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Prognostic value of colonic and peripheral venous lactate measurements in horses with large colon volvulus

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Abstract

Objective: To determine the prognostic value of (1) colonic venous lactate or peripheral lactate values obtained before and after manual correction of a large colon volvulus and (2) a combination of variables including pelvic flexure biopsy.

Study design: Prospective clinical study.

Animals: Forty adult horses in which large colon volvulus was diagnosed intraoperatively.

Methods: Colonic venous, peripheral venous, and arterial blood samples were collected to measure lactate values before and after manual correction. Mucosal biopsy samples were obtained in cases that underwent enterotomy or colonic resection and anastomosis. Interstitium to crypt (I:C) ratio and hemorrhage scores were measured. Optimal cutoff values were determined by receiver operator curve analysis, and associations between variables and short-term outcome were determined by univariable regression. Short-term survival was defined as horses being discharged from the hospital. $P < .05$ was considered significant.

Results: No association was found between colonic venous lactate values before ($P = .011$) or after ($P = .201$) manual correction of large colon volvulus and determination of short-term outcome. Peripheral venous lactate at admission 3.2 mmol/L and after manual correction 5 mmol/L, arterial lactate postmanual correction 3.53 mmol/L, and histomorphometric measurements of mucosal hemorrhage 3 and I:C ratio > 1 were associated with poor short-term outcome.

Conclusion: Peripheral lactate values, histomorphometric measures of I:C ratio, and hemorrhage score provided prognostic information that could help guide recommendations made to owners.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this report.

Clinical significance: Peripheral lactate values after manual correction provide important intraoperative diagnostic information to assist in predicting case outcome in the operative and immediately postoperative period.

1 | INTRODUCTION

Strangulating large colon volvulus (LCV) is a serious manifestation of colic that has been classically associated with mortality rates of 35% to 86%.¹⁻⁹ However, an 88% survival to discharge has been described that was attributed to early recognition and prompt surgical intervention.¹⁰ Despite this improvement, no objective intraoperative measurement to determine colonic viability exists in cases in which prolonged ischemia has occurred. Determining colonic viability is critical to intraoperative decision making, such as the requirement to perform large colon resection and anastomosis, and to predict patient outcome. Multiple objective methods to determine colonic viability have been studied and include fluorescein fluorescence,^{11,12} surface oximetry,¹³ luminal pressure,^{14,15} dark field microscopy,¹⁶ and histopathology.^{4,8,17} However, all of these methods are predominantly limited in use to academic institutions. Histopathologic evaluation of pelvic flexure biopsies have been shown to have the most promise as measurements of interstitium to crypt (I:C) ratio, and degree of mucosal hemorrhage has been shown to predict outcome in cases of LCV.¹⁷ Nonetheless, histopathologic biopsy evaluation is rarely available for immediate interpretation intraoperatively or in clinical practice.

The methods that are commonly used in clinical practice to determine colonic viability and to make clinical decisions are subjective and include evaluation of colonic arterial pulse, enterotomy site hemorrhage, motility, resolution of colon wall edema after manual correction of the volvulus, and serosal and mucosal appearance.¹⁸ In fact, visual assessment was shown in a recent survey to be the most commonly used method by surgeons to make clinical decisions.¹⁹ However, these methods do not provide a reliable, repeatable, and objective determination of case outcome.

Peripheral and peritoneal lactate measurements are objective, are rapidly available, and have been associated with outcome in cases of colic.^{6,20-24} The elevated peripheral lactate values measured in cases of strangulating LCV are predominantly attributed to the ischemic large colon, although systemic hypoperfusion, endotoxic shock, and hypotension contribute to the increased values.^{6,21,25} Venous plasma lactate concentrations measured in cases of LCV at the time of admission were found to be strongly associated with colonic viability and survival.⁶ Nonsurvival in cases of LCV was also associated with elevated peripheral venous lactate levels measured immediately postoperatively.²¹

In a model of experimentally induced hemorrhagic colonic ischemia, lactate values measured from colonic venous blood was found to predict the degree of mucosal damage.²⁶ However, the potential prognostic value of colonic venous lactate levels from clinical cases of LCV or associated lactate changes immediately before and after correction of the volvulus with case outcome requires additional study. We hypothesized that in clinical cases of LCV, lactate values measured directly from the colonic vasculature could be associated with case outcome. More specifically, we hypothesized that lactate values measured from

colonic vasculature and from peripheral vessels, after manual correction, would more clearly indicate the severity of tissue hypoxia and more accurately predict case outcome compared with peripheral lactate samples taken prior to manual correction of the LCV.

Therefore, the objective of this study was to determine the prognostic value of (1) colonic venous lactate or peripheral lactate values obtained before and after manual correction of a large colon volvulus and (2) a combination of variables including histomorphometric evaluation of pelvic flexure mucosal biopsy.

2 | MATERIALS AND METHODS

2.1 | Study population and inclusion criteria

This was a prospective study performed between April 1, 2007 and January 1, 2010 from clinical cases admitted to Peterson and Smith Equine Hospital. Client consent was obtained from all clinical cases. Only horses 1 year of age presenting for colic in which a 360 degree strangulating LCV was diagnosed at the time of surgery were included in the study. Only animals for whom recovery from general anesthesia was attempted were included in the statistical analysis to minimize potential bias associated with surgeon subjectivity or the economic constraints of owners.

2.2 | Surgical protocol and perioperative medical management

After admission to the hospital, heart rate and preoperative hematological values (packed cell volume [PCV], total protein [TP], white blood cell count [WBC], and venous lactate) were recorded. The venous blood was collected from the jugular vein, and lactate values were measured on a portable lactate analyzer (i-STAT; Abbott Laboratories, Princeton, New Jersey). An exploratory celiotomy was performed, and, after manual correction of the LCV, serosal color, wall thickness, friability, motility, appearance of the mesentery, and presence of a detectable pulse were used to determine the viability of the large colon.² When the colon was deemed viable, it was replaced manually. A pelvic flexure enterotomy was variably performed according to surgeon preference. If the colon was deemed nonviable, the client was consulted and given the option of a large colon resection. In cases of repeat celiotomy for LCV, the owner was given the option of prophylactic large colon resection to prevent recurrence. When prophylactic large colon resection was performed, the large colon was resected at the most proximal, easily exteriorizable extent by using the end-to-end technique, as previously described by Hughes et al.²⁷ In addition, intraoperative abdominal drain placement and postoperative abdominal lavage were performed in all horses that underwent large colon resection. All horses were administered gentamicin (6.6 mg/kg IV every 24 hours) and potassium penicillin G (22 000 U/kg IV every 6 hours) preoperatively and for a minimum of 5 days postoperatively. Flunixin meglumine (1.1 mg/kg IV every 12 hours or 0.5 mg/kg IV every 8 hours) was administered preoperatively and for a minimum of 3 days postoperatively. Continued antibiotic and anti-inflammatory therapy was based on clinical assessment and hematological results. Some horses received polymyxin B (6000 U/kg IV every 8 to 12 hours) and/or plasma (Immuno-glo; MG Biologics, Ames, Iowa), depending on the degree of endotoxemia and/or hypoproteinemia. Other medications used on a case by case basis included metronidazole (15 mg/kg orally every 8 hours) and

lidocaine HCl (1.3 ug/kg IV loading dose followed by 0.05 mg/kg/min constant rate IV infusion).

2.3 | Collection of lactate values

After exteriorization of the colon and diagnosis of volvulus, a 0.75- to 1-mL blood sample was drawn from the accessible colonic veins supplying the pelvic flexure with a 25-gauge needle attached to a 3-mL heparinized syringe. To prevent hematoma formation, manual pressure was applied to the site of venipuncture for 3 to 5 minutes until hemostasis was established. Concurrently, a 1-mL blood sample was taken from either the jugular vein or the facial venous sinus. Both samples were run consecutively on a portable lactate analyzer (i-STAT, Abbott Laboratories). The colon was then manually corrected, and proper orientation was confirmed by palpation. A second 1-mL blood sample was taken from both the colonic vasculature and the jugular vein or the facial venous sinus in the same manner as previously described. At this time, 1 mL of blood was also drawn from the facial artery for lactate measurement.

2.4 | Histopathology

A 5-cm mucosal biopsy of the pelvic flexure was obtained from all horses that underwent large colon resection and anastomosis and those horses that had a pelvic flexure enterotomy performed. Samples were placed in 10% neutral buffered formalin solution. Tissues were processed, embedded in paraffin, sectioned at 5 μ m, and stained with hematoxylin and eosin by the same histopathology laboratory (NC State University College of Veterinary Medicine). Four visual fields were evaluated by light microscopy ($\times 10$ and $\times 40$ objective lenses), and the I:C ratio and mucosal hemorrhage score were determined by two of the authors (L.M.G and C.A.F.). The evaluators performed histological evaluation independently and were blinded to horse and short-term outcome data. Interstitium to crypt ratio was defined as the ratio of the measured lamina propria space occupied by the interstitium and the space occupied by the crypts. A normal value for the I:C ratio was considered to be 1.²⁸ Hemorrhage within the lamina propria was assigned a score from 0 to 4 based on a scale published by Gonzalez et al.¹⁷ A score of 0 indicated no hemorrhage, and a score of 4 was consistent with confluent red blood cells obscuring the demarcation of the lamina propria.

2.5 | Statistical analysis

Short-term survival was defined as horses being discharged from the hospital. Thirteen variables were evaluated for association with survival: HR, PCV, WBC, TP, I:C ratio, mucosal hemorrhage score, venous lactate at admission, premanual correction colonic lactate and peripheral venous lactate, postmanual correction colonic lactate, venous lactate, arterial lactate, and surgical correction (resection and anastomosis versus manual correction alone). The cutoff points for dichotomizing the quantitative variables entered in the logistic regression model were either referenced values or determined by using a receiver operator curve that maximized sensitivity and specificity to the best predictive value associated with short-term outcome (alive or dead). The test variables without previously published or universally accepted cut-off values included venous lactate at admission, premanual correction colonic lactate and peripheral venous lactate, postmanual correction colonic lactate, peripheral venous lactate, and arterial lactate (Tables 1 and 2). For these variables,

the optimal cutoff value was determined by using the equation $d = (1 - \text{sensitivity})^2 + (1 - \text{specificity})^2$, where the cutoff value was associated with the smallest value of d .²⁹ To test for potential multivariable models that might be significant, backward stepwise regression was performed by using those variables found in the univariable regression with $P < .20$ to build a potential multivariable association model. Results are presented as odds ratios and 95% confidence intervals.

The sensitivity (the probability that a truly affected individual would test positive), specificity (the probability of a surviving horse having a negative test result), and accuracy (the sum of the number of true positives and true negatives divided by the sum of the number of true positives, false positives, false negatives, and true negatives) of predicting death was determined for those variables found to be associated with outcome in the univariable regression models and for those retained in a multivariable logistic regression. Statistical analyses were performed in Prism (GraphPad Software, La Jolla, California) and SigmaStat (Systat Software, San Jose, California). $P < .05$ was considered to be statistically significant.

3 | RESULTS

During the study period of April 01, 2007 to January 01, 2010, a total of 373 horses had an exploratory celiotomy performed at Peterson and Smith Equine Hospital. Among the surgery cases, 108 horses were identified as having LCV, and colonic lactate samples were collected from 54 of these horses. However, 14 of 54 (25.9%) horses were euthanized intraoperatively. Seven of 14 (50%) were euthanized because of grave prognosis, five of 14 (35.7%) horses were euthanized at the owner's request, and two of 14 (14.3%) horses had an intraoperative large colon rupture. All horses euthanized intraoperatively were excluded from the study. Forty horses met all inclusion criteria and were therefore included for further analysis. The mean age of the study population was 8.03 years (range, 1-10). There were 35 mares, four geldings, and one intact male. Represented breeds included thoroughbred ($n = 28$ [70%]), warmblood ($n = 6$ [15%]), quarter horse ($n = 4$ [10%]), Arabian ($n = 1$ [2.5%]), and paso fino ($n = 1$ [2.5%]). Twenty one of 40 (52.5%) horses had manual correction of the volvulus, and pelvic flexure enterotomy was performed in six of these horses. Nineteen of 40 (47.5%) horses underwent an end-to-end resection and anastomosis of the large colon. There were no complications associated with blood collection from the colonic vasculature.

In total, 33 of 54 (61.1%) horses survived until hospital discharge. Excluding horses euthanized intraoperatively ($n = 14$), 33 of 40 (82.5%) horses survived until hospital discharge. Among the horses that had manual correction of the volvulus only (with no resection of the large colon), 18 of 21 (85.7%) survived until hospital discharge. Fifteen of 19 (78.9%) horses that had a large colon resection and anastomosis survived until hospital discharge. All of the horses that did not survive ($n = 7$) were euthanized because of deteriorating clinical signs. Four of seven (57.1%) horses were euthanized because of clinical signs consistent with systemic inflammatory response syndrome,³⁰ two of seven (28.6%) were euthanized because of peritonitis, and one of seven (14.3%) was euthanized because of colitis and laminitis.

Factors that were associated with short-term outcome on univariable regression were preoperative PCV $\geq 50\%$ ($P = .003$) and venous lactate ≥ 3.2 mmol/L ($P = .035$; Table 1); intraoperative venous lactate postmanual correction ≥ 5 mmol/L ($P = .012$) and arterial lactate postmanual correction ≥ 3.53 mmol/L ($P = .018$; Table 2); and histomorphometric measures of hemorrhage score ≥ 3 ($P = .017$) and I:C ratio > 1 ($P = .050$; Table 3). Six of 11 (54.5%) horses with a PCV of $\geq 50\%$ died. Twenty-seven of 28 (96.4%) horses with a PCV $< 50\%$ survived. Packed cell volume was 85% accurate in the prediction of short-term outcome (sensitivity, 86%; specificity 84%; PPV, 55%; NPV, 96%). Six of 19 (31.6%) horses with a venous lactate of ≥ 3.2 mmol/L at admission died. Fourteen of 15 (93.3%) horses with venous lactate of < 3.2 mmol/L at admission survived. Venous lactate measured at admission was 59% accurate in predicting short-term outcome (sensitivity, 86%; specificity, 52%; PPV, 32%; NPV, 93%).

Among LCV cases with a venous lactate postmanual correction ≥ 5 mmol/L, three of four (75%) horses died. Twenty-seven of 30 (90%) horses with a venous lactate postmanual correction < 5 mmol/L survived. Venous lactate values measured after manual correction were 88% accurate in predicting short-term outcome (sensitivity, 50%; specificity, 96%; PPV, 75%; NPV, 90%). Five of 12 (41.6%) horses with an arterial lactate postmanual correction ≥ 3.53 mmol/L died, whereas 26 of 28 (92.9%) horses with an arterial lactate postmanual correction < 3.53 mmol/L survived. Arterial lactate measured after manual correction was 78% accurate in predicting short-term outcome (sensitivity, 71%; specificity 79%; PPV, 42%; NPV, 93%). Among LCV cases with an I:C ratio > 1 , four of 11 (36.4%) horses died. Among those cases with an I:C ratio of ≤ 1 , 12 of 13 (92.3%) horses survived. Interstitium to crypt ratio measurement was 67% accurate in predicting short-term outcome (sensitivity, 80%; specificity, 63%; PPV, 36%; NPV, 92%). Four of seven (57.1%) LCV cases with a mucosal hemorrhage score of ≥ 3 died. Sixteen of 17 (94.1%) horses with a mucosal hemorrhage score of < 3 survived. Hemorrhage score was 83% accurate in predicting short-term outcome (sensitivity, 80%; specificity, 84%; PPV 57%; NPV 94%).

In horses with LCV, lactate levels measured from the colonic vasculature before and after manual correction of the volvulus were not found to be associated with outcome. Resection was also not associated with short-term outcome (Table 2). When all variables identified in a univariable regression with $P < .2$ were included in the multivariable backward stepwise regression model, no combined variables (interactions) were found to strengthen the model in predicting outcome. Instead, of all the measured variables, venous lactate measurement collected after manual correction was the most accurate predictor of death.

4 | DISCUSSION

Determining outcome in clinical cases of LCV continues to remain elusive. This study is unique in that, in addition to variables measured preoperatively and intraoperatively, peripheral and colonic lactate concentrations were evaluated before and after correction of the volvulus. Among the variables measured, PCV and peripheral venous lactate at the time of admission, peripheral venous and arterial lactate values after manual correction of volvulus, and histomorphometric measures of I:C ratio and hemorrhage score were all associated with case outcome. The results of this study did not provide evidence

to support our hypothesis that lactate concentrations measured directly from the colonic venous vasculature could be used to determine case outcome. However, the data did provide evidence to support the use of peripheral lactate measurements to predict case outcome. In fact, the variable that most reliably predicted case outcome in this study was the peripheral venous lactate measurement obtained immediately after manual correction of the volvulus.

As we had previously stated, we found no association between colonic venous lactate values before or after manual correction of LCV and short-term outcome. This is in contrast to a study that measured venous and arterial lactate concentrations from colonic vessels in an experimentally induced hemorrhagic ischemia model in horses.²⁶ In that study, the increased lactate values measured from colonic veins sampled distal to the vascular occlusion were predictors of the severity of histologic damage. Although this was our expected outcome, it was not surprising that a research model would not perfectly recapitulate the results of a study in which clinical samples were used. In the hemorrhagic ischemia model, the blood flow to the pelvic flexure was controlled by maintaining approximately 20% to 30% arterial flow and completely occluding the colonic vein and lumen associated with either the dorsal or ventral colon over a period of 3 hours followed by an hour of reperfusion.²⁶ In most clinical cases of LCV, the colon rotates near the base of the cecum, and there is variability in the degree of vascular occlusion. This likely impacts the amount of lactate sequestered within the colonic veins compared to the amount that reaches the systemic circulation. Because of the short referral time in our case population, it is possible that the duration of colonic ischemia was less than 3 hours and that the veins were not completely occluded.

Increased PCV at admission was associated with nonsurvival in horses with LCV, as was the case in other studies.^{5,7,10,31} Increased PCV is reflective of the duration of the colic and the degree of systemic compromise of the horse.⁵ Moreover, elevated PCV is a marker of systemic inflammatory response syndrome, hypovolemia, pain, and increased sympathetic tone, all of which may be made worse with more severe LCV. With an increasing PCV there is decreased tissue perfusion which decreases oxygen availability for cellular metabolism leading to lactic acidosis from anaerobic respiration. Measurements of lactate concentrations have consequently been associated with outcome in clinical cases of colic and specifically those with intestinal strangulation.^{6,20-24,32}

In a retrospective study of peripheral plasma lactate collected preoperatively in horses with 360-degree LCV, Johnston et al⁶ found that cases with venous plasma lactate concentration < 6 mmol/L at admission had a > 90% chance of survival; in comparison, only 30% of horses survived with a plasma lactate concentration > 7 mmol/L. In that study, lactate measurements had a positive predictive value of 96%, thereby supporting the use of lactate measurements to predict short-term survival. In similar samples collected in our study, although peripheral venous lactate values measured at admission were statistically associated with death, the PPV was only 32% with a 59% accuracy. Variability between the duration of colic prior to admission between the two study populations may have contributed to differences in the lactate concentrations measured. Nevertheless, another study found no association between preoperative lactate values and case outcome, which may be evidence that peripheral lactate is unreliable in predicting outcome in cases of LCV.²¹ Taken together, these studies support the requirement for additional investigation

into the utility of preoperative lactate values as a tool to predict outcome. In addition, the discrepancies may be attributed to the samples having been obtained at a single time point instead of serially. Sequential sampling of plasma lactate concentrations has been shown to increase the predictive value of this test.^{33,34}

Peripheral venous lactate values measured after manual correction of the volvulus were the most accurate predictor of death and survival in the postoperative period in our study population. Moreover, a horse with a venous lactate of ≥ 5 mmol/L after manual correction of volvulus was 27 times more likely to die in the postoperative period than a horse with a lactate of <5 mmol/L. This finding is supported by Dallap et al²¹ who found that horses with lactate levels greater than 3.7 mmol/L immediately postoperative were more likely to be euthanized. The persistently increased systemic lactate values in the early postoperative period are likely the result of prolonged tissue hypoxia compounded by systemic effects of endotoxemia, dehydration, and circulatory shock and is reflective of the severity of disease.^{6,21} The association of increased arterial lactate concentration after manual correction and death in our study was likely because of these same physiologic effects. An arterial lactate postmanual correction of ≥ 3.53 mmol/L was associated with nonsurvival ($P = .018$). Similar to our findings, McCoy et al³⁵ found that a recovery arterial lactate of >5 mmol/L had a 2.02 times higher relative risk of developing postoperative complications. While this was a trend that was not statistically significant in their study, the doubled risk of developing postoperative complications was considered to be potentially of clinical importance.

One of the limitations of this study was the small sample size. This was exacerbated by the exclusion of horses that were euthanized intraoperatively. These cases were excluded because the decision to euthanize is subjective and based on a variety of factors, including perceived prognosis and owner's financial constraints. A second limitation of this study was that there was not a set protocol for which horses received a large colon resection and anastomosis vs those horses that had manual correction of the volvulus. This underscores how, within the field of equine veterinary medicine, case selection to perform resection and anastomosis in cases of LCV remains controversial and clinician dependent.

In conclusion, peripheral lactate values obtained at admission and after manual correction can provide prognostic information to help guide recommendations to owners. Peripheral venous lactate concentrations obtained after manual correction, in particular, were 88% accurate in predicting short-term outcome. In addition, the importance of histopathology of the pelvic flexure in the prediction of short-term outcome is further supported. However, at this time, there is no intraoperative measurement of I:C ratio and mucosal hemorrhage score. Until such a test is developed, systemic lactate values after manual correction can provide important intraoperative diagnostic information to assist in outcome prediction in cases of LCV.

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Univariable logistic regression analysis of admission variables associated with death in cases of LCV

TABLE 1

Variable	Category	Horses, n (%)	OR	95% CI	P value
Heart rate, beats/min	>48	29/38 (79.3)	0.729	0.115-4.607	.737
	48	9/38 (23.7)			
PCV% ^a	50	11/39 (28.2)	32	3.177-330.394	.003
	<50	28/39 (71.8)			
WBC/ μ L	>12 000	12/39 (30.8)	0.9	0.145-5.335	.889
	12 000	27/39 (66.7)			
TP, g/dL	>7.2	8/38 (21.1)	0.6	0.059-5.58	.630
	7.2	30/38 (78.9)			
Lactate initial, mmol/L ^{a,b}	3.2	19/34 (55.9)	1.8	1.044-3.136	.035
	<3.2	15/34 (44.1)			

Abbreviations: CI, confidence interval; LCV, large colon volvulus; OR, odds ratio; PCV, packed cell volume; TP, total protein; WBC, white blood cell.

^aVariable found to be statistically significant, $P < .05$.

^bVariable cutoff determined by receiver operator curve.

Univariable logistic regression analysis of intraoperative variables associated with death in cases of LCV

TABLE 2

Variable	Category	Horses, n (%)	OR	95% CI	P value
Colonic venous lactate Pre, mmol/L ^a	5.45	12/39 (30.8)	4.0	0.733-21.838	.109
	<5.45	27/39 (69.2)			
Peripheral venous lactate Pre, mmol/L ^a	2.95	17/33 (51.5)	6.3	0.641-60.938	.115
	<2.95	16/33 (48.5)			
Colonic venous lactate Post, mmol/L ^a	3.35	21/36 (58.3)	4.4	0.455-42.08	.201
	<3.35	15/36 (41.7)			
Peripheral venous lactate Post, mmol/L ^{a,b}	5	4/34 (11.8)	27	2.091-348.661	.012
	<5	30/34 (88.2)			
Peripheral arterial lactate Post, mmol/L ^{a,b}	3.53	12/40 (30.0)	9.3	1.475-58.467	.018
	<3.53	28/40 (70.0)			
Surgical correction	Resection	19/40 (47.5)	1.6	0.308-8.301	.576
	Manual	21/40 (52.5)			

Abbreviations: CI, confidence interval; LCV, large colon volvulus; OR, odds ratio; Post, postoperatively; Pre, preoperatively.

^aVariable cutoff determined by receiver operator curve.

^bVariable found to be statistically significant, $P < .05$.

Univariable logistic regression analysis of histopathology associated with death in cases of LCV

TABLE 3

Variable	Category	Horses (%)	OR	95% CI	P value
I:C ratio ^a	>1	11/24 (45.8)	32	0.985-1046.3	.050
	1	13/24 (54.2)			
Mucosal hemorrhage score ^a	3	7/24 (29.2)	21	1.726-263.675	.017
	<3	17/24 (70.8)			

Abbreviations: CI, confidence interval; I:C, interstitium to crypt; LCV, large colon volvulus; OR, odds ratio.

^aVariable found to be statistically significant, *P* < .05.