Dis* 2009;24(5):569–576
- Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JL. The human microbiome project. *Nature* 2007;449(7164):804–810
- Morowitz MJ, Babrowski T, Carlisle EM, et al. The human microbiome and surgical disease. *Ann Surg* 2011;253(6):1094–1101
- Lederberg J. Infectious history. *Science* 2000;288(5464):287–293
- Cohn I Jr, Rives JD. Antibiotic protection of colon anastomoses. *Ann Surg* 1955;141(5):707–717
- Blain A III, Kennedy JD. The effect of penicillin in experimental intestinal obstruction; studies on strangulated low ileal obstruction. *Alexander Blain Hosp Bull* 1946;5(4):144–159
- Cohn I Jr, Gelb A, Hawthorne HR. Strangulation obstruction; the effect of pre- and post-operative antibacterial agents. *Ann Surg* 1953;138(5):748–758
- Shogan BD, Smith DP, Christley S, Gilbert JA, Zaborina O, Alverdy JC. Intestinal anastomotic injury alters spatially defined microbiome composition and function. *Microbiome* 2014;2:35
- Stern JR, Olivas AD, Valuckaite V, Zaborina O, Alverdy JC, An G. Agent-based model of epithelial host-pathogen interactions in anastomotic leak. *J Surg Res* 2013;184(2):730–738
- Wu L, Holbrook C, Zaborina O, et al. *Pseudomonas aeruginosa* expresses a lethal virulence determinant, the PA-I lectin/adhesin, in the intestinal tract of a stressed host: the role of epithelia cell contact and molecules of the Quorum Sensing Signaling System. *Ann Surg* 2003;238(5):754–764
- Seal JB, Alverdy JC, Zaborina O, An G. Agent-based dynamic knowledge representation of *Pseudomonas aeruginosa* virulence activation in the stressed gut: Towards characterizing host-pathogen interactions in gut-derived sepsis. *Theor Biol Med Model* 2011;8:33
- Olivas AD, Shogan BD, Valuckaite V, et al. Intestinal tissues induce an SNP mutation in *Pseudomonas aeruginosa* that enhances its virulence: possible role in anastomotic leak. *PLoS ONE* 2012;7(8):e44326

- 19 Pasternak B, Rehn M, Andersen L, et al. Doxycycline-coated sutures improve mechanical strength of intestinal anastomoses. *Int J Colorectal Dis* 2008;23(3):271–276
- 20 Siemonsma MA, de Hingh IH, de Man BM, Lomme RM, Verhofstad AA, Hendriks T. Doxycycline improves wound strength after intestinal anastomosis in the rat. *Surgery* 2003;133(3):268–276
- 21 Agren MS, Andersen TL, Mirastschijski U, et al. Action of matrix metalloproteinases at restricted sites in colon anastomosis repair: an immunohistochemical and biochemical study. *Surgery* 2006;140(1):72–82
- 22 Donohoe DR, Holley D, Collins LB, et al. A gnotobiotic mouse model demonstrates that dietary fiber protects against colorectal tumorigenesis in a microbiota- and butyrate-dependent manner. *Cancer Discov* 2014;4(12):1387–1397
- 23 Roediger WE. The starved colon—diminished mucosal nutrition, diminished absorption, and colitis. *Dis Colon Rectum* 1990;33(10):858–862
- 24 Bloemen JC, Schreinemacher MH, de Bruine AP, Buurman WA, Bouvy ND, Dejong CH. Butyrate enemas improve intestinal anastomotic strength in a rat model. *Dis Colon Rectum* 2010;53(7):1069–1075
- 25 Mathew AJ, Wann VC, Abraham DT, et al. The effect of butyrate on the healing of colonic anastomoses in rats. *J Invest Surg* 2010;23(2):101–104
- 26 Levison ME. Effect of colon flora and short-chain fatty acids on growth in vitro of *Pseudomonas aeruginosa* and Enterobacteriaceae. *Infect Immun* 1973;8(1):30–35
- 27 Gilbert JA, Alverdy J. Stool consistency as a major confounding factor affecting microbiota composition: an ignored variable? *Gut* 2016;65(1):1–2
- 28 Leone V, Gibbons SM, Martinez K, et al. Effects of diurnal variation of gut microbes and high-fat feeding on host circadian clock function and metabolism. *Cell Host Microbe* 2015;17(5):681–689
- 29 Mangram AJ, Horan TC, Pearson ML, Silver LC, Jarvis WR; Centers for Disease Control and Prevention (CDC) Hospital Infection Control Practices Advisory Committee. Guideline for prevention of surgical site infection, 1999. *Am J Infect Control* 1999;27(2):97–132, quiz 133–134, discussion 96
- 30 Contant CM, Hop WC, van't Sant HP, et al. Mechanical bowel preparation for elective colorectal surgery: a multicentre randomised trial. *Lancet* 2007;370(9605):2112–2117
- 31 Slim K, Vicaut E, Launay-Savary MV, Contant C, Chipponi J. Updated systematic review and meta-analysis of randomized clinical trials on the role of mechanical bowel preparation before colorectal surgery. *Ann Surg* 2009;249(2):203–209
- 32 Jung B, Pahlman L, Nyström PO, Nilsson E; Mechanical Bowel Preparation Study Group. Multicentre randomized clinical trial of mechanical bowel preparation in elective colonic resection. *Br J Surg* 2007;94(6):689–695
- 33 Güenaga KF, Matos D, Wille-Jørgensen P. Mechanical bowel preparation for elective colorectal surgery. *Cochrane Database Syst Rev* 2011;(9):CD001544
- 34 Fry DE. Colon preparation and surgical site infection. *Am J Surg* 2011;202(2):225–232
- 35 Hayashi MS, Wilson SE. Is there a current role for preoperative non-absorbable oral antimicrobial agents for prophylaxis of infection after colorectal surgery? *Surg Infect (Larchmt)* 2009;10(3):285–288
- 36 Kiran RP, Murray AC, Chiuzan C, Estrada D, Forde K. Combined preoperative mechanical bowel preparation with oral antibiotics significantly reduces surgical site infection, anastomotic leak, and ileus after colorectal surgery. *Ann Surg* 2015;262(3):416–425, discussion 423–425
- 37 Chen M, Song X, Chen LZ, Lin ZD, Zhang XL. Comparing mechanical bowel preparation with both oral and systemic antibiotics versus mechanical bowel preparation and systemic antibiotics alone for the prevention of surgical site infection after elective colorectal surgery: a meta-analysis of randomized controlled clinical trials. *Dis Colon Rectum* 2016;59(1):70–78