Experimental Study of Mechanical Integrity Testing in Stapled Large Bowel: Methylene Blue Leak Test Is Not Inferior to Air Leak Test

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Keywords

Colon · Anastomosis · Stapling · Anastomotic leak · Porcine colon · Air leak test · Methylene blue test

Abstract

Background/Objectives: Anastomotic leakage remains the most devastating postoperative complication in colorectal surgery. The mechanical integrity of the newly formed colorectal anastomosis can be evaluated by visual inspection intraoperatively; both air leak and liquid leak tests are also used to evaluate the integrity of stapled colorectal anastomoses. It is not clear whether double-stapled anastomoses are more prone to leaks than single-stapled anastomoses. The aim of our study was to compare the methylene blue and the air leak test in the experimental setting of single-stapled and double-stapled porcine bowels. **Methods:** Twenty-four distal colons were excised from slaughtered pigs without delay. The proximal bowel end was closed with a linear stapler using blue cartridges. The bowels were randomly divided into single-stapled or double-stapled groups. Air leak and methylene blue leak tests were performed. A digital pressure monitor with a gradual pressure increase function was used to both gradually increase pressure within the bowel and to determine the pressure at which the stapler line disintegrated. **Results:** Air leakage occurred at a

mean pressure of 51.62 (± 16.60) mm Hg and methylene blue leakage occurred at 46.54 (± 16.78) mm Hg ($p = 0.31$). The air and methylene blue leaks occurred at comparable pressures in single-stapled bowels and in double-stapled bowels (47.21 [± 14.02] mm Hg vs. 50.96 [± 19.15] mm Hg, $p = 0.6$).

Conclusions: The methylene blue solution leak test is not inferior to the air leak test. There is no significant difference in bursting pressure between single-stapled and double-stapled anastomoses.

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Introduction

Anastomotic leakage (AL) remains the most devastating postoperative complication in colorectal surgery. It results in significant morbidity and mortality, prolonged hospital stay, and increased costs and demands on health services [1, 2]. There is an inevitable interplay between patient physiology and technical factors that predispose a patient to AL occurrence [3]. The AL rate after anterior resection varies considerably among published reports and ranges from 3 to 23% [4].

The anastomotic technique plays an important role in AL rates [5]. Most of the low colorectal anastomoses are stapled, as suturing in the low pelvis is difficult or even

Fig. 1. Double-stapled colon.

impossible. Adequate blood supply of the bowel ends, as well as tension-free and mechanically impermeable (water-tight) anastomoses, are prerequisites for good anastomotic healing. The mechanical integrity of a newly formed colorectal anastomosis can be evaluated by visual inspection intraoperatively; however, this is impossible in most low colorectal anastomoses.

An air leak test of mechanical integrity was proposed immediately after the first description of stapled colorectal anastomosis [6]. It remains the test of choice for an overwhelming majority of surgeons who test anastomoses. Other tests, such as intraoperative sigmoidoscopy (which could also be described as a type of air leak test) and the methylene blue leak test, are occasionally being used [7–14]. We found 5 studies where the mechanical integrity of colorectal anastomosis was checked by methylene blue, Patent Blue dye, or intraluminal saline [11, 12, 15–17]. Our own recent experience suggested that the methylene blue leak test was positive in 20% of patients after a negative air leak test [18]. This was seen mostly in the area of intersection of two staple lines.

The aim of our study was to compare the methylene blue and the air leak test in the experimental setting of single-stapled and double-stapled porcine bowels.

Materials and Methods

Twenty-four distal colons were excised from slaughtered pigs without delay. All further manipulations were performed within 20 min from excision to prevent tissue degradation. All specimens were randomly divided into four groups. The proximal large bowel edge was separated from the mesentery at the stapler transection point. The proximal bowel end was closed with a linear stapler using blue cartridges (ECHELON FLEX GST, Ethicon, 60 mm blue reload with gripping surface technology, closed –1.5 mm, open –3.6 mm, 60 mm staple line). Each bowel was compressed for 15 s using the stapler.

The bowels were randomly divided into single-stapled or double-stapled groups. The proximal end was closed with a single cartridge or with two cartridges (Fig. 1), depending on the group. Therefore, single-stapled bowels and double-stapled bowels were created. The flowchart of the study is presented in Figure 2.

After that, air leak and methylene blue leak tests were performed. The experimental model described by Schwab et al. [19] for bursting strength measurement was used. A digital pressure monitor with a gradual pressure increase function (BioTek® Instruments, Inc.) was used to both gradually increase pressure within the bowel and to determine the pressure at which the stapler line disintegrated.

A Foley catheter was introduced intraluminally from the distal end. After that, the distal end was occluded around the catheter to prevent the escape of air or methylene blue solution. The bowels were submerged in water to identify leaks. The Foley catheter was connected to the water-tight container. A 0.9% sodium chloride solution, dyed with methylene blue, was used to test the bursting strength of half of the stapled bowels in the experiment. A water-tight container filled with air was used for the other half of the stapled bowels in the experiment. The water-tight container was attached to the digital pressure monitor with the gradual pressure increase function. The pressure within the container was increased gradually by 4 mL with each stroke of airflow until leakage of air or of methylene blue dye was noted. The pressure at which the leak occurred was recorded.

In order to ascertain whether different pressures are generated within the bowel by injecting the same quantity of methylene blue and air, the experimental system was modified by adding an additional Foley catheter at the free end of the bowel and connecting it to a syringe. A digital pressure manometer was used only to measure the pressure within the lumen of the bowel. The first round of testing included 400 mL of air injection into the bowel. The second round of measurement on the same bowel was performed using 400 mL of methylene blue solution. During both tests, the maximum pressure achieved was recorded.

Statistical Analysis

Statistical analyses were performed using the R statistical software package version 3.6.1 (© The R Foundation for Statistical Computing), RStudio version 1.2.1335 (© 2009–2019; RStudio, Inc.), IBM SPSS Statistics version 23, and G*Power version 3.1.9.4 (Universität Düsseldorf, Germany).

Interval and ratio variables are described by means and SD, medians, and median absolute deviations (MAD), as well as first (Q1) and third (Q3) quartiles. The Shapiro-Wilk test was used to check for data normality. The statistically significant relationship between two independent groups was tested using the Mann-Whitney U test. Once, statistically significant dependencies between groups were determined for rank variables, or when the normality assumption for our data was not satisfied, the Spearman correlation coefficient and Cliff's delta effect size were used to assess the strength of the relationships. The effect size of the Kruskal-Wallis, Mann-Whitney, or Wilcoxon test was calculated using the Rosenthal (1994) formula (Cohen's *d* alternative). Hedges' *g* is recommended rather than Cohen's *d* effect size for very small sample sizes (<20).

Boxplots (left sides of the figures) were used for graphical comparison of the data. In addition, the means in the groups and the overall mean (longer dash) are shown on the right side of the figures.

The relationship between groups was rated as statistically significant when the *p* value was <0.05 and the power of the statistical tests was $1 - \beta = 0.95$.

Fig. 2. Experimental study flowchart.

Fig. 3. Histograms (left) and Q-Q plots (right) for the air and methylene blue dye groups.

Results

Thirty-six stapler cartridges were used to create 24 experiments: 12 in the single-stapled and 12 in the double-stapled groups. All 24 experiments were successful. The differences between air leak and methylene blue leak pressures are presented in Figures 3 and 4 and in Table 1. The Shapiro-Wilk normality test result for air samples was $W = 0.89, p = 0.1 (>0.05)$, and that for methylene blue dye samples was $W = 0.85, p = 0.03 (<0.05)$.

A p value >0.05 implies that the distribution of the data is not significantly different from the normal distribution. In other words, we can assume normality. However, we can see from the graph (especially from the density graph; Fig. 3) that the condition of normality is not satisfied. The differences in stapled bowel bursting pressures between the methylene blue leak and the air leak test group are presented in Figure 4 and Table 1.

The observed difference between the two methods in detecting stapled bowel leaks was not statistically significant.

Fig. 4. Box plots of bowel bursting pressure (mm Hg) in the air leak and methylene blue leak test groups.

Table 1. Descriptive and quantitative statistics for stapled bowel bursting pressure (mm Hg) in the methylene blue leak and air leak test groups

	Air leak test group	Methylene blue leak test group
Mean (SD)	51.62 (16.60)	46.54 (16.78)
Median (MAD)	49.75 (18.16)	41.50 (11.12)
[Q1;Q3]	[37.00;54.00]	[33.00;46.00]
[Min.;max.]	[34.00;84.00]	[30.00;82.00]
<i>p</i> value (Mann-Whitney U test)	0.312 No statistically significant dependence was found	
Spearman's correlation coefficient/ <i>p</i> value	0.653 No statistically significant correlation was found	
Cohen's <i>d</i> effect size	0.3044	
Hedges' <i>g</i> effect size	0.2941	
Cliff's delta effect size	0.2431	
Rosenthal effect size	0.2087	

cant. The effect size can make up for the weak point by providing information on the actual effect, which is independent of the sample size. In our case, we had a clinically significant – though small – dependency. The same can be said about the correlation coefficient. We calculated that if the same difference between the groups persisted, it would be significant with a sample size of 299, as calculated by the Rosenthal (1994) power size.

The differences between air leak and methylene blue leak pressures in single-stapled bowels are presented in Table 2 and Figure 5. The differences between air leak and methylene blue leak pressures in double-stapled bowels are presented in Table 3 and Figure 6. Overall, we found

that the methylene blue leak test was not inferior to the air leak test.

Four additional bowels were used to test the pressure created by injection of air and methylene blue solution. There were no significant differences between created pressures after injecting 400 mL of air or methylene blue solution into the leak-proof system (Table 4; Fig. 7).

We found that there was no significant difference in burst pressure between single-stapled and double-stapled anastomoses (47.21 [±14.02] mm Hg vs. 50.96 [±19.15] mm Hg, $p = 0.6$). The comparison of bursting pressures between single-stapled and double-stapled bowels is presented in Figure 8.

Fig. 5. Box plots of air leak and methylene blue pressure (mm Hg) in single-stapled bowels.

Table 2. Descriptive and quantitative statistics for bowel leak pressures (mm Hg) in single-stapled bowels

	Air leak test group	Methylene blue leak test group
Mean (SD)	48.58 (11.62)	45.83 (17.13)
Median (MAD)	49.75 (11.86)	38.50 (10.38)
[Q1;Q3]	[38.00;52.50]	[33.00;39.00]
[Min.;max.]	[34.00;66.00]	[30.00;69.00]
<i>p</i> value	0.630	
Mann-Whitney U test	No statistically significant dependence was found	
Spearman's correlation coefficient/ <i>p</i> value	0.222	
	No statistically significant correlation was found	
Cohen's <i>d</i> effect size	0.1879	
Hedges' <i>g</i> effect size	0.1734	
Cliff's delta effect size	0.1667	
Rosenthal effect size	0.1391	

Based on our data, methylene blue solution leakage occurs at least at the same and possibly at a lower pressure than air leakage; thus, the methylene blue leak test is not inferior to the air leak test. Double-stapled bowels may be more resistant to pressure increases than single-stapled bowels.

Discussion

We compared the air leak and the methylene blue leak test in the experimental setting of single-stapled and double-stapled porcine bowels. We found that the methylene blue solution leak test is not inferior to the air leak test in detecting leaks, even showing a tendency towards lower pressure; therefore, both methods are comparable. We

also discovered that bursting pressures do not differ statistically significantly between single-stapled and double-stapled anastomoses.

It is important to note that liquids and gases have different physical properties. The two most dominant factors contributing to the different leaking properties are viscosity and surface tension. The leakage of liquids is governed by the viscosity of the liquid. The higher the viscosity of the liquid, the more slowly will it leak through a particular hole. The viscosity of a gas is generally much lower than that of a liquid. The viscosity of air, for example, is roughly 50 times lower than that of water. This means that if we consider the influence of viscosity only, a particular leak that passes 20 mm³ of air per second would only pass 0.4 mm³ of water per second. The surface tension sets a limit to how small a leak can be and still pass

Fig. 6. Box plots of air leak and methylene blue leak pressure (mm Hg) in double-stapled bowels.

Table 3. Descriptive and quantitative statistics for bowel leak pressures (mm Hg) in double-stapled bowels

	Air leak test group	Methylene blue leak test group
Mean (SD)	54.67 (21.19)	47.25 (18.02)
Median (MAD)	47.50 (17.05)	44.50 (8.15)
[Q1;Q3]	[37.00;54.00]	[35.00;45.00]
[Min.;max.]	[35.00;84.00]	[31.50;82.00]
<i>p</i> value	0.575	
Mann-Whitney U test	No statistically significant dependence was found	
Spearman's correlation coefficient/ <i>p</i> value	0.282	
	No statistically significant correlation was found	
Cohen's <i>d</i> effect size	0.3771	
Hedges' <i>g</i> effect size	0.3481	
Cliff's delta effect size	0.1944	
Rosenthal effect size	0.1619	

water (or another liquid). The actual size of the “limit” leak depends on a large number of properties such as pressure, temperature, material, and the shape of the leak and can therefore not be theoretically calculated [20, 21]. However, our study seems to show that methylene blue dye passes through small defects in the staple line with the same reliability as does air.

The main strength of this study is that porcine colons, which are very similar to human colons, were used [22]. Moreover, this experiment was conducted immediately after each colon had been removed to prevent ischemic tissue degradation. Another benefit was that we used a validated experimental model developed in previous studies [19]. The main difference from earlier experiments was that we compared air to methylene blue solu-

tion and included in the analysis single-stapled versus double-stapled bowels, but not circularly stapled anastomoses of two bowel ends.

Nevertheless, our study has some weaknesses. It must be taken into consideration that the ex vivo model that was used in this study may be less hydrated than an in vivo model with no blood circulation.

Current studies show that the degree of mucosal capture differs between staplers. Leaks in stapled bowels occur at a point where mucosal capture is poor [21]. Uncaptured mucosa may represent a source of intraluminal bleeding in live tissue. Poor mucosal capture may also expose the submucosa to more luminal contents including bacteria. Different studies have linked luminal bacteria to intestinal anastomotic failure [19]. In practice, we

Fig. 7. Pressures (mm Hg) created by air and methylene blue solution.

Fig. 8. Box plots of bursting pressures (mm Hg) of single-versus double-stapled bowels.

Table 4. Descriptive and quantitative statistics for pressures (mm Hg) created by air and methylene blue insufflation

	Air	Methylene blue solution
Mean (SD)	31.25 (18.61)	24.62 (18.20)
Median (MAD)	28.00 (15.57)	24.00 (16.31)
[Q1;Q3]	[13.00;34.00]	[3.00;25.00]
[Min.;max.]	[13.00;56.00]	[3.00;47.50]
<i>p</i> value	0.773	
Mann-Whitney U test	No statistically significant dependence was found	
Spearman's correlation coefficient/ <i>p</i> value	0.137	
	No statistically significant correlation was found	
Cohen's <i>d</i> effect size	0.36	
Hedges' <i>g</i> effect size	0.313	
Cliff's delta effect size	0.125	
Rosenthal effect size	0.1022	

perform both air leak and methylene blue leak tests to check the mechanical integrity of colorectal anastomosis [18, 23]. First, we perform air leak testing of the newly formed anastomosis. Saline fluid is filled into the low pelvis and air is injected through the rectum in order to check the integrity of the anastomosis. After that, the saline is removed, and the solution, dyed with methylene blue, is injected through the anus to check the mechanical integrity of the anastomosis too. Our study differs from surgical practice in that the methylene blue test was done underwater, whereas in practice it is usually performed in atmospheric air (open surgery) or under CO₂ pressure (laparoscopic surgery).

It is current practice to check the mechanical integrity of a colorectal anastomosis by standard air leak test, insufflating air through a drain or Foley catheter. Also, some authors present intraoperative sigmoidoscopy as a method of checking the mechanical integrity of a colorectal anastomosis. We found two randomized controlled trials which presented a positive intraoperative endoscopy test for 23.2–25% of the patients. Postoperative AL occurred in 4–10% of the patients despite correction of insufficient colorectal anastomosis [7, 24]. Ricciardi et al. [25] found positive intraoperative air leak test results in 7.9% of the tested anastomoses. Postoperative AL occurred in 7.7% of the anastomoses with a positive air leak test result, compared to 3.8% of the anastomoses with a negative air leak test result. The postoperative AL rate was 8.1% for the untested anastomoses. Another study confirmed similar results. Allaix et al. [26] tested anastomoses by standard air leak test and found positive test results for 5% of the patients. The rate of postoperative AL was significantly lower in the study group than in the untested anastomoses (2.5 vs. 5.8%; $p = 0.025$). Moreover, no postoperative AL occurred in the patients with a positive air leak test result. Schmidt et al. [8] tested the mechanical integrity of anastomoses in combination with intraoperative endoscopy followed by a blue-stained saline test. The rate of postoperative AL after a negative test was 10.3%. These findings might be similar to those of our experimental study.

We found five studies where the mechanical integrity of colorectal anastomosis was checked by methylene blue, Patent Blue dye, or intraluminal saline [11, 12, 15–17]. Gilbert and Trapnell [15] presented positive intraluminal test results for 24% of their patients, whereas Wheeler and Gilbert [17] found 20.6% of their patients with a positive test result. The postoperative leakage rate was 9.5% in the study by Wheeler and Gilbert [17]. Smith et al. [11] tested colorectal anastomoses with methylene blue dye and found a positive test result for 7% of the anastomoses, with a 3.3% postoperative AL rate. Moreover, there was no detected postoperative AL in patients with positive methylene blue test results. Also Chen et al. [12] found

similar results. A positive Patent Blue dye test result was noticed in 14.5% of their anastomoses. The postoperative AL rate was 1.3%. Similar to the Smith study, there was no confirmed postoperative AL in patients with positive intraoperative test results.

The air leak test and the methylene blue test can equally identify insufficient colorectal anastomosis. Nevertheless, there are not enough studies to answer which is the better mechanical integrity test, and they may possibly complement one another.

Conclusions

Our experiment showed that the methylene blue solution leak test is not inferior to the air leak test. Double-stapled bowels and single-stapled anastomoses were equally resistant to pressure increases in our experimental setting.

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Statement of Ethics

The research presented in the paper was conducted ethically in accordance with the World Medical Association Declaration of Helsinki as well as according to animal welfare regulations and was approved by the appropriate institutional review bodies.

Conflict of Interest Statement

The authors declare no conflicts of interest.

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Author Contributions

T.P., E.P., and M.K.: conceptualization; T.P., M.K., and E.J.: methodology; T.P., E.P., and K.S.: validation; E.J.: formal analysis; M.K., V.A., A.E.D., and B.L.: investigation; T.P. and E.P.: resources; T.P., V.A., and A.E.D.: data curation; V.A., A.E.D., M.K., and B.L.: writing – original draft preparation; M.K., T.P., and K.S.: writing – review and editing; V.A.: visualization; E.P., T.P., and K.S.: supervision; E.P., T.P., and K.S.: project administration.

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