



Dear Editor,

We greatly appreciate your interest in our manuscript and are delighted to have been offered the opportunity of resubmitting our paper.

We have made our best efforts to improve and revise the manuscript according to each of the peer-reviewers' comments and suggestions. You can find below an itemized, point-by-point response to the reviewer's comments.

Also, the paper has been carefully revised for language by our English native speaker editorial assistant. If the manuscript language should not meet your requirements, we will request editing services provided by the suggested companies.

Thank you very much for your cooperation.

We look forward to hearing from you.

Best regards,

Umberto Bracale

Comments to Authors	Answers
<b>Reviewer 1</b>	
Dear authors, Thank you for a thorough review of a topic of interest to every colorectal surgeon. The review is well written and does not need any revision	We thank the reviewer for his appreciation of our review.
<b>Reviewer 2</b>	
This is an interesting systematic review aiming to assess risk factors for anastomotic leakage in laparoscopic colorectal resection. There has been a great deal of interest on this topic and much effort has been devoted to evaluating the predictive factors	We thank the reviewer for his evaluation of our work. We appreciate him for highlighting this limitation with the manuscript and for providing suggestions to enhance the paper. Including information about the suggested issues is

<p>of the devastating complication. This review article seems useful to update our knowledge of the published evidence concerning this topic. Nonetheless, I would prefer to be informed about potentially important issues as follows, which may be difficult to be addressed by the papers selected systematically in this review.</p>	<p>useful to make the review more thorough and of interest to every laparoscopic colorectal surgeon.</p>
<p>#1 Is intraoperative assessment of perfusion at the site of anastomosis, such as indocyanine green fluorescence angiography, associated with a reduced risk of anastomotic leakage?</p>	<p>Intraoperative assessment of perfusion at the site of anastomosis with indocyanine green (ICG) fluorescence angiography has been increasingly considered a potential intraoperative tool that could be used to ensure adequate perfusion. Most published studies focused on the change of surgical strategy (site of resection and/or anastomosis) due to the subjective recording of hypoperfusion after ICG. Across the studies, there is some initial evidence that ICG fluorescence angiography may reduce the incidence of AL. In the paper, this issue has been addressed as follows:</p> <p><u>Indocyanine green fluorescence angiography</u></p> <p><i>Intraoperative assessment of perfusion at the site of anastomosis with indocyanine green (ICG) has been increasingly considered a potential intraoperative tool that could be used to ensure adequate perfusion, possibly leading to a reduction in the AL rate. Most published studies focused on the change of surgical strategy (site of resection and/or anastomosis) due to the subjective recording of hypoperfusion after ICG fluorescence angiography (FA). However, its capacity to reduce AL incidence needs to be confirmed in large RCTs. Boni et al<sup>[61]</sup> compared 42 patients undergoing LAR with ICG angiography to a historical control group of 38 patients operated on without the use of angiography. No clinically relevant leaks were observed in the FA group, whereas two AL were reported in the case-matched group. This difference is not likely to be statistically significant due to the limited number of patients analyzed. Jafari et al<sup>[62]</sup> published a prospective multicenter clinical trial including 139 patients who had undergone</i></p>

	<p><i>laparoscopic left-sided colectomy and anterior resection. The overall AL rate was 1.4%. FA changed surgical plans in 11 (7.9%) patients, with the majority of changes occurring at the time of transection of the proximal margin (7%). No AL was recorded amongst this subgroup of patients. In a prospective single-institution study of 68 patients undergoing laparoscopic resection for left-sided colorectal cancers, AL occurred in 16.7% of the poor perfusion group based on ICG fluorescence imaging, whereas none of the patients in the good perfusion group had AL. When further focusing on LAR, the AL rate was 10.7%. Leak occurred in 30% of the poor perfusion group, whereas no leak took place in the good perfusion group<sup>[63]</sup>.</i></p> <p><i>Ris et al<sup>[90]</sup> recently conducted a prospective phase II study of 504 patients undergoing elective bowel resection of which 85.3% were operated on by laparoscopy. The overall leak rate for colorectal operations not involving ICG fluorescence was 5.8%, compared with 2.6% with the use of ICG imaging (P = 0.009). Statistical significance was confirmed for left-sided resections (6.9% vs 2.6%, P = 0.005) and for LARs alone (10.7 vs 3%), but not for right-sided operations (2.6% vs 2.8%, P = 0.928).</i></p>
<p>#2 Recently, there has been several studies for intracorporeal anastomosis in laparoscopic surgery of the right colon. Is there any difference in the incidence of leakage between intracorporeal and extracorporeal anastomosis?</p>	<p>Laparoscopic right colectomy with intracorporeal anastomosis (IA) is reported to have some benefits in terms of enhanced postoperative recovery in comparison with laparoscopic-assisted right colectomy with extracorporeal anastomosis (EA). Published studies suggest that both approaches appear to achieve similar results in terms of AL occurrence. In the paper, this issue has been addressed as follows:</p>

### Intracorporeal anastomosis

Laparoscopic right colectomy with intracorporeal anastomosis (IA) is reported to have some benefits in terms of enhanced postoperative recovery in comparison with laparoscopic-assisted right colectomy with extracorporeal anastomosis (EA)<sup>[25]</sup>. Both approaches appear to achieve similar results in terms of AL occurrence. Definitive conclusions are difficult to draw, however due to the nature of the published studies and the heterogeneity of surgical techniques used in fashioning the EA, including both manual, totally-stapled, and stapled-manual<sup>[26]</sup>. Vignali et al<sup>[26]</sup> published an interim analysis of the first RCT analyzing the role of intracorporeal stapled versus extracorporeal stapled anastomosis following laparoscopic right colectomy using a standardized approach. In their series of 60 patients (30 EA vs 30 IA) no significant difference was observed between the two groups with respect to AL (6.6% in the IA group versus 0% in the EA group,  $P = 0.39$ ). In the largest multicenter study comparing IA and EA for 512 right-sided colorectal cancers, the incidence of leak or dehiscence was 4.19% (12 patients) in the IA group and 5.50% (12 patients) in the EA group ( $P = 0.53$ )<sup>[3]</sup>. Similarly, in a case-matched study, Vignali et al<sup>[27]</sup> compared the outcomes of IA (64 patients) versus EA (64 patients) in an obese population ( $BMI > 30 \text{ kg/m}^2$ ). Clinically evident anastomotic leaks occurred in 4.7% of the patients in the IA group vs. 7.8% in the EA group ( $P = 0.71$ ). Also, in a retrospective multicentric comparative study including 195 patients, multivariate analysis revealed a trend towards lower risk of clinically AL (requiring percutaneous or operative intervention) with IA that failed to reach statistical significance (adjusted OR 0.29,  $P > 0.05$ )<sup>[28]</sup>. Other retrospective series found no significant differences in incidence of anastomotic leaks between the two techniques<sup>[29-32]</sup>. With regards to

	<p><i>IA, a single-centre retrospective series of 162 patients found that double-layer closure of enterotomy was associated with a significantly lower incidence of AL compared to single-layer closure (1.2% in DL vs 7.8% in SL, <math>p = 0.044</math>) after mechanical ileocolic anastomosis [33].</i></p>
<p>#3 Some harmful species or strains of the gut microbiota may be implicated in the pathogenesis of leakage. Is there any clinical evidence to support the hypothesis?</p>	<p>Some experimental studies have implicated intraluminal microbes as causative agents in pathogenesis of AL although extensive clinical evidence on the impact of gut microbiota on postoperative anastomotic complications is lacking. Two studies have been conducted in patients undergoing colorectal surgery. Moreover, recent data suggest that use of oral antibiotics in preoperative bowel preparation could lower the incidence of AL after colorectal surgery. This finding further supports a role of the gut microbioma in anastomotic integrity. In the paper, this issue has been addressed as follows:</p> <p><u>Gut microbiota</u></p> <p><i>Intestinal flora near the anastomotic site has been proposed to interact with intestinal tissue and likely affects intestinal healing<sup>[10]</sup>. Some experimental studies suggest that cues released by surgically injured tissues can lead to phenotype transformation of intraluminal microbes, turning them into pathogens. These may play a causative role in the development of AL by increased collagenase production and activation of host metalloproteinase-9<sup>[72]</sup>. Nonetheless, extensive clinical evidence on the impact of gut microbiota on postoperative anastomotic complications is lacking<sup>[73]</sup>. A pilot study compared the intestinal microbiota of 8 patients who had developed AL with 8 matched patients with healed circular stapled colorectal anastomoses without any clinical signs of AL<sup>[74]</sup>. The abundance of the Lachnospiraceae family was found to be significantly higher in patients who had developed AL when compared to patients who had not (<math>P = 0.001</math>),</i></p>

	<p><i>while microbial diversity levels were higher in the latter group (P = 0.037). Also, BMI was positively associated with the abundance of the Lachnospiraceae family (P = 0.022). The same study group further investigated the role of gut microbiota in the development of AL in a series of 123 “donuts” of patients where a stapled colorectal anastomosis was made<sup>[75]</sup>. In 63 patients this anastomosis was covered with a C-seal; a bioresorbable sheath stapled to the anastomosis. In the group of non-C-seal samples a high abundance of Lachnospiraceae and Bacteroidaceae and lower microbial diversity were confirmed to be strongly associated with AL. A bacterial composition that consisted of 60% or more of these 2 families seemed to be predictive for AL. On the contrary, other species such as Prevotella copri and the Streptococcus genus were both negatively associated with AL. The authors speculated that a disturbed microbial composition which is more easily associated with low microbial diversity<sup>[10]</sup> due to preoperative or surgical processes, may affect the metabolic balance and lack colonization resistance to pathogenic bacteria that could play a role in the development of AL. In C-seal patients where AL rates were slightly higher, it seemed that any potential protective benefits or harmful consequences of the gut microbiota composition were negated, as progression to AL was independent of the dominant bacterial composition before surgery. These observations suggested that the C-seal influences the microbial composition after introduction and that this may ultimately impair anastomotic healing.</i></p>
<b>Reviewer 3</b>	
Thank you for the opportunity to review this paper. Overall, it is a review of factors for anastomotic leakage (AL) after laparoscopic colorectal surgery. Much effort has been made to prevent and reduce the leakage rate in colorectal surgery. Several risk factors for AL have been reported, yet the cause and ideal	We thank the reviewer for his evaluation of our work and for providing suggestions to enhance the paper.

methods for prevention of AL remain controversial and unclear. I have some suggestions:	
In left sided anastomosis, it should be of interest to distinguish outcomes between colon and rectal surgery	We agree with the reviewer that it would be of interest to distinguish outcomes between colon and rectal surgery in left-sided anastomoses. However, the included studies did not allow to make this distinction as data about left colectomies/sigmoid resections are reported together with those of either rectal resections or all other colonic localizations.
Regarding factors influencing AL, some significant issues are lacking: the use of abdominal or pelvic drainage;	<p>Many studies have assessed the interest of drainage after colorectal surgery and confirmed its ineffectiveness after colonic procedure, whereas, to date, the effect of pelvic drain after rectal excision remains controversial. The evidence to support its use is low. In the paper, this issue has been addressed as follows:</p> <p><u>Pelvic drainage</u></p> <p><i>Routine prophylactic drainage after colorectal anastomoses is debatable and the evidence to support its use is low<sup>[69]</sup>. A recent RCT analyzed 469 patients who underwent rectal resection with intraperitoneal anastomosis, of whom 93.6% were operated on by laparoscopy. There was no significant difference in terms of pelvic sepsis between drained and nondrained patients, either during hospital stay or at 30 days after surgery (16.1% vs 18.0%, <math>P = 0.58</math>). Early (&lt;5 days) versus late (&gt;5 days) pelvic drain removal did not affect significantly the risk of pelvic sepsis (11.6% vs 18.6%, <math>P = 0.122</math>)<sup>[70]</sup>. Two retrospective studies found pelvic drainage associated with lower rates of AL after LAR, though without reaching statistical significance. Kawada et al<sup>[42]</sup> reported AL in 10.8% of drained patients versus 20.8% of non-drained patients (<math>P = 0.18</math>) in a series of 154 low LARs without diverting stoma. Similarly, in a series of 363 LARs, 2.6% of drained patients had clinical AL compared to 6.3% of non-</i></p>



	<p><i>drained patients (<math>P = 0.11</math>). Nonetheless lack of pelvic drain was found to be independently predictive (<math>P = 0.0225</math>, <math>OR = 3.814</math>) of leakage at a multivariate analysis<sup>[49]</sup>. Pelvic drain may prevent hematomas or seromas that constitute a fertile medium for bacteria and may promote infection which can involve the anastomosis thereby causing dehiscence. Moreover, pelvic drain may help control leaks if they do take place, leading to a less severe clinical course<sup>[71]</sup>.</i></p>
the role of defunctioning stoma in rectal surgery;	<p>Although evidence regarding the clinical benefit of fecal diversion is conflicting, it is generally agreed that creation of a diverting stoma (DS) can reduce the clinically adverse effects of AL, including fecal peritonitis and septicemia, rather than preventing leakage. Indeed, several studies did not find any difference between DS groups and no-DS group in terms of AL incidence. However, DS may reduce the occurrence of AL in high-risk patients. In addition, among TaTME registry cases, significantly more patients that did not have a defunctioning stoma developed early symptomatic AL compared with those that were defunctioned. In the paper, this issue has been addressed as follows:</p> <p><u><i>Diverting stoma</i></u></p> <p><i>Although evidence regarding the clinical benefit of fecal diversion is conflicting, it is generally agreed that creation of a diverting stoma (DS) can reduce the clinically adverse effects of AL, including fecal peritonitis and septicemia, rather than preventing leakage. In a retrospective series of 69 patients undergoing LAR<sup>[36]</sup>, no significant difference between DS groups and no-DS group in terms of AL incidence (15.4% versus 16.3%) was noted. Although AL was observed in four patients in the DS group, none of them developed AL grade C. In contrast, 57.1% (4/7 cases) of the patients in the no-DS</i></p>

	<p>group developed AL grade C, but this difference did not reach statistical significance<sup>[36]</sup>.</p> <p>In the series from Park et al<sup>[34]</sup> (1609 patients) defunctioning stoma did not significantly reduced risk of AL (OR = 0.649, P = 0.154 at multivariate analysis). Similarly, in a series of 363 LARS, the incidence of AL was 4.8% in patients with covering stoma versus 3.3% in patients without stoma (P = 0.4718)<sup>[49]</sup> . Other studies reported similar findings <sup>[37, 41]</sup>.</p> <p>In a series of 296 low LARs for cancer<sup>[35]</sup>, AL was observed in 5.5% of patients with DS and in 8.7% of patients without DS (OR = 0.60, P = 0.4243 at univariate analysis). Based on the two risk factors (sex and anal verge distance) patients were stratified according to risk for AL occurrence. The incidence of AL was 8.1% in the overall population compared to 23% in high-risk patients (males with tumors less or equal than 7 cm from the anal verge). Within this group, diverting stoma creation significantly reduced the AL rate (P = 0.0363) as the rate of AL occurrence was 10.7% in patient for whom a DS was created compared to 33.3% in patients without a DS. The occurrence of AL in the low-risk group was not influenced by DS creation (P = 0.2443). Based on the findings of this study, DS may help prevent the occurrence of AL in a high-risk population.</p>
the impact of immunofluorescence in colorectal surgery;	<p>Intraoperative assessment of perfusion at the site of anastomosis with indocyanine green (ICG) fluorescence angiography has been increasingly considered a potential intraoperative tool that could be used to ensure adequate perfusion. Most published studies focused on the change of surgical strategy (site of resection and/or anastomosis) due to the subjective recording of hypoperfusion after ICG. Across the studies, there is some initial evidence that ICG fluorescence angiography may reduce the incidence of AL. In the paper, this issue has been addressed as follows:</p>

#### Indocyanine green fluorescence angiography

Intraoperative assessment of perfusion at the site of anastomosis with indocyanine green (ICG) has been increasingly considered a potential intraoperative tool that could be used to ensure adequate perfusion, possibly leading to a reduction in the AL rate. Most published studies focused on the change of surgical strategy (site of resection and/or anastomosis) due to the subjective recording of hypoperfusion after ICG fluorescence angiography (FA). However, its capacity to reduce AL incidence needs to be confirmed in large RCTs. Boni et al<sup>[61]</sup> compared 42 patients undergoing LAR with ICG angiography to a historical control group of 38 patients operated on without the use of angiography. No clinically relevant leaks were observed in the FA group, whereas two AL were reported in the case-matched group. This difference is not likely to be statistically significant due to the limited number of patients analyzed. Jafari et al<sup>[62]</sup> published a prospective multicenter clinical trial including 139 patients who had undergone laparoscopic left-sided colectomy and anterior resection. The overall AL rate was 1.4%. FA changed surgical plans in 11 (7.9%) patients, with the majority of changes occurring at the time of transection of the proximal margin (7%). No AL was recorded amongst this subgroup of patients. In a prospective single-institution study of 68 patients undergoing laparoscopic resection for left-sided colorectal cancers, AL occurred in 16.7% of the poor perfusion group based on ICG fluorescence imaging, whereas none of the patients in the good perfusion group had AL. When further focusing on LAR, the AL rate was 10.7%. Leak occurred in 30% of the poor perfusion group, whereas no leak took place in the good perfusion group<sup>[63]</sup>.

Ris et al<sup>[90]</sup> recently conducted a prospective phase II study of 504 patients undergoing elective bowel resection of which 85.3% were operated on by laparoscopy. The overall leak rate for colorectal operations not involving ICG fluorescence was 5.8%, compared with

	<p>2.6% with the use of ICG imaging (<math>P = 0.009</math>). Statistical significance was confirmed for left-sided resections (6.9% vs 2.6%, <math>P = 0.005</math>) and for LARs alone (10.7 vs 3%), but not for right-sided operations (2.6% vs 2.8%, <math>P = 0.928</math>).</p>
<p>manual vs stapled anastomosis, and intracorporeal anastomosis in right sided resections;</p>	<p>Laparoscopic right colectomy with intracorporeal anastomosis (IA) is reported to have some benefits in terms of enhanced postoperative recovery in comparison with laparoscopic-assisted right colectomy with extracorporeal anastomosis (EA). Published studies suggest that both approaches appear to achieve similar results in terms of AL occurrence. Definitive conclusions are difficult to draw, however due to the nature of the published studies and the heterogeneity of surgical techniques used in fashioning the EA, including both manual, totally-stapled, and stapled-manual. In the paper, this issue has been addressed as follows:</p> <p><u>Intracorporeal anastomosis</u></p> <p><i>Laparoscopic right colectomy with intracorporeal anastomosis (IA) is reported to have some benefits in terms of enhanced postoperative recovery in comparison with laparoscopic-assisted right colectomy with extracorporeal anastomosis (EA)<sup>[25]</sup>. Both approaches appear to achieve similar results in terms of AL occurrence. Definitive conclusions are difficult to draw, however due to the nature of the published studies and the heterogeneity of surgical techniques used in fashioning the EA, including both manual, totally-stapled, and stapled-manual<sup>[26]</sup>. Vignali et al<sup>[26]</sup> published an interim analysis of the first RCT analyzing the role of intracorporeal stapled versus extracorporeal stapled anastomosis following laparoscopic right colectomy using a standardized approach. In their series of 60 patients</i></p>

	<p>(30 EA vs 30 IA) no significant difference was observed between the two groups with respect to AL (6.6% in the IA group versus 0% in the EA group, <math>P = 0.39</math>). In the largest multicenter study comparing IA and EA for 512 right-sided colorectal cancers, the incidence of leak or dehiscence was 4.19% (12 patients) in the IA group and 5.50% (12 patients) in the EA group (<math>P = 0.53</math>)<sup>[31]</sup>. Similarly, in a case-matched study, Vignali et al<sup>[27]</sup> compared the outcomes of IA (64 patients) versus EA (64 patients) in an obese population (<math>BMI &gt; 30 \text{ kg/m}^2</math>). Clinically evident anastomotic leaks occurred in 4.7% of the patients in the IA group vs. 7.8% in the EA group (<math>P = 0.71</math>). Also, in a retrospective multicentric comparative study including 195 patients, multivariate analysis revealed a trend towards lower risk of clinically AL (requiring percutaneous or operative intervention) with IA that failed to reach statistical significance (adjusted OR 0.29, <math>P &gt; 0.05</math>)<sup>[28]</sup>. Other retrospective series found no significant differences in incidence of anastomotic leaks between the two techniques<sup>[29-32]</sup>. With regards to IA, a single-centre retrospective series of 162 patients found that double-layer closure of enterotomy was associated with a significantly lower incidence of AL compared to single-layer closure (1.2% in DL vs 7.8% in SL, <math>p = 0.044</math>) after mechanical ileocolic anastomosis <sup>[33]</sup>.</p>
influence of oral antibiotic in bowel preparation;	<p>Recent studies suggest that use of oral antibiotics in preoperative bowel preparation could lower the incidence of AL after colorectal surgery. However, data on the impact of this measure in patients specifically undergoing minimally invasive surgery are still limited. In the paper, this issue has been addressed as follows:</p>

	<p><u>Oral antibiotics</u></p> <p>Recent studies<sup>[85, 86]</sup> suggest that use of oral antibiotics in preoperative bowel preparation could lower infectious complications and also incidence of AL after colorectal surgery. This finding further supports a role of the gut microbiota in anastomotic integrity<sup>[67]</sup>. However data on the impact of this measure in patients specifically undergoing minimally invasive colorectal surgery are still limited<sup>[86]</sup>. In a retrospective ACS-NSQIP database analysis, in which 5291 (62.5%) patients underwent minimally invasive surgery, oral antibiotic preparation was associated with lower rates of SSI and AL for both minimally invasive and open cohorts<sup>[87]</sup>. A recent RCT by Hata et al<sup>[88]</sup> revealed that patients undergoing laparoscopic colorectal procedures for cancer had a lower incidence of overall SSIs (7.3% versus 12.8%, OR = 0.536, P = 0.028) when receiving oral antibiotic prophylaxis in addition to mechanical bowel preparation. However, incidence of organ/space infection was comparable to that of patients receiving mechanical bowel preparation and intravenous prophylaxis where 6/290 (2.1%) leaks took place in the IV group compared to 5/289 (1.7%) in the oral-IV group. In another single-center RCT including 515 colorectal cancer patients undergoing elective laparoscopic resection, intravenous perioperative antimicrobial prophylaxis alone was not inferior to combined pre-operative oral and intravenous perioperative prophylaxis with regards to SSI. AL was observed in 2.5 % of the IV-only group and in 1.2% of the oral-IV group (OR = 2.01, P = 0.504). The authors speculated that the study was evidently underpowered to provide any conclusions regarding the contribution of oral microbial prophylaxis in reducing AL<sup>[89]</sup>.</p>
gut microbiota or even intraoperative leak management;	<p>Some experimental studies have implicated intraluminal microbes as causative agents in pathogenesis of AL although extensive clinical evidence on the impact of gut microbiota on postoperative anastomotic complications is lacking. Two studies have been conducted in patients undergoing colorectal</p>

surgery. Moreover, recent data suggest that use of oral antibiotics in preoperative bowel preparation could lower the incidence of AL after colorectal surgery. This finding further supports a role of the gut microbioma in anastomotic integrity. In the paper, this issue has been addressed as follows:

#### Gut microbiota

*Intestinal flora near the anastomotic site has been proposed to interact with intestinal tissue and likely affects intestinal healing<sup>[10]</sup>. Some experimental studies suggest that cues released by surgically injured tissues can lead to phenotype transformation of intraluminal microbes, turning them into pathogens. These may play a causative role in the development of AL by increased collagenase production and activation of host metalloproteinase-9<sup>[72]</sup>. Nonetheless, extensive clinical evidence on the impact of gut microbiota on postoperative anastomotic complications is lacking<sup>[73]</sup>. A pilot study compared the intestinal microbiota of 8 patients who had developed AL with 8 matched patients with healed circular stapled colorectal anastomoses without any clinical signs of AL<sup>[74]</sup>. The abundance of the Lachnospiraceae family was found to be significantly higher in patients who had developed AL when compared to patients who had not ( $P = 0.001$ ), while microbial diversity levels were higher in the latter group ( $P = 0.037$ ). Also, BMI was positively associated with the abundance of the Lachnospiraceae family ( $P = 0.022$ ). The same study group further investigated the role of gut microbiota in the development of AL in a series of 123 “donuts” of patients where a stapled colorectal anastomosis was made<sup>[75]</sup>. In 63 patients this anastomosis was covered with a C-seal; a bioresorbable sheath stapled to the anastomosis. In the group of non-C-seal samples a high abundance of Lachnospiraceae and Bacteroidaceae and lower microbial diversity were confirmed to be strongly associated with AL. A bacterial composition that consisted of 60% or more of these 2 families seemed to be predictive for AL. On the*

	<p><i>contrary, other species such as Prevotella copri and the Streptococcus genus were both negatively associated with AL. The authors speculated that a disturbed microbial composition which is more easily associated with low microbial diversity<sup>[10]</sup> due to preoperative or surgical processes, may affect the metabolic balance and lack colonization resistance to pathogenic bacteria that could play a role in the development of AL. In C-seal patients where AL rates were slightly higher, it seemed that any potential protective benefits or harmful consequences of the gut microbiota composition were negated, as progression to AL was independent of the dominant bacterial composition before surgery. These observations suggested that the C-seal influences the microbial composition after introduction and that this may ultimately impair anastomotic healing.</i></p> <p>After a careful search, we did not find relevant studies to substantially address intraoperative leak management.</p>
<p>It should also be of interest in the review (avoiding multiple linear stapler firings in rectal surgery) recent data about AL in transanal rectal surgery (TaTME Registry).</p>	<p>TaTME is being implemented into clinical practice in order to overcome the technical drawbacks and limitations of standard laparoscopic TME with intracorporeal anastomosis. Recent data from the TaTME registry have been reported. AL rates as well as risk factors for leak seem to be comparable between both techniques. In TaTME patients, anastomotic technique (manual versus stapled) was not identified as a risk factor for early AL, although the manual technique significantly increased the risk of late stricturing. In the paper, this issue has been addressed as follows:</p> <p><u>Transanal TME (TaTME)</u></p> <p><i>TaTME represents the latest advanced surgical access technique for pelvic dissection and anastomosis during rectal resection and is being implemented in clinical practice in order to overcome the technical drawbacks and limitations of standard laparoscopic TME<sup>[50]</sup>. For</i></p>



instance, the distal rectal transection does not involve multiple stapler firings and therefore eliminates this potential risk factor for leakage. Recently, Penna et al<sup>[50]</sup> analyzed 1594 TaTME cases with an anastomosis recorded on the international TaTME registry<sup>[51]</sup>. The overall anastomotic failure rate was 15.7%. This included early (within 30-days; 7.8%) and delayed (after 30-days; 2.0%) leak, pelvic abscess (4.7%), anastomotic fistula (0.8%), chronic sinus (0.9%), and anastomotic stricture in 3.6% of cases. Of 250 patients diagnosed with anastomotic failure, 219 had a defunctioning stoma created at the index operation. The reported early leak rate of 7.8% was higher than the previously published rate of 5.4% in the initial 720 registry cases<sup>[52]</sup>. The authors suggested that this value could be explained by an increased complexity of cases performed transanally, wider adoption of TaTME by surgeons at the start of their learning curve, or improved recording and reporting of adverse events on the registry. Nonetheless, the leak rate was comparable to previously reported incidences in colorectal surgery. Upon multivariate analysis, male sex, obesity, smoking, diabetes, larger tumors (>25 mm maximum diameter), tumor height > 4 cm from anorectal junction on magnetic resonance imaging, and intraoperative blood loss of  $\geq 500$  mL were risk factors for early AL. These factors are similar to those identified in previous studies on laparoscopic rectal resections. Significantly more cases that did not have a defunctioning stoma developed early symptomatic AL compared with those that were defunctioned (12.4% vs. 7.2%, OR = 0.547, P = 0.015). However, the presence of a defunctioning stoma did not appear to significantly influence incidence of anastomotic failure in this cohort. Anastomotic technique (manual versus stapled) was not identified as a risk factor for early AL, although the manual technique significantly increased the risk of late stricturing. A few published studies have compared laparoscopic and transanal TME with respect to AL rates. A RCT including 100 patients found a leak rate of 2% in the transanal group compared to 10% in the laparoscopic group, without a significant difference (P =

	<p>0.204)<sup>[53]</sup>. Other retrospective matched case-control trials did not find any statistically significant difference in terms of AL rates between the two approaches<sup>[54-57]</sup>. Results from the recently commenced RCTs comparing TaTME with laparoscopic TME may provide some robust data in the future<sup>[58, 59]</sup>.</p>
<p>You must include some comments about the limitations of the review (ie, the absence of randomized controlled trials should introduce some bias).</p>	<p>Some limitations of this review have to be addressed, including retrospective nature of the included studies; different definitions of AL across the studies; heterogeneity in terms of type of patients, study period, and operative practice; small sample size of some studies. In the paper, this issue has been addressed as follows:</p> <p><b><i>LIMITATIONS</i></b></p> <p><i>Some limitations of this study have to be addressed. The major limitation lies in the retrospective nature and consequent lack of randomization of the included studies, that may lead to patient and surgeon selection bias. Second, different definitions of AL were used across the studies, which is a general problem in the literature dealing with this postoperative complication. Moreover, some series are heterogeneous in terms of type of patients, study era, surgical technique, and perioperative practice. The variable presence of diverting stoma across studies dealing with rectal resections should also be considered. Finally, some studies have relatively small sample size.</i></p>
<p>Some grammatical and syntax errors should be corrected.</p>	<p>The paper has been carefully revised for language by our English native speaker editorial assistant.</p>

