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Review

Current evidence on the relation between gut microbiota and intestinal anastomotic leak in colorectal surgery

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ABSTRACT

Background: Anastomotic leak (AL) is a major complication in colorectal surgery. It worsens morbidity, mortality and oncological outcomes in colorectal cancer. Some evidence suggests a potential effect of the intestinal microbiome on wound healing. This review aims to provide a comprehensive review on historical and current evidence regarding the relation between the gastrointestinal microbiota and AL in colorectal surgery, and the potential microbiota-modifying effect of some perioperative commonly used measures.

Data sources: A comprehensive search was conducted in Pubmed, Medline and Embase for historical and current clinical and animal studies addressing perioperative intestinal microbiota evaluation, intestinal healing and AL.

Conclusions: Evidence on microbes' role in AL is mainly derived from animal experiments. The microbiota's composition and implications are poorly understood in surgical patients. Elaborate microbiota sequencing is required in colorectal surgery to identify potentially beneficial microbial profiles that could lead to specific perioperative microbiome-altering measures and improve surgical and oncological outcomes.

Background

Anastomotic leak (AL) is one of the major complications in colorectal surgery. One of the proposed definitions of AL is "a defect of the intestinal wall at the anastomotic site leading to a communication between the intra- and extraluminal compartments".[1] Anastomotic leak significantly impacts on postoperative mortality.[2,3] There is also evidence that AL is associated with negative oncological outcomes and a decrease of overall and cancer-specific survival in colorectal cancer patients through mechanisms that are yet to be elucidated.[2,3] The risk of developing AL varies greatly depending on several factors, but an approximate estimate of 10% seems to be widely accepted in recent medical literature.[1–3] As AL impacts significantly on immediate and delayed postoperative morbidity and mortality, it increases health costs and seems to alter quality of life and bowel function even when anastomotic continuity is maintained.[2–4]

Risk factors for anastomotic leak

Anastomotic insufficiency is an unpredictable complication and serious efforts have been made to identify patients at a higher risk of developing one after a colorectal procedure. The lack of consensus on possible risk factors in its development was due until very recently to the relative lack of large and strong observational studies. Recent large database evaluations and population-based data have led to the identification of strong associations between patient factors, technical aspects and the occurrence of AL in intestinal surgery.

Parthasarathy et al. have identified, using the American College of Surgeons National Surgical Quality Improvement Program (ACS-NSQIP) database, male sex, smoking, elective rectal cancer surgery, preoperative albumin level $\leq$ 40 g/L, ASA classification $\geq$ 3, diabetes

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mellitus and urgent surgery for bleeding as strong predictors for AL.[2] Interestingly, preoperative use of prophylactic oral antibiotics was found in this same paper to be a protective factor against this complication.[2] In low anterior resection (LAR) of the rectum *Kawada* et al. have identified male gender, increased body mass index (BMI), lower anastomotic location, ischemia and possibly neoadjuvant chemoradiotherapy, tension and corticosteroids use as potential factors for AL.[3] A proximal diverting stoma did not decrease the rate of anastomotic leak but seemed to protect patients against symptomatic and significant intra-abdominal spillage of intestinal content.[3] Many of these factors, such as radiation therapy and corticosteroids, have underlying mechanisms that are not fully understood and many were reported to have conflictual results in different publications.[3] In this same study, a combination of oral and intravenous antibiotics has been reported as having a possible protective effect against surgical site infections (SSIs) and AL.[3] Intestinal microbes have been mentioned in this paper as emerging potential factors in intestinal healing.[3]

Anastomotic leak has intrigued surgeons for decades and endless efforts have been made to prevent it. Despite steady attempts to perfectly understand its pathophysiology, even the most experienced surgeons have not succeeded to eradicate it from their practice. Even with a perfectly conceived intestinal anastomosis with no tension, torsion or ischemia, in healthy patients with no identifiable factors that could impair tissue healing, anastomotic leak may still occur. The inconsistency of some elements as being causal factors of this complication has raised serious doubts over our understanding of its entire pathophysiology.

The emergence of the impact of the microbiome as a common denominator in several medical conditions and pathophysiological processes have made it recently the center of attention in certain gastrointestinal pathologies; the bowel being the largest pool of microbiota in the human body.[5]

Data sources

A comprehensive search was conducted in Pubmed, Medline and Embase for historical and current studies, published between 1950 and 2019, addressing potential links between the gut microbiota, wound healing and AL. Relevant clinical, animal, quantitative and qualitative studies were included.

Gastrointestinal microbiota

The human microbiota contains 100 trillion microorganisms with the greatest density present in the terminal ileum and large bowel.[6] It encompasses bacteria, fungi and viruses.[6,7] The gastrointestinal tract microbiota contains more than a thousand species and is primarily composed of: *Firmicutes* and *Bacteroidetes*, which are the dominant phyla, *Actinobacteria* and *Proteobacteria*.[7] The role of the gut microbiota seems to be well established in physiological and metabolic processes important to the host, such as degradation of complex polysaccharides, synthesis of short-chain fatty acids (SCFAs, namely butyrate, acetate and propionate), indispensable amino acids and vitamins, and maintenance of normal immune function.[8] A healthy gut microbiota is characterized by a dominance of obligate anaerobic bacteria of the *Firmicutes* and *Bacteroidetes* phyla that should prevent the expansion of facultative aerobic and potentially pathogenic members of the *Enterobacteriaceae* family belonging to the *Proteobacteria* phylum. Imbalance in the microbial community, or dysbiosis, has been associated with several medical conditions such as obesity, diabetes and inflammatory bowel disease (IBD).[6] Dysbiosis may involve a relative increase of potential pathogenic bacteria, a reduced proportion of beneficial bacteria, and a decrease in species diversity.[9]

Clinical and fundamental scientific evidence has potentially suggested over the last few decades that the gut microbiota may have a significant role in infectious events and surgical wound healing. Studies seemed however to be more centered on interventions that improved clinical surgical outcomes rather on the understanding of the molecular mechanisms relating intestinal microflora to the host's tissues. The reverse strategy of understanding microbiota's effects on surgical outcomes through already well-established practices, such as prophylactic antibiotics, is probably due in part to the relative difficulty that elaborate microorganisms' characterization requires. In fact, evaluation of the gut flora relied until very recently on long-established bacterial cultures, then the use of the more precise and elaborate amplification via polymerase chain reaction (PCR), and finally detailed and extensive genomic sequencing.[10]

Historical glimpse

The first recorded literature on the potential impact of gastrointestinal microbiota on intestinal healing, and most specifically anastomotic healing, was reported more than 60 years ago by *Cohn* and *Rives*.[11] The authors have shown that the use of intraluminal antibiotics was associated with a complete healing of a devitalized colonic anastomosis and with decreased mortality in dogs. This striking discovery is probably one of the earliest illustrations of the fundamental roles of prophylactic antibiotic therapy in gastrointestinal surgery, and the first suggestion that microbiota may be implicated in anastomotic healing even when ischemia, an adverse outcome predictor, is present.

The findings of *Cohn* and *Rives* have probably led the way to the systematic use of prophylactic antibiotics in colorectal surgery. The arrival of intravenous antibacterial agents, which have generally better bioavailability and systemic effect, has probably led to its use as the preferred route for perioperative prophylaxis.[12] This transition from prophylactic oral to intravenous antibiotics is however poorly documented and has probably occurred gradually over several decades. Despite several studies on the benefits of oral antibiotics as a prophylaxis prior to colorectal surgery, it isn't until very recently that this practice has regained more recognition for its positive effects on surgical outcomes when combined to a mechanical bowel preparation (MBP). The use of MBP as a preoperative bowel cleansing practice is still strongly present in surgical routine. Many conflictual papers have been published however on its effect on deep wound infection.[13] Despite the lack of objectified advantages for the use of MBP alone, it remains a very solidly anchored habit in surgical practice, especially in rectal surgery where overt fecal contamination could complicate elemental steps of the surgical technique.

In the last few decades, especially with the advancements in microbiota's analysis technique, many animal and human experiments have been conducted with the objective of trying to understand the shifts in microbiota composition that perioperative measures induce, and to explain their effects on clinical outcomes.

Animal experiments

Studies evaluating the direct effect of perioperative antibiotics or bowel preparation regimens on anastomotic healing are scarce. This section aims at reviewing scientific evidence on the potential effect of such perioperative interventions on microbiota composition and clinical outcomes, and the biomolecular pathways that potentially related some bacterial species to tissue healing. Table 1 displays studies on perioperative microbiota modifying strategies, such as oral and/or intravenous antibiotics, MBP or povidone-iodine lavage, and their effects on the intestinal microflora in

Table 1
Animal studies on perioperative microbiota modification strategies and effects.

Author	Year	Population	Exposure	Effect(s) on Microbiota[a]	Comment(s)
Orsay & al.[15]	1986	N = 52 (2 groups; dogs)	Colonic povidone-iodine Oral neomycin-erythromycin combination	Greatest decrease of aerobic counts with povidone-iodine	Equal bursting pressures at 3 weeks Elevated blood iodine levels with no systemic toxicity
Groner & al.[16]	1989	N = 28 (4 groups; dogs)	No bowel preparation Three-day clear liquid diet MBP MBP + oral neomycin and erythromycin	Significant decrease of colonic mucosal flora with oral neomycin and erythromycin; no significant change in colonic mucosal flora without oral antibiotics	–
Lindsey & al.[17]	1990	N = 35 (5 groups including a control group; rats)	MBP MBP + IM Cefoxitine Colonic povidone-iodine MBP + oral neomycin sulfate and erythromycin base	No significant decrease in aerobic nor anaerobic counts Decrease in aerobic and anaerobic counts Most important suppression of luminal and mucosal flora	–
Smith & al.[18]	1991	N = 10 (rats)	Intraoperative colonic washout	1000-fold to 10 000-fold decrease in aerobic and anaerobic intraluminal microflora; no significant change in mucosal microflora	–

MBP: mechanical bowel preparation; IM: intramuscular.
[a] Microbiota analysis was performed via bacterial cultures and SEM (scanning electron microscopy).

surgical animal models. It is worth noting that povidone-iodine colonic preparations have been tested in rats and showed injuries to the colonic mucosa.[14] Unlike MBP and antibiotics, iodine can affect thyroid function and hormone levels, and is not commonly used in routine surgical practice. It is important to note that bacterial characterization in these studies is based on culturing techniques.

Studies on animal models have the advantage of working in a controlled and customizable setting. As of the 1990s, they were successful in specifically identifying several microbial species in the gut that could be related to sepsis, impaired wound healing and possibly anastomotic leak. *Okada* et al. have shown in 1999 that germ-free rats had a lower intestinal anastomotic bursting pressure than those with an intestinal flora.[19] Experiments on piglets have shown that small bowel resection and anastomosis was associated with long-term intestinal inflammation and modification of the colonic microbiota.[20] One of the experiments that were successful in linking a specific microbial exposure to anastomotic leak is that of *Schardey* et al. who have demonstrated in 1994 that post-gastrectomy anastomotic insufficiency was higher in rats inoculated with oral *Pseudomonas aeruginosa*, and that anastomotic bursting pressure was higher in those decontaminated with oral tobramycin, polymyxin B and vancomycin.[21] These observations were yet to be corroborated by molecular characterization of the mechanisms underlying these outcomes.

Subsequent studies have focused on the pathophysiological and molecular apparatus of the virulence some germs acquire when exposed to a surgical or a systemic stress. *Pseudomonas aeruginosa* (phylum: *Proteobacteria*) is a well-known opportunistic pathogen that is usually involved in nosocomial infections, especially in immunocompromised patients.[22] Its presence in gut-derived infections and expected absence from the gut of healthy individuals have made it a research prototype to better understand the physiological modifications the intestinal environment undergoes when subjected to a local or a systemic stress. A study published in 1969 describing oral ingestion of *Pseudomonas aeruginosa* in 3 healthy individuals reported 1 case of colonization but no cases of lethal sepsis.[23] This finding raised a question on the host's role in inducing, or protecting against, a harmful phenotype of this pathogen.

Being a commonly encountered pathogen in chronic respiratory conditions, such as cystic fibrosis, *Pseudomonas aeruginosa* has already been the subject of experiments aiming to determine specific factors associated with its virulence in stressful situations. The expression of the galactophilic internal lectin of *Pseudomonas aeruginosa* (PA-I lectin), which is a potentially cytotoxic microbial lectin that gets discharged upon bacterial disruption, has been suggested in 1994 as a virulence factor that contributes to epithelial damage in chronic respiratory infections.[24] In gut-derived sepsis, *Laughlin* et al. have reported in 2000 this same factor as a detrimental element in surgically stressed mice.[25] Its association with lethal gut-derived sepsis seems to be related to an alteration of the intestinal barrier function.[25] Another study published in 2005 has moreover shown that PA-I and exotoxin A, virulence factors of *Pseudomonas aeruginosa*, display an increased expression in an intestinal environment submitted to a surgical and metabolic stress.[26] The expression of PA-I lectin has been the subject of several experiments trying to define, beyond systemic stress, local intestinal factors and mechanisms that were involved in the harmful effects of *Pseudomonas aeruginosa*. *Kohler* et al. have demonstrated in 2004 that epithelial hypoxia, which is usually observed when a surgical stress is present, increases PA-I expression and thus the pathogen's virulence.[27] PA-I lectin expression has also been related to other local factors in the intestine of surgically stressed mice.[28] Binding of interferon-gamma (IFNγ), a pro-inflammatory cytokine, to a porin protein on the outer membrane of *Pseudomonas aeruginosa* has also been suggested as a trigger for PA-I expression.[29]

These findings have led to the investigation of possible preventive measures that could protect against gut-derived sepsis due to this pathogen. Chronic acid water ingestion in mice has been suggested as a possible strategy in 2006.[30] The same study stated that the cecum's barrier function was enhanced in mice ingesting acidified water.[30] Similarly, high-molecular-weight polyethylene glycol was proposed by *Wu* et al. in 2004 as a possible adjunct to the epithelial mucin barrier in stressed mice, protecting thus against *Pseudomonas aeruginosa* sepsis.[31]

More recently, the premise of severe hypophosphatemia being an independent mortality predictor in septic patients have led to the investigation of this factor as a possible trigger of gut-derived sepsis in animal models.[32] *Long* et al. have demonstrated in 2008 that a phosphate-depleted intestinal environment was associated with an increase in PA-I lectin expression that altered epithelial barrier function. It was also associated with biofilm and pyocyanin

production that likely protect the bacterium against immune destruction.[33] Maintaining an appropriate local amount of phosphate at a specific pH was shown to protect against its harmful virulent effects.[34] *Babrowski* et al. have shown that a specific morphotype of *Pseudomonas aeruginosa*, described as having a "wrinked shape", was able to cause lethal peritonitis in comparison with the "smooth shaped" one.[35] Interestingly, virulent strains with a wrinked shape were harvested from mice with a surgical injury and caused 100% mortality.[35] The same author reported in another study that intestinal inoculation with *Pseudomonas aeruginosa* and morphine was associated with less mucus, altered epithelial barrier, higher mortality, increased local and systemic IL-10 level, and once again increased expression of PA-I.[36]

The capacity of *Pseudomonas aeruginosa* to shift to a hypervirulent strain when subjected to a surgical or metabolic stress has been well established and its contribution to gut-derived local and systemic sepsis is evident. The pathogen's effect on intestinal tissues' healing and specifically anastomotic leak was yet to be better defined. *Olivas* et al. have shown in 2012 that irradiated colonic tissue displayed a higher rate of anastomotic leak only when inoculated with *Pseudomonas aeruginosa*.[37] The germ displayed after inoculation in an irradiated territory a shift in its phenotype toward a collagenolytic profile via a single nucleotide mutation (SNP) in the *mexT* gene, which encodes the MexT protein that regulates the mexE-mexF-oprN multidrug efflux system.[37] This damaging phenotype is reported to be harmful to the epithelial barrier via alteration of apoptosis and cytolysis. What is striking in this paper is that this deleterious phenotype could be prevented by a polyethylene glycol and potassium phosphate solution, combing thus both already proven protective strategies.[37]

These new findings on collagenolytic activity have led to the examination of the intestinal microbiota as a whole in an aim to target other potential pathogens that were possibly involved in anastomotic insufficiency. *Shogan* et al. have shown in 2014 that anastomotic injury was associated with significant changes in tissue-associated microbiota with 200-fold and 500-fold increase in the relative abundance of *Enterecoccus* (phylum: *Firmicutes*) and *Escherichia/Shigella* (phylum: *Proteobacteria*) respectively.[38] The paper reported also altered microbial functions with production of hemolysin, cytotoxic necrotizing factors and fimbriæ.[38] The same author reported in 2015 a collagenolytic phenotype of *Enterococcus fæcalis*, along with a capacity to activate the metalloprotease 9 (MMP9) in the host's intestinal tissue.[39] MMP9 is a member of the family of zinc-dependent endopeptidases.[40,41] It contributes to the degradation of extracellular matrix (ECM) in a wide array of pathophysiological processes, and is further believed to delay wound healing.[40,41] In that same study, *Shogan* et al. have obtained swabs from colonic segments after surgical resection in 11 patients.[39] Microbial analysis of these segments displayed disturbed composition and collagenolytic activity along with MMP9 cleavage capacity in *Pseudomonas aeruginosa* and *Enterococcus fæcalis*.[39] This probably constitutes the first proper attempt to link directly microbial function and anastomotic leak in human patients. One central message in this paper is that anastomotic leak could be prevented in rats with suppression of MMP9 or with *Enterococcus fæcalis* elimination via topical intestinal antibiotics, while intravenous antibiotics in the 11 recruited patients failed to eliminate those virulent germs from human samples.[39]

In keeping with the previously published data, more recent papers have demonstrated that morphine enhances colonization of intestinal tissues with collagenolytic *Enterococcus fæcalis* contributing to anastomotic leak in rats, and oral polyphosphate in mice prevents anastomotic insufficiency by inhibiting collagenase production by *Pseudomonas aeruginosa* and *Serratia marcescens* (*Proteobacteria*).[42,43]

Human experiments

Research on human subjects is neither as detailed nor elaborate especially when it comes to interactions between microbes and the host's cells. Very few studies exist on the potential relation between the microbiota composition, perioperative antibiotic and mechanical bowel preparation use and intestinal healing. Table 2 displays studies on microbiota modification with commonly used perioperative therapeutic or pharmacological agents.

The effects of probiotics on postoperative clinical outcomes have also been occasionally tested. Probiotics are defined, according to the Food and Agriculture Organization of the United Nations World Health Organization, as "live microorganisms which when administered in adequate amounts confer a health benefit on the host".[54] *Mizuta* et al. showed via microbial 16S rRNA MiSeq sequencing that probiotics were associated with increased *Actinobacteria* and decreased blood inflammatory parameters, while their absence was associated with increased *Bacteroidetes* and *Proteobacteria* and inflammatory blood markers.[55] *Actinobacteria* represent a small percentage of all bacteria in the gut, yet they are pivotal in the maintenance of gut homeostasis, with some classes of this phylum being widely used as probiotics, for example, *Bifidobacteria*.[56] While some studies have succeeded in finding a positive impact of these supplements on postoperative outcomes, namely anastomotic leak, results still seem to be conflictual and too heterogeneous to support formal clinical recommendations.[55,57]

Van Praagh et al. have tried to evaluate the direct relationship between mucosal microbiota at the time of bowel resection and the rate of postoperative anastomotic leak. They retrieved resection doughnuts from 29 patients undergoing colorectal resection who have subsequently developed AL and compared the microbial 16S rRNA via MiSeq sequencing to those retrieved from patients matched for sex, age and neoadjuvant chemoradiotherapy.[58,59] This experiment showed that AL was associated with lower microbial diversity and a high abundance of anaerobic *Lachnospiraceae* and *Bacteroidaceae* families, with mucin-degrading bacteria.[58,59] These patients were included in the C-seal trial, the aim of which was to evaluate the efficacy of an intraluminal biodegradable sheath in the prevention of anastomotic leak in colorectal anastomoses.[60] It is worth noting that this sheath did not reduce AL, but eliminated the association and effect that were observed between colonic microbiota and AL.[58–60] It is important to note that the *Lachnospiraceae* are deemed unharmful, and the findings were attributed by the author to a disparity in the abundance of *Ruminococcus obeum*, a mucin-degrading bacteria.[58] It is worth noting that microbial characterization was not performed before and after surgery to determine if differences in patients' microbiota were already present before the procedure, as a result of personal and environmental factors, or were the consequence of preoperative MBP and prophylactic antibiotics.

Clinical practice

In surgical practice, several measures are implemented to improve postoperative outcomes and reduce patients' hospital stay. In light of recent evidence of the strong impact intestinal bacteria have on local wound healing, a question is raised over the pathophysiological benefit of some well-established clinical interventions and the possible impact they have on the perioperative intestinal microbiota. Such practices include "enhanced recovery after surgery" (ERAS) protocols, which emphasize the importance of early ambulation, rapid reintroduction of diet and limitation of opioid drugs' use.[61] Another re-emerging trend is the use of prophylactic oral antibiotics before colorectal procedures. Several studies have shown that a preoperative combination of oral

Table 2
Human studies on perioperative microbiota modification strategies and effects.

Author	Year	Population	Exposure		Effect(s) on Microbiota	Comment(s)
Arabi & al.[44]	1978	N = 88 (elective surgery for diverticular disease or colorectal cancer) versus 21 controls	3 days of low-residue diet + oral magnesium sulfate + enemas 5 days of elemental diet (Vivonex standard) + single rectal whashout the day before the surgery Bowel cleansing with normal saline infusion via a nasogastric tube until clear fluid is passed per rectum	+/− Oral neomycin and metronidazole	Cultures: Elemental diet was associated with a small decrease in the number of *Escherichia coli*; addition of oral antibiotics was associated with a significant decrease in *Escherichia coli* and *Bacteroides fragilis* counts	–
van den Boogard & al.[45]	1986	N = 15 (volunteers)	Gut irrigation without antibiotics Gut irrigation with neomycin and metronidazole in the irrigation liquid Oral mannitol + gut irrigation + IV gentamycin + oral metronidazole	+ Oral contamination with *Escherichia coli*	Cultures: All 3 methods of preparation decreased the colonization resistance of the intestinal tract	Systemic antibiotics suggested as the required antimicrobial prophylaxis due to its scant effect on colonization resistance of the gastrointestinal tract
Smith & al.[46]	1990	N = 31 (colonoscopic biopsies or operative samples)	MBP + oral neomycin/erythromycin IV cefoxitin or cefotetan MBP + oral neomycin/erythromycin + IV cefoxitin		Cultures/SEM: greatest reduction in mucosal aerobic and anaerobic counts with MBP and both oral and parenteral antibiotics	–
Bleday & al.[47]	1993	N = 10 (colonoscopic brush samples)	Polyethylene glycol-electrolyte lavage preparation		Cultures: Significant increase in mucosal counts of aerobes, anaerobes, enterics, Gram +, *Bacteroides fragilis*, and *Escherichia coli* with progression from distal to proximal large bowel	–
Jung & al.[48]	2010	N = 37 (elective colorectal surgery)	MBP + oral sulfamethoxazole-trimethoprim/metronidazole or IV cephalosporin/metronidazole		Cultures: MBP did not affect counts of *Escherichia coli*, *Bacteroides* or total bacteria	A significantly higher count of *Escherichia coli* was noted in patients who received oral antibiotics
Watanabe & al.[49]	2010	N = 42 (elective colorectal cancer surgery)	MBP		RT-qPCR: Lower intraoperative and postoperative *Bifidobacterium* and *Lactobacillus* with MBP, as well as lower levels of SCFAs and increased level of lactic acid in postoperative faecal material	MBP suggested as causing an imbalance of the microflora and as having no advantages in colonic cancer resection
Wu & al.[50]	2012	N = 60 (colorectal resection)	1 day of bowel preparation 3 days of bowel preparation		PCR: decreased *Bifidobacterium* and *Lactobacillus* (more significant with 1 day of bowel preparation); increased *Escherichia coli* and *Staphylococcus* (more significant with 3 days of bowel preparation)	Less postoperative infections with 1 versus 3 days of bowel preparation (9.1% versus 29.6%)
Harrell & al.[51]	2012	N = 12 (healthy subjects)	Polyethylene glycol-based bowel preparation; 24-h clear liquid diet		16S rRNA: modification of the mucosal associated microbiota with bowel preparation (significant changes at the genus level but not at the phylum level)	–
Ohigashi & al.[52]	2013	N = 81 (elective colorectal cancer surgery)	MBP (polyethylene glycol–electrolyte solution) + oral kanamycin/metronidazole + IV Cefmetazole before the start of surgery and until 24 h after surgery		16S rRNA-targeted RT-qPCR: Significant reduction in total bacterial counts and 6 groups of obligate anaerobes after surgery; significant increase in *Enterobacteriaceae*, *Enterococcus*, *Staphylococcus* and *Pseudomonas*; decrease in SCFAs	Results showed increase in pathogenic bacteria; perioperative stabilization if the intestinal microflora is suggested by the author to prevent infectious complications
Ralls & al.[53]	2014	N = 15 (small bowel resection)	TPN		454 pyrosequencing: low level of microbial diversity is correlated to gut-derived infections and anastomotic complications	–

IV: intravenous; MBP: mechanical bowel preparation; SEM: scanning electron microscopy; RT-qPCR: reverse transcription quantitative polymerase chain reaction; SCFAs: short-chain fatty acids; PCR: polymerase chain reaction; rRNA: ribosomal RNA; TPN: total parenteral nutrition.

antibiotics and a MBP reduces postoperative surgical site infections (SSIs) including AL and deep wound abscesses.[62,63] Despite these studies, no level 1 recommendation has been issued on the use of oral antibiotics regimens, and guidelines published in 2017 by the *Society of American Gastrointestinal and Endoscopic Surgeons* (SAGES) and the *American Society of Colon and Rectal Surgeons* (ASCRS) have recommended the use of preoperative oral antibiotics and MBP at a 2B level.[64,65] Nonetheless, this practice seems to be increasingly popular without a higher risk of the potential antibiotic-associated *Clostridium difficile* colitis.[63]

Moreover, previously reported studies seem to link opioids and enteral feeding to distinct gut microbial diversity and composition.

Oral antibiotics seem also to have a well-established relationship with microbial shifts in the bowel. These findings might suggest that these favorable clinical measures could have been ultimately acting via a modulating effect on the patient's microbiota at a moment where the concept of microbiota was much less recognized.

Related avenues in colorectal surgery

The intestinal microbiota has been suggested very recently as contributing probably to the carcinogenesis of colorectal cancer (CRC).[66] CRC has been repeatedly associated to red meat consumption. Development of colonic adenoma or adenocarcinoma seems to be related to heme consumption in a dose-dependent manner.[67] This association is definitely not difficult to believe especially that CRC is more prevalent in North America where red meat consumption is very common.[68] A study from our research center suggests that intraluminal heme coming from diet or bleeding due to IBD may induce a dysbiosis and aggravate colitis.[69] These findings might suggest that colorectal surgery patients, suffering mainly from IBD and CRC, may potentially benefit from preventive measures aiming to shift dysbiosis preoperatively in order to insure better postoperative outcomes. This same experiment has shown that lower fecal butyrate levels were present in mice with heme-supplemented diet.[69] The latter notion opens another potential path in preventive strategies for anastomotic healing, namely butyrate supplementation.

Butyric acid is a short-chain fatty acid (SCFA) that is sometimes produced by some intestinal bacteria. Butyrate is an energy source for epithelial cells, solidifies tight junctions and decreases thus epithelial permeability.[58] Several animal experiments have even evaluated oral or rectal butyrate administration in the perioperative period of an intestinal resection and showed that anastomoses were stronger.[70,71] A recent study has even suggested that anastomotic healing was enhanced by oral pectin, was associated to a higher intestinal SCFAs that could have been produced by fermentation of pectin, and contributed thus to better intestinal healing.[72]

Although most of the recent evidence on the role of microbiota in anastomotic healing is described with bacterial communities, fungal microbiota, or “mycobiota”, might have a role to play. *Romanowski* et al. have reported in 2012 that *Candida albicans* expressed a lethal phenotype in critical illness with a depleted phosphate environment.[73] This is not unlike the harmful phenotype expressed by *Pseudomonas aeruginosa* when subjected to radiotherapy or a surgical stress. Nonetheless, fungal dysbiosis has already been described with IBD and is still relatively a poorly understood avenue in host's health and disease.[74]

The role of these avenues in anastomotic leak still require better characterization before targeted clinical interventions could be used to alter their effect on intestinal healing.

Insight and future directions

The gastrointestinal microbiome is the center of attention in surgical recovery and tissue healing more than ever before. Studies going back half a century ago suggest that luminal antibiotics not only prevent anastomotic leak, but also counteract the effects of ischemia.[11]

Novel technologies in microbiome's characterization have led to a significantly better understanding of its composition and function. Animal experiments have become a mainstay in the understanding of cellular and molecular implications of gut microflora on local intestinal inflammation, wound healing, and anastomotic leak in surgical practice. These controlled experimentations focus on specific germs and pathways that help us understand pathophysiological mechanisms that might lead to therapeutic strategies to prevent AL in clinical practice.

It is worth noting that microbiota research in recent years emanated frequently from clinical interventions that has proved to be efficient in preventing postoperative complications like oral antibiotics and limited opioid use. This reverse strategy of going back from the outcome to the understanding of the underlying pathophysiological microbial process is useful as it can potentially shed light not only on microbiota's effect on anastomoses' healing but also on its extensive interactions with human cells and its clinical local and systemic impacts. If we take for instance oral antibiotics and MBP, an abandoned practice that has recently resurfaced, one may wonder with recent evidence if luminal antibiotherapy, similarly to *Cohn* and *Rives* experiments, acts by decimating at least temporarily mucosal microbiota and requires MBP to maximize its effect by flushing fecal intraluminal material. One might argue however that this might have adverse outcomes such as proliferation of less desirable germs that could lead ultimately to inconveniences rather than advantages. Nevertheless, clinical outcomes seem to rather suggest a positive effect on the rate of AL and postoperative infections.

The gastrointestinal microbiota seems to have a serious impact on anastomotic healing (see Table 3). Studies on animal models are succeeding in constantly unveiling new pathways and relationships

Table 3
Actual evidence on microbes potentially involved in intestinal wound healing and anastomotic leak.

Author	Year(s)	Experimental population	Finding(s)	Comment(s)
Shardey et al.[21]	1994	Animal	*Pseudomonas aeruginosa* associated with post-gastrectomy anastomotic insufficiency	Tobramycin, polymyxin B and vancomycin increased anastomotic bursting pressure
Olivas et al.[37]	2012	Animal	*Pseudomonas aeruginosa* is associated with anastomotic leak in an irradiated bowel	Collagenolytic profile via a single nucleotide mutation (SNP) in the mexT gene
Shogan et al.[38]	2014	Animal	Anastomotic injury is associated with an increase in the relative abundance of *Enterecoccus* and *Eschericia/Shigella*	Microbial production of hemolysin, cytotoxic necrotizing factors and fimbriæ
Shogan et al.[39]	2015	Animal	Collagenolytic phenotype of *Enterococcus fæcalis*	Activation of metalloprotease-9 (MMP9)
Shakhsheer et al.[42]	2016	Animal	Colonization of anastomotic tissues with collagenolytic *Enterococcus fæcalis* is enhanced by morphine	ERAS protocols promote limited opioid use
Hyoju et al.[43]	2017	Animal	Polyphosphate prevents anastomotic insufficiency by inhibiting collagenase production by *Pseudomonas aeruginosa* and *Serratia marcescens*	Hypophosphatemia is an independent predictor of mortality[35]
Van Praagh et al.[58,59]	2016, 2019	Human	Lower microbial diversity and a high abundance of *Lachnospiraceae* and *Bacteroidaceae*	Possible involvement of mucin-degrading bacteria
Reddy et al.[75]	2018	Human	Increased preoperative variance of oral and gastric flora associated with post-esophagectomy anastomotic leak	–

between intestinal germs and host's health. Human experiments and better characterization of surgical patients' microbiota is still required more than ever to better understand the microbiota's signature and its effects on anastomotic healing. *Van Praagh* et al. has remarkably described a correlation between intraoperative colonic microbiota and anastomotic leak.[58,59] This correlation however needs to be complemented by preoperative and postoperative microbiota's description. While a characterization of the mucosal microbiota would theoretically be the best-suited strategy, understanding of the intraluminal microbiota and its relation to intestinal healing is yet to be clarified. These observations and correlations in surgical patients will undeniably lead to strong hypothesis that would help us recreate human microflora conditions and better target pathways analysis in special populations such as CRC or IBD patients. The ultimate purpose would be the identification of a "signature" microbiota predicting poor clinical outcomes and thus opening the way for patient-specific interventions that could prevent the adverse effects of generalized drastic antibacterial decontamination.

Finally, anastomotic leak is every surgeon's concern and every patient's apprehension. Further studies are required to better understand perioperative shifts in luminal and mucosal intestinal microbiota, and their impact on immediate and long-term clinical outcomes. The impact of perioperative factors, such as antibiotic therapy, MBP and diet, on intestinal microbiota has also to be elucidated in order to understand the true impact of these measures on clinical outcomes. The question of whether the perioperative period modifies the patient's microbiota for a significant period of time is yet to be clarified, especially in IBD and CRC patients where chronic induced dysbiosis could possibly alter disease evolution. Recent breakthroughs and advances in clinical practice and extensive evaluation of the gastrointestinal microbiota points toward imminent breakthroughs in the prevention of this complication in colorectal surgery.

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