Reducing gastrointestinal anastomotic leak rates: review of challenges and solutions

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Abstract: Various techniques and interventions have been developed in an effort to obviate gastrointestinal anastomotic leaks. This review is intended to delineate potential modifications that can be made to reduce the risk of anastomotic leaks following gastrointestinal surgery. It may also serve to aid in identifying patients who are at increased risk of anastomotic leak. Modifiable risk factors for leak discussed include malnutrition, smoking, steroid use, bowel preparation, chemotherapy, duration of surgery, use of pressors, intravenous fluid administration, blood transfusion, and surgical anastomotic technique. Based upon literature review, operative techniques should include minimizing operative time, reducing ischemia, and utilizing stapled anastomoses. Buttressing of anastomoses with omentum has proven utility for esophageal surgery. Further recommendations include 5–7 days of immune-modifying nutritional supplementation for malnourished patients, discontinuation of smoking in the perioperative period, limiting steroid use, utilization of oral antibiotic preparation for colorectal surgery, avoidance of early operations (<4 weeks) following chemotherapy, limiting pressor use, and the utilization of goal-directed fluid management.

Keywords: anastomosis, anastomotic, leak, dehiscence, gastrointestinal, complications

Background
Anastomotic leakage has been the bane of intestinal surgery for over a century. Various surgical techniques and interventions have been developed in an effort to obviate these leaks. Unfortunately, to date, the issue remains and will likely persist into the foreseeable future. Anastomotic leaks are often difficult to manage and add a degree of frustration to surgeons. They have a great clinical impact as well. Patients with anastomotic leaks have higher lengths of stay, higher mortality rates, higher readmission rates, more reoperations, and an overall greater impact on quality of life. Patients who have anastomotic leaks following cancer operations have a higher risk of distant recurrence. These patients also experience large delays in receiving indicated adjuvant chemotherapy. In addition, in the new world of tighter health care spending, we must also be aware that anastomotic leaks have a significant financial impact on hospitals as well.

This review is intended to delineate the potential modifications that can be made to reduce the risk of anastomotic leaks following gastrointestinal (GI) surgery. It may also serve to aid in identifying patients who are at increased risk of anastomotic leak.

Risk factors for anastomotic leak
In general, anastomotic leaks occur in varying frequencies depending upon the tissue that is being anastomosed (Table 1). As GI surgeons, these rates are of utmost
importance as they have significant impact on our index of suspicion for detection of leaks. Leak rates are high for very proximal (esophageal) and very distal (low rectal) anastomoses.

There are clearly many patient and care factors that contribute to anastomotic leaks. Unfortunately, many risk factors are not under the control of the medical team and are relatively unalterable (Table 2). When faced with patients who have these risk factors, leak rates will clearly be higher. It should go without saying that in these circumstances, surgeons should have a high index of suspicion for leak, consider avoiding anastomoses and using end stomas (where appropriate) or consider placing proximal diverting stomas (where appropriate).

### Adjustable risk factors for anastomotic leak

There are a number of factors that may be adjustable and lead to lower rates of anastomotic leak (Table 3). Some interventions to address these issues may be difficult to undertake while others can be utilized as standard practice for many surgeons. These factors are discussed further in the Modifying patient factors to reduce anastomotic leaks section.

### Table 1 Incidence of anastomotic leaks

<table>
<thead>
<tr>
<th>Type of anastomosis</th>
<th>Incidence of anastomotic leak</th>
</tr>
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<tbody>
<tr>
<td>Esophageal</td>
<td>9.6%–14%</td>
</tr>
<tr>
<td>Stomach</td>
<td>1.1%–3.3%</td>
</tr>
<tr>
<td>Small intestine</td>
<td>1%–3.8%</td>
</tr>
<tr>
<td>Ileocolic</td>
<td>2%–6.5%</td>
</tr>
<tr>
<td>Colocolonic</td>
<td>3%–5.4%</td>
</tr>
<tr>
<td>Colorectal</td>
<td>7%–13%</td>
</tr>
<tr>
<td>Ileorectal</td>
<td>5%–19%</td>
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</tbody>
</table>

**Notes:** Data from these studies.4–16,24

### Table 3 Adjustable risk factors for anastomotic leak

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Recommendation</th>
</tr>
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<tbody>
<tr>
<td>Malnutrition</td>
<td>5–7 days of immune-modifying nutritional supplementation recommended for malnourished patients. TPN is only indicated for postoperative patients unable to tolerate enteral feeding</td>
</tr>
<tr>
<td>Smoking</td>
<td>Discontinuation of smoking (4–8 weeks of cessation preop and for 4 weeks postop)</td>
</tr>
<tr>
<td>Steroid use</td>
<td>If possible, discontinue use prior to surgery (single dose on induction of anesthesia is acceptable)</td>
</tr>
<tr>
<td>Bowel preparation</td>
<td>Use of oral antibiotic preparation for colorectal surgery</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>Avoid early operations (&lt;4 weeks) following chemotherapy but long-term delay times (&gt;7 weeks) are likely unnecessary</td>
</tr>
<tr>
<td>Surgery duration</td>
<td>Minimize the length of surgery</td>
</tr>
<tr>
<td>Pressor agents</td>
<td>Limit use of pressors and when pressors are necessary consider proximal diversion or end stoma in colorectal surgery</td>
</tr>
<tr>
<td>Intraoperative fluids/blood transfusion</td>
<td>Utilize goal-directed fluid management and restrictive blood transfusion guidelines</td>
</tr>
<tr>
<td>Anastomotic technique</td>
<td>Limit tension, optimize perfusion, use the technique most comfortable with (stapled may be preferable)</td>
</tr>
<tr>
<td>Buttressing anastomosis</td>
<td>Omentoplasty should be utilized for esophageal anastomoses after transhiatal esophagectomy</td>
</tr>
</tbody>
</table>

**Abbreviations:** postop, postoperative; preop, preoperative; TPN, total parenteral nutrition.

### Table 2 Nonadjustable risk factors for anastomotic leak

<table>
<thead>
<tr>
<th>Abdominal ascites</th>
<th>Obesity*</th>
<th>Radiation therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advanced malignancy (metastatic disease)</td>
<td>History of cardiovascular disease</td>
<td>Renal failure</td>
</tr>
<tr>
<td>Alcohol abuse*</td>
<td>Previous history of smoking</td>
<td>Site of anastomosis (esophageal and low rectal have the highest risk)</td>
</tr>
<tr>
<td>Anemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>Male sex</td>
<td></td>
</tr>
<tr>
<td>Emergency surgery</td>
<td>Medical comorbidity (high ASA)</td>
<td>Tumor size</td>
</tr>
</tbody>
</table>

**Note:** *Denotes potentially partially adjustable risk factor.

**Abbreviation:** ASA, American Society of Anesthesiologists Physical Status Classification System.

### Modifying patient factors to reduce anastomotic leaks

#### Nutrition

It is clear that nutritionally depleted patients are more prone to complications, and there have been a number of studies, which have shown that preoperative weight loss is associated with increased risk of anastomotic leakage.4,17-19 Studies evaluating nutritional supplementation tend to evaluate overall complications (including anastomotic leak), but few break out anastomotic leak alone. In addition, we must strongly consider the type (parenteral vs enteral) and timing (preoperative or postoperative) of nutritional supplementation.

There is evidence that total parenteral nutrition (TPN) administration may reduce overall complications of surgery in malnourished patients.20 This has been evaluated a number of times in malnourished patients undergoing gastric surgery, and it does appear that the addition of TPN reduces complications in this patient population.21 However, these studies mainly evaluate the administration of TPN in the postoperative period. Most practitioners are well-aware of the benefits of TPN in patients who are unable to tolerate a diet in the postoperative period. However, it is unclear if there is
any benefit to preoperative parenteral supplementation. The data are sparse and in at least one study on the preoperative administration of TPN in malnourished ulcerative colitis patients undergoing colectomy, TPN did not improve anastomotic leak rates.

In a recent Cochran review on the subject, the authors concluded that although there is some evidence that preoperative parenteral nutrition can reduce complications after GI tract surgery in malnourished patients, the data may not be applicable to most patients. Currently, it is difficult to make sweeping recommendations regarding preoperative TPN administration except in cases where patients are unable to tolerate enteral nutrition.

At the same time, there is a growing body of evidence that suggest enteral nutritional supplementation may aid in reducing surgical complications. There is clearly a body of evidence that demonstrates decreased complication rates in malnourished patients when they undergo preoperative nutritional supplementation. Further, there is growing sentiment that immune-enhancing nutrition may be even more beneficial for the malnourished patient. Immune-enhancing enteral nutrition typically entails a high-protein nutritional supplement with the addition of “immune-enhancing” components, which have been shown to augment the immune response (glutamine, arginine, n-3 fatty acids, and ribonucleic acids).

One would imagine that there is a possibility that these immune-enhancing formulas might be beneficial to non-malnourished patients as well. To address this question, there have been a number trials of immune-enhancing supplements in non-malnourished patients undergoing upper GI surgery and, to date, there does not appear to be an advantage to supplementation in non-malnourished patients.

To summarize, there are strong recommendations for the administration of immune-enhancing oral supplementation for 5–7 days preoperatively in malnourished patients in an effort to reduce complications. Although anastomotic leakage has not been individually evaluated in most studies, major morbidity and mortality has and, it is safe to extrapolate that lower infection rates and complications encompasses anastomotic leakage as well.

Smoking

Smoking has been shown to be a risk factor for anastomotic leak in multiple studies on various types of GI anastomoses. This increase has been shown to be approximately fourfold compared to nonsmokers. As surgeons, we are often left with the difficult task of counselling smoking cessation. Unfortunately, short-term smoking cessation has not been shown to reduce anastomotic leak complications. Therefore, recommendations for preoperative smoking cessation entail discontinuation of smoking for 4–8 weeks prior to surgery and through the postoperative healing phase as well. This may be something of a “tough sell” but, for the motivated patient undergoing semi elective surgery, it is well-worth counselling. The greatest success will likely come from practitioners who utilize a smoking cessation team rather than a go-it-alone strategy.

Medications

Steroids/immune modulators

There are myriad publications describing the use of steroid is an independent risk factor for impaired healing of many types of wounds across multiple specialties. In animal models, steroid administration over short-term and long-term at both high and low dosages result in impaired intestinal healing. Not surprisingly, long-term administration of high-dose steroids has been shown to cause the most impairment in healing. In systemic analysis, steroids have been shown to increase anastomotic leak rate. Muddying the waters a bit is a study on patients with inflammatory bowel disease, which demonstrated no increase in anastomotic leak in patients who are on low- and high-dose steroids at the time of surgery. Bearing this in mind, the association between impaired wound healing has been well established and, if at all possible, patients should be steroid free at the time of intestinal surgery.

Increasingly, our patients are being given steroids upon induction of anesthesia. As part of enhanced recovery pathway, it is becoming standard to administer a single dose of dexamethasone perioperatively to reduce nausea. To date, this practice does not appear to cause an impairment in wound healing but further study is pending. The DREAMS trial to analyze the potential risk of dexamethasone on GI surgery completed recruitment of over 1,300 patients in the UK in January 2015, and the final data analysis is pending at the time of this publication.

Finally, it is worth mentioning anastomotic leak risk with biologic therapy (anti-tumor necrosis factor agents). Logically, one would assume that these agents would cause an increase in anastomotic leakage. To date, there have been multiple retrospective studies on the subject. In some studies, it appears that biologic therapy increases postoperative complications while others have reached the opposite conclusion. Currently, it is unclear if biologic therapy does, in fact, cause an increase in anastomotic leak rates. Until more definitive data are available, no clear recommendation regarding these agents can be made.
Chemotherapy
Preoperative chemotherapy and radiation therapy are most commonly utilized for esophageal and rectal cancers. Therefore, these locations of GI anastomoses are the most studied. Although some have reported no increase in anastomotic leak in colorectal surgery following chemoradiation therapy, others have demonstrated that there is evidence that preoperative chemotherapy increases the risk of anastomotic leak in colorectal surgery. At the same time, neoadjuvant chemotherapy has not been shown to increase anastomotic leak rate in esophageal surgery. Although logically, one would assume there would be an association between neoadjuvant therapy and anastomotic leak, to date, there is no consensus about this relationship.

Some have considered the possibility that it is the timing of resection following neoadjuvant therapy that may have influence on anastomotic leak. In one study evaluating the timing of rectal resection after neoadjuvant chemoradiation therapy, there was no difference in leak rate between patients who had earlier vs later surgery following therapy (<7 vs >7 weeks). In a similar study on esophagectomy patients, there was also no difference in earlier vs later surgery following chemoradiation therapy either (<8 vs >8 weeks). From these studies, we may be able to conclude that longer wait times are unlikely to reduce the risk of anastomotic leakage.

In addition to neoadjuvant therapy, the newest forms of chemotherapy are causing increasing concerns for surgery. There have been a number of reports of late anastomotic leaks following the administration of vascular endothelial growth factor (VEGF) inhibitors (eg, bevacizumab). In a recent trial on patients treated with neoadjuvant chemotherapy (with bevacizumab) there was a higher than the expected anastomotic leak rate. These particular chemotherapeutic agents pose a particular challenge for surgeons. Current recommendations are to hold VEGF inhibitors a minimum of 28 days prior to and 28 days after intestinal surgery to reduce the risk of anastomotic leakage. However, others have pointed out that VEGF inhibitors may be active up to 12 weeks following administration and have recommended delaying surgery longer.

NSAIDS
There are emerging data, which suggest that nonsteroidal anti-inflammatory drugs (NSAIDs) may contribute to anastomotic leaks. This issue has gained importance with the emergence of early recovery bundles following GI surgery. Initial evidence in 2009 pointed to an increase in leaks from 3.3% to 15.1% with the addition of celecoxib, but this finding was with small numbers in a retrospective evaluation. However, it was further supported by another retrospective study on leaks, which identified an increase in leak rate from 7.6% to 13.2% with NSAID use a few years later. Studies continue to find an association between NSAIDS and leaks. Interestingly, in rat models, the addition of the NSAID carprofen significantly impaired the healing in ileal anastomoses (but not colocolonic) in two studies. Of course, there have also been papers that have concluded that there is no increase in anastomotic leak with NSAID usage. In a recent meta-analysis of NSAID usage and anastomotic leaks, the authors concluded that most studies, which indicate an association of NSAIDS with anastomotic leak are flawed but, that there is still concern regarding NSAID use and leak. It is this thought that has prompted prospective multicenter studies on the topic, which are currently underway.

Unfortunately, to date, we can draw no clear conclusion about the association of NSAIDS and anastomotic leakage. It is likely to remain a hot topic as surgeons continue to balance the push for early discharge, cost savings, and enhanced recovery against potential increased morbidity, which may be associated with some of these regimens.

Medical therapy/future research
Although we do not currently possess medications/therapies that have been proven to improve GI wound healing and decrease anastomotic leaks, there are numerous ongoing studies investigating potential therapeutic possibilities. Based primarily on animal models, strategies include local application (topical placement, injection at anastomotic site, or coated suture materials) or systemic administration (intravenous, subcutaneous, and intraperitoneal) of growth factors or coated suture materials) or systemic administration (intravenous, subcutaneous, and intraperitoneal) of growth factors directed specifically at the phases of intestinal healing. This burgeoning field may become increasingly relevant as we work to leverage technology to reduce anastomotic leaks.

Technical considerations for reducing anastomotic leaks
Stapled vs handsewn anastomosis
Disputes over the superiority of either handsewn or stapled anastomoses have been going on since the advent of stapled techniques. In almost all fora, there does not appear to be clear evidence of superiority with regard to anastomotic leak. A recent metaanalysis of emergency GI surgery found no significant difference between stapled and handsewn anastomoses. The authors concluded that surgeons should use the technique of their choice. A recent Cochrane review
on colorectal anastomoses also found no difference between handsewn and stapled anastomoses.73

Interestingly, there are a few studies from the 1990s, which demonstrated a higher anastomotic leak rate in cancer surgeries following ileocolic resection with handsewn anastomoses.74,75 However, more recent studies have failed to identify a difference in leak rate between the two techniques.76 In gastric bypass surgery, there has also been no demonstration of reduced leak with either technique in Roux-en-Y anastomoses77 nor has there been a difference been seen for ileostomy closure.78,79

Although there does not appear to be any major difference in anastomotic leak rate with stapled vs handsewn anastomoses (with the potential exception of ileocolic anastomoses for cancer demonstrated in the 1990s). A number of authors had pointed out the advantages of faster surgery with the lower obstruction rate when stapled anastomoses are made.78,80 In the end, the decision of type of anastomosis is likely a matter of surgeon preference as the techniques appear to be essentially equivalent with regard to leak rate.

Bioabsorbable staple-line reinforcement

The use of bioabsorbable staple-line reinforcing material is appealing to some. A number of reinforcing materials placed on anastomotic staplers are currently on the market. Although studies have shown that these reinforcements are safe, and there have been a number of randomized studies on the subject, to date, there have been no compelling studies, which have demonstrated a decrease in anastomotic leak rates when they are used.81–83 Consequently, advocates of bioabsorbable staple-line reinforcement should likely site potential reduction in staple line anastomotic stricture (which has been demonstrated) as their impetus for use rather than decreasing leak rate.84,85

Suture reinforcement

A number of authors have advocated placing reinforcing sutures about an anastomosis. There sutures are typically placed around the anastomosis, but intraluminal reinforcement with sutures has also been described.86,87 To date, there is no compelling evidence indicating that suture reinforcement reduces anastomotic leak, yet these techniques may improve a surgeon’s confidence regarding the strength of one’s anastomosis.

Fibrin glues

These topical sealants appear to have the most popularity in obesity surgery. However, to date, a randomized study has failed to show a decrease in anastomotic leak rate with the use of fibrin glue.88 At the same time, there have been a number of case series of obesity surgeries published, which had very low leak rates with the use of fibrin glues and consequently, it has remained popular in this area of the GI anastomosis despite a lack of hard comparison evidence.89

** Buttressing anastomoses with native tissue**

There are many different techniques described to buttress one’s anastomosis.90,91 These include the use of omentum and mesentery. Advocates of these buttressing techniques often describe low anastomotic leak rates. No large comparative studies are available for GI anastomoses except for esophagectomy.87 In the esophagus, omentoplasia of anastomoses has been prospectively evaluated and has lead to a decrease in anastomotic leak with an odds ratio of 0.26 in metaanalysis.87 However, there is no convincing evidence to date that this technique results in reduced leak rates of other GI anastomoses.

**Type of suture material/layers of sewn anastomoses**

Few studies have evaluated the type of suture material utilized or type of sutured construction in GI tract anastomoses. However, in the interest of cost-containment and time management, some authors have published their data comparing single-layer (either monofilament absorbable suture) vs double-layer (classically internal absorbable layer and outer interrupted silk suture layer) anastomoses. The majority of these studies exclude esophageal and colorectal anastomoses. Invariably, the authors have concluded that single-layer anastomoses are faster and easier to construct with an equivalent complication rate when compared to double-layered anastomoses.92–95

Simplification of suturing techniques has become increasingly relevant with the advent and increasing popularity of laparoscopic suturing techniques and laparoscopic obesity surgery. In keeping with this, a number of recent studies have evaluated the utility of utilizing self-locking “barbed” sutures to create single-layered upper GI anastomoses. Single-layered anastomoses with absorbable “barbed” sutures have been found to be more efficient with no increase in anastomotic leak rates.96–99

**Bowel preparation**

The standard preoperative preparation for colorectal surgery is to administer oral and mechanical bowel preparation on
the day prior to surgery. Over the past decade, this dogma has been brought into question. There have been numerous studies focused on the risks of bowel preparation and their appropriateness and numerous studies have found no evidence that mechanical bowel preparation has an effect on anastomotic leak.\textsuperscript{100–102} Bearing this in mind, recommendations have been made to avoid mechanical bowel prepping patients due to concerns regarding patient satisfaction, potential electrolyte disturbances, and potential risk of \textit{Clostridium difficile} infection.

Avoidance of mechanical bowel preparation should not lead one to delete the oral antibiotic portion of bowel preparation, as the digestive tract decontamination with oral antibiotics on the day prior to surgery has been clearly demonstrated to reduce anastomotic leaks.\textsuperscript{103} There is actually increasing evidence that the makeup and the concentration of the intestinal microbiome contributes to anastomotic leak. Therefore, reducing the bacterial load rests soundly on the basis of scientific data.\textsuperscript{104} Recently, large NSQIP evaluation studies have found a lower incidence of anastomotic leak in patients who received combined oral and mechanical bowel preparation prior to colon surgery.\textsuperscript{105,106} Larger prospective studies are certainly needed but, in general, one can recommend the use of non-absorbable oral antibiotics with/without a mechanical preparation on the day prior to colonic surgery.

**Intraluminal devices**

A number of intraluminal devices have been employed in an effort to control anastomotic leaks, including stents, transanal tubes, and condoms. To date, no large randomized studies have clearly demonstrated any advantage to utilizing intraluminal devices.\textsuperscript{107}

**Reducing ischemia**

Ischemia to one’s anastomosis will invariably lead to complications such as leak or stricture. Traditional evaluation of viability is based upon palpable pulse, bleeding, and the appearance of duskeness. There are newer technological advances that are specifically geared toward the evaluation of perfusion. Although there are a number of companies working on real-time tissue perfusion evaluation, the two main players today are Firefly infrared perfusion technology performed during robotic surgery (Firefly\textsuperscript{TM}, Intuitive Surgical Inc., Sunnyvale, CA, USA) and the SPY System (LifeCell Corp, Branchburg, NJ, USA). Both of these systems rely on injection of a fluorescence dye and intraoperative fluorescence imaging to evaluate tissue perfusion. In one study on the use of fluorescence technology intraoperatively during robotic colectomy, fluorescence imaging resulted in a change of the proximal transection location in 40% of patients and a 5% change in distal transection location.\textsuperscript{108} A similar recent retrospective evaluation of SPY technology resulted in a less modest change in transection rate. In this study, two patients had anastomotic leaks and, interestingly, they both had well-documented perfusion at the time of surgery using the SPY system.\textsuperscript{109} Although intraoperative perfusion technology is intriguing and appears to give a more objective way to evaluate perfusion, to date, there is no clear evidence that this technology leads to a lower anastomotic leak rate or has superiority to tradition observational evaluations of potentially ischemic tissue.

**Resuscitative factors (crystalloid administration, hypotension, blood loss, and duration of surgery)**

**Fluid restriction**

There is very good evidence in the trauma literature that over-resuscitation is directly associated with anastomotic leak after colectomy.\textsuperscript{108} On the other hand, there is also evidence that excessive fluid restriction in the operating room also leads to anastomotic leak after GI surgery.\textsuperscript{111,112} As part of most enhanced recovery pathway bundles, goal-directed fluid administration is advocated. It is quite likely that this method of fluid administration will result in a decrease in anastomotic leak but this data have yet to be demonstrated. An appropriate fluid administration that is goal directed should be part of all intraoperative and postoperative care following GI surgery.

**Hypotension**

Most surgeons know well the postoperative implications of intraoperative and postoperative cardiopulmonary issues. Hypotension is one of these. It has been demonstrated that patients with prolonged diastolic blood pressure drops have a significantly increased risk of anastomotic leak.\textsuperscript{113} Interestingly, the method of treating hypotension may be very important. Patients who have postoperative treatment with vasopressors have an over three- to fourfold increase in anastomotic leak rate and the longer the exposure to vasopressors, the higher the risk of anastomotic leak.\textsuperscript{114,115} If possible, avoidance of vasopressors is prudent unless they are absolutely necessary.

**Blood transfusion**

Higher blood loss intraoperatively is associated with increased anastomotic leak rates.\textsuperscript{104,116} In addition, blood transfusion in
the perioperative period has a very high association with anastomotic leak (odds ratio > 10). Both of these factors (blood loss and blood transfusion) may reflect more complicated surgeries with more intraoperative and postoperative difficulties. However, one must also consider the immunologic consequences of blood transfusion. Increasingly, we are seeing evidence that blood transfusion alone can be a risk factor for hospital acquired infections and many have concluded that restrictive rather than liberal utilization of blood transfusion leads to fewer complications.

Conclusion
Anastomotic leak remains one of the most relevant complications following GI surgery. Therefore, it is the obligation of GI surgeons to do their best to reduce the risk of anastomotic leaks. Unfortunately, despite one’s best efforts, these complications are bound to occur and we cannot underestimate the importance of a high index of suspicion leading to early diagnoses of leaks. In addition, we should try to recognize high-risk individuals and consider the avoidance of anastomosis, proximal diversion, and very close observation when possible.

Disclosure
The author reports no conflicts of interest in this work.

References


