

Evolution of Surgical Management of Complicated Left Colon Cancer

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Rezumat

Evoluția managementului chirurgical al cancerului de colon stâng complicat

Introducere: Cancerul de colon complicat reprezintă până la 40% dintre pacienții cu cancer de colon. În timp ce pentru managementul cancerului de colon drept complicat sunt câteva recomandări standard, pentru cancerul de colon stâng complicat există numeroase controverse privind utilizarea procedurilor într-un singur timp sau a procedurilor seriata. **Scop:** Studiarea tipurilor de proceduri și a morbidității și mortalității postoperatorii la pacienții cu cancer de colon stâng complicat internați la Clinica I Chirurgie a Spitalului Clinic Județean de Urgență Craiova în ultimii 23 de ani. Ne-am propus să prezentăm evoluția managementului chirurgical în procedurile de urgență pentru colonul stâng complicat.

Material și metodă: studiu retrospectiv al pacienților cu cancer de colon stâng complicat internați în clinica noastră între 2001 și 2023. Am analizat morbiditatea și mortalitatea postoperatorie a fiecărui tip de procedură de urgență (într-un singur timp sau în două etape) și le-am comparat pe parcursul a trei perioade distincte de timp.

Rezultate: Trei loturi urmărite: G1 – 2001-2010, (96 pacienți); G2 – 2011-2016, (65 pacienți); G3 – 2017-2023, (77 pacienți). Am înregistrat o creștere semnificativă a procedurilor într-un singur timp de la G1 la G2 (11,2% față de 33,8%). În G3, rata procedurilor într-o singură etapă a scăzut semnificativ (20,8% față de 33,8%). Morbiditatea și mortalitatea postoperatorie au fost semnificativ mai mici în G2 comparativ cu G1 atât în procedurile cu o singură etapă, cât și în două etape. G3 comparativ cu G2 a înregistrat o scădere semnificativă a ratei procedurilor într-un singur timp, dar

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a rămas similară pentru procedurile în două etape.

Concluzie: Pentru chirurgia de urgență a cancerului de colon stâng, procedurile în două etape sunt mai sigure, în timp ce rezecțiile colice cu anastomoză primară, chiar și în cazuri selectate și având experiență chirurgicală, încă asociază morbiditate și mortalitate postoperatorie mai ridicate.

Cuvinte cheie: cancer de colon stâng complicat, intervenții chirurgicale de urgență, anastomoză primară, proceduri într-un singur timp

Abstract

Complicated colon cancer accounts for up to 40% of colon cancer patients. While the management of complicated right colon cancer has some standard recommendations, for complicated left colon cancer single stage or two-stage procedures are subject to controversies. *Aim:* To study the types of procedures and postoperative morbidity and mortality for complicated left colon cancer patients admitted to the 1st Surgical Clinic of the County Clinical Emergency Hospital of Craiova during the past 23 years. We aimed to present the evolution of the surgical management in the emergency procedures for complicated left colon.

Material and method: retrospective study of patients with complicated left colon cancer admitted to our clinic between 2001 and 2023. We analysed the postoperative morbidity and mortality of each type of emergency procedure (single stage or two-stage) and compared them throughout three periods of time.

Results: Three groups observed: G1 – 2001-2010, (96 patients); G2 – 2011-2016, (65 patients); G3 – 2017-2023, (77 patients). We registered significant increase in single stage procedures from G1 to G2 (11.2% vs. 33.8%). In G3, single stage procedure rate decreased significantly (20.8% vs. 33.8%). Postoperative morbidity and mortality was significantly lower in G2 compared to G1 in both single stage and two-stage procedures. G3 compared to G2 registered significant decrease for single stage procedures but similar for two-stage procedures.

Conclusion: For left colon emergencies, two-stage procedures seem safer, as resections with primary anastomosis, even with selected cases and experienced surgeons, still associate higher postoperative morbidity and mortality.

Key words: complicated left colon cancer, emergency surgery, primary anastomosis, single stage procedures

Introduction

Despite advancements in colon cancer screening and diagnostic techniques, a considerable proportion of patients continue to present with complications of colon tumours. These emergency complications include perforation and obstruction. Between 3% and 10% of cases diagnosed with colon cancer exhibit signs of perforation upon initial presentation, while a higher range, spanning from 8% to 40%, display manifestations of colon obstruction. Notably,

there appears to be a certain predilection towards emergency colorectal interventions among female patients (1-3).

Studies show that emergency surgery for complicated colon cancer can have an adverse impact on patient's postoperative evolution and also on long term survival with poorer oncologic outcome (3-6). The altered general status of patients presenting with complications such as perforation, obstruction, or bleeding can be associated with an unfavourable postoperative outcome. In addition, emergency

colon cancer surgery usually reveals advanced tumour stages thus leading to worse oncologic results (7,8).

Choosing an adequate procedure for complicated colon cancer involves taking into consideration the site of the tumour (right or left colon), the type of complication (perforation, obstruction, bleeding), the patient's general status, age, and nutritional state. The standard treatment for complicated right colon cancer is right colectomy with ileo-colic anastomosis (9,10). For complicated left colon cancer there are still numerous controversies regarding emergency surgery treatment. One stage resections with anastomosis and two-stage procedures are currently considered. Other non-surgical interventions can be applied to resolve the complication (laser, colonic stent, emergency colonoscopy) followed by resection and anastomosis during the second stage (11-13).

Performing an anastomosis on a distended colon with vascular deficit of the colonic wall is associated with a high risk of postoperative morbidity. Still, primary resection with anastomosis can be safely performed for selected cases. For right colon cancer patients the distention of the distal ileum must be moderate at best or the resection may include the distended segment. For left colon cancer it may be required to perform a subtotal or total colectomy with ileo-colic or ileo-rectal anastomosis. These procedures may be challenging for unexperimented surgeons and would best be avoided in the absence of a colorectal specialist (7,12,14,15).

Material and Methods

A prospective and retrospective analysis was conducted involving patients diagnosed with complicated left colon cancer who were admitted to the 1st Surgery Clinic of the County Clinical Emergency Hospital of Craiova, and subsequently undergoing emergency surgical intervention between 2001 and 2023.

The study adhered to the ethical tenets outlined in the World Medical Association Declaration of Helsinki. Prior to participation,

all participants provided written informed consent. Approval for the study protocol was obtained from the Ethics Committee of the University of Medicine and Pharmacy of Craiova.

Inclusion criteria encompassed emergency admission, a confirmed diagnosis of complicated left colon cancer, and the requirement for emergency surgery. *Exclusion criteria* comprised diagnoses of benign colon pathology, instances of ambulatory admission, and elective surgical procedures.

The primary objective of this investigation was to track and allow a better understanding of the progression of surgical strategies used in the treatment of complicated left colon cancer, specifically focusing on the ongoing debate surrounding the choice between primary anastomosis and two-stage procedures.

We conducted an analysis of the surgical interventions employed, noting the variations in treatment protocols across the three defined temporal intervals: an initial phase spanning from 2001 to 2010, an intermediate phase spanning from 2011 to 2016, and a subsequent period from 2017 to 2023.

The study was carried out in our department since 2011 when we conducted a retrospective analysis of colon cancer patients admitted between 2001 and 2010. The following 6 year interval, 2011-2016 was studied prospectively with partial results observed at the end of the period. These results determined a revision of our surgical strategy for the next time interval that we documented using a prospective analysis between 2017 and 2023.

Additional variables under investigation comprised of postoperative complications, overall morbidity, and mortality rates. Categorical variables were presented as absolute values alongside their corresponding percentages.

Research data was uploaded to the electronic database and the statistical analysis was performed using Microsoft Excel (Microsoft, Redmond, USA). For normally distributed values, the t-Student's test and chi2 test were used to assess potential statistically

significant differences between the two groups. The statistical significance level was considered for $p < 0.05$.

Results

We divided the 23-year timeframe into three discrete intervals, thereby establishing three distinct cohorts of patients. The first cohort, designated as Group 1 (G1), comprised individuals with complicated left colon cancer who were admitted and underwent emergency surgery between 2001 and 2010. Group 2 (G2) included patients diagnosed with complicated left colon cancer admitted for emergency surgical intervention during the period spanning from 2011 to 2016. Lastly, Group 3 (G3) consisted of patients with complicated left colon cancer admitted and operated upon in an emergency setting from 2017 to 2023.

Between 2001 and 2010, a total of 368 patients diagnosed with colon cancer were admitted to our institution, comprising both emergency and elective admissions. Emergency cases (119 patients) accounted for 32.3% of all colon cancer admissions during this period. Among these, 96 patients met the inclusion criteria for complicated left colon cancer and were consequently selected for inclusion in Group 1.

During the second time interval investigated, spanning from 2011 to 2016, a total of 265 patients diagnosed with colon cancer were admitted to our department. Among these, 85 patients were identified as having complicated colon cancer and consequently underwent emergency surgery, representing 32% of all colon cancer patients treated during this period. There were 65 patients with complicated left colon cancer. These patients were included in Group 2.

Between 2017 and 2023, 317 patients diagnosed with colon cancer were admitted to our clinic. Among these cases, 104 cases were complicated colon cancer patients accounting for 32.8% of all colon cancer admissions. 77 patients with complicated left colon cancer met the criteria for admission in Group 3.

In Group 1 (96 patients), 11 single-stage procedures were conducted, representing 11.4% of cases within the group, while 85 two-staged procedures were performed, accounting for 88.6% of the group. Specifically, 11 colectomies with primary anastomosis were performed, comprising 4 left colectomies, 2 sub-total colectomies, and 5 segmental sigmoidectomies. Additionally, the Hartmann procedure was performed in 27 cases (28.1%), with only 17 patients (62.9%) readmitted for reversal. Proximal colostomies were performed in 41 cases (42.7%), followed by left or segmental colectomy in 22 patients (53.6%). Other procedures included caecostomy in 7 cases (7.3%) and internal diversions in 10 cases (10.4%). This data is presented in *Tables 1 and 2, Fig. 1*.

In Group 2 there were 65 patients with complicated left colon cancer admitted to our department between 2011 and 2016.

In Group 2 there were 22 patients that underwent single stage procedures represented by colectomy with primary anastomosis (33.8% of the subgroup). We noted 10 left colectomies, 8 rectosigmoidectomies and 4 subtotal colectomies. In 43 cases two-stage procedures were preferred representing 66.2% of the cases in the subgroup. There were 27 Hartmann procedures and 16 proximal colostomies. Only 17 patients with Hartman procedure returned for reversal (63%). 11 patients with proximal colostomy returned for the second stage

Table 1. Emergency surgery treatment strategy during the three consecutive timeframes

Topography No of pts. %	Single-stage procedures			Two-stage procedures					
	G1	G2	G3	Stage I			Stage II		
				G1	G2	G3	G1	G2	G3
Left colon	11/96a 11.2%	22/65 33.8%	16/77 20.8%	85/96 88.5%	43/65 66.2%	61/77 79.2%	48/85 56.4%	29/43 67.4%	38/61 62.3%

Table 2. Emergency procedures for complicated left colon cancer patients

Procedure – Stage I	G1	G2	G3	Procedure – stage II	G1	G2	G3
Hartmann	27 28.1%	27 41,5%	28 36,4%	Anastomosis	17/27 63%	17/27 63%	19/28 67,8%
Proximal colostomy	41 42.7%	16 24,6%	33 42,8%	Segmental colectomy	11/41 22.9%	5/16 31.2%	6/33 18,2%
				Left colectomy	11/41 22.9%	6/16 37.5%	13/33 39,4%
Caecostomy	7 7.3%	-	-	Left colectomy	2/7 28.5%		
Internal diversion	10 10.4%	-	-	Left colectomy	7/10 70%%		
Colectomy with primary anastomosis	11 11.4%	22 33,8%	16 20,8%	-	-	-	-
Total	96	65	77		48/85 56.4%	28/43 65.1%	38/61 62.3%

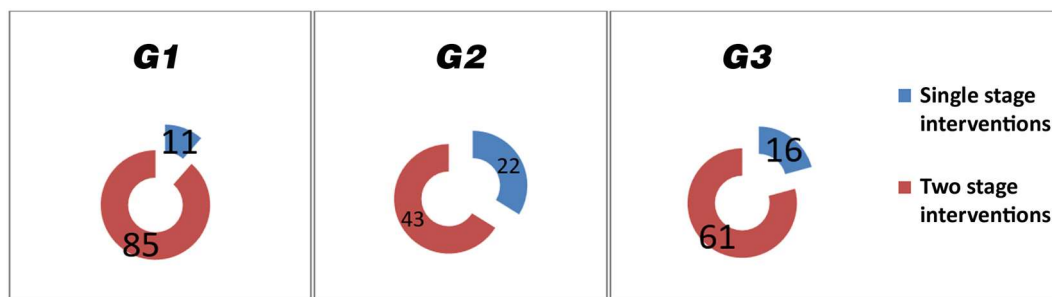


Figure 1. Surgical strategy in the three groups

intervention (68.7%). We performed 5 segmental colectomies and 6 left colectomies (Tables 1 and 2, Fig. 1).

In Group 3, there were 77 patients with complicated left colon cancer. Within Group 3, only 16 patients underwent resections with primary anastomosis, accounting for 20.8% of complicated left colon cancer cases. Specifically, seven cases required left hemicolectomies, 4 involved sigmoidectomies, and 5 necessitated subtotal colectomies. A substantial majority of 61 patients underwent two-stage procedures, accounting for 79.2% of the subgroup. Among these, 28 patients underwent Hartmann operations, with 19 individuals subsequently returning for reversal, indicating a return rate of 67.8%. Additionally, 33 patients received colostomies, among whom subsequent segmental colectomy was performed in 6 cases and left colectomy in 13

cases, resulting in a return rate of 57.5%. The overall return rate for patients within the left colon subgroup was 62.3% (Tables 1 and 2, Fig. 1).

We conducted a comparative analysis of the surgical approach across the three patient groups. We noted an escalation of single-stage procedures from Group 1 to Group 2, with rates increasing from 11.2% to 33.8%. However, within Group 3, we documented a decline in the proportion of single-stage procedures (20.8%), in favour of the two-stage interventions. (Table 1, Fig. 1)

Postoperative morbidity for patients from Group 1 was significantly higher for the single stage procedure cases compared to two-stage procedures (chi² test, p<0.05). We registered 5 patients with anastomotic leak out of the 11 patients with primary anastomosis. This represents 45.4% rate of anastomotic leak. For

comparison, only 2 cases out of the 48 cases that were readmitted in our service for the second stage procedure developed an anastomotic leak (4.1% rate of fistula). There were 3 cases of postoperative peritonitis after primary anastomosis. Two cases had poor evolution with MODS and death. One more patient died through a massive pulmonary embolism thus establishing a 27.2% mortality rate for patients with primary anastomosis (3 out of 11 cases). Mortality rates for two-stage procedure cases were 5.9% for stage one procedures and 2.08% for stage two procedures. Other mild complications included wound infection (6 cases for single stage procedures – 54.5%, 29 cases for stage I of two-stage procedures – 34.1%, 17 cases for stage II of two-stage procedures – 35.4%) and urinary infection (36.3% for single stage procedures, 11.7% for stage I of two stage procedures and 10.4% for stage II of two stage procedures. Also 2 cases developed acute pneumopathy after the first stage of a two-stage intervention (*Table 3*).

Postoperative morbidity analysis in Group 2 showed significantly higher rates for patients with primary anastomosis compared with the two-stage procedure cases (χ^2 test, $p < 0.05$). We noted 5 cases of anastomotic leak representing 22.7% of patients with single stage procedures. 3 patients were treated conservatively but 2 cases developed peritonitis (9.1%) and there was 1 case of sepsis. Other mild complications included 4 wound infections and 2 urinary infections. Mortality rate for single stage procedures was 9.1% (2 cases of 22). In the two-stage procedure group we registered 5 wound infections (11.6%) and 3 urinary infections (6.9%) with a 4.6% mortality rate (2 cases of 43) for the first stage procedures and 1 anastomotic leak (3.5%), 3 wound infections and 2 urinary infections for the second stage procedures (*Table 3*).

In Group 3, the mortality and morbidity rates were higher for single stage procedures compared to two-stage procedures (χ^2 test, $p < 0.05$). We found 2 cases of anastomotic leak

Table 3. Group 1 vs. Group 2 vs. Group 3: postoperative morbidity and mortality

Complication	Single-stage procedures			Two-stage procedures					
	Gr. 1 11 pts.	Gr. 2 22 pts.	Gr. 3 16 pts.	Stage I			Stage II		
	Gr. 1 11 pts.	Gr. 2 22 pts.	Gr. 3 16 pts.	Gr. 1 85 pts.	Gr. 2 43 pts.	Gr.3 61 pts.	Gr. 1 48 pts.	Gr. 2 28 pts.	Gr. 3 38 pts.
Wound infection	6/11 54.5%	4/22 18.1%	3/16 18.7%	29/85 34.1%	5/43 11.6%	6/61 9.8%	17/48 35.4%	3/28 10.7%	4/38 10.5%
Anastomotic leak	5/11 45.4%	5/22 22.7%	2/16 12.5%	-	-	-	2/48 4.1%	1/28 3.5%	2/38 5.2%
Peritonitis	3/11 27.3%	2/22 9.1%	1/16 6.25%	-	-	-	-	-	-
Urinary infection	4/11 36.3%	3/22 13.6%	2/16 12.5%	10/85 11.7%	3/43 6.9%	3/61 4.9%	5/48 10.4%	2/28 7.1%	3/38 7.9%
Thromboembolism	1/11 9.1%	-	-	-	-	-	-	-	-
Sepsis (MODS)	2/11 18.1%	1/22 4.5%	1/16 6.25%	-	-	3/61 4.9%	-	-	-
Global morbidity	8/11 72.7%	7/22 31.8%	4/16 25%	34/85 40%	6/43 13.9%	7/61 11.4%	20/48 41.6%	3/28 10.7%	5/38 13.1%
Mortality	3/11 27.2%	2/22 9.1%	1/16 6.25%	5/85 5.9%	2/43 4.6%	3/61 4.9%	1/48 2.08%	-	-
P (χ^2 test) Comparison between groups	G1/G2 – $p < 0.05$ – signif.			G1/G2 – $p < 0.05$ – signif.					
	G2/G3 – $p < 0.05$ – signif.			G2/G3 – $p > 0.05$ – non signif.					
P (χ^2 test) Comparison between strategies within each group	G1 – Single stage/II stage – $p < 0.05$ – signif.								
	G2 – Single stage/II stage – $p < 0.05$ – signif.								
	G3 – Single stage/II stage – $p < 0.05$ – signif.								

(12.5%), one case of peritonitis (6.25%) and one case of sepsis (6.25%) for the single stage procedures. Other mild complications included 3 wound infections (18.7%) and 2 urinary infections (12.5%). Mortality was 6.25% (one case). For the two-stage procedures there were 6 wound infections (9.8%) and 3 urinary infections (4.9%) in the first stage surgery, as well as 3 cases of prolonged sepsis (4.9%), with a mortality rate of 4.9% (3 cases). For second stage procedures we registered 2 anastomotic leaks (5.2%), 4 wound infections (10.5%), and 3 urinary infections (7.9%) (*Table 3*).

For patients with complicated left colon cancer, a notable decrease in morbidity and mortality rates was observed between Group 1 and Group 2 across both single-stage and two-stage procedures (χ^2 test, $p < 0.05$). Furthermore, a decrease in morbidity and mortality rates was evident between Group 2 and Group 3 for single-stage procedures (χ^2 test, $p < 0.05$), while rates for two-stage procedures remained relatively similar (χ^2 test, $p > 0.05$) (*Table 3*).

Discussion

Emergency surgery for complicated left colon cancer remains a formidable aspect of surgical practice, presenting challenges not only due to the technical difficulties associated with colorectal procedures but also to the intricate decision-making process regarding the most appropriate surgical strategy. In contrast, for cases of complicated right colon cancer, there appears to be a reduced level of controversy regarding the optimal surgical technique. Specifically, right colectomy with primary anastomosis is widely acknowledged as the standard therapeutic procedure. Notably, even in emergency scenarios, the incidence of anastomotic leakage remains below 5%, underscoring the favorable risk profile associated with this approach (10,16).

Regarding complicated left colon cancer there is still a great level of controversy surrounding the choice of single stage or two-stage procedures. The discourse appears ostensibly straightforward, with single-stage

interventions being associated with elevated postoperative morbidity and mortality rates (17). However, this does not necessarily imply an exclusive preference for two-stage procedures. Indeed, single-stage approaches offer distinct advantages, such as the avoidance of additional surgical interventions, thereby mitigating the risk of further morbidity and mortality. Furthermore, the absence of tumour progression during the interval between first-stage and second-stage procedures is a notable benefit. Moreover, empirical evidence suggests that approximately 30% of patients who undergo diverting stoma creation never undergo stoma reversal, thereby potentially exacerbating long-term morbidity concerns. These considerations underscore the nuanced nature of the decision-making process in selecting the most appropriate surgical strategy for complicated left colon cancer (18-20).

Previous research has indicated comparable postoperative evolution and long-term oncological outcomes between patients undergoing emergency procedures and those undergoing elective surgery (21-23). Moreover, a growing body of literature suggests an increased trend towards undertaking single-stage curative interventions in emergency scenarios. This trend extends to cases of significant colonic distension, where preference is often given to single-stage procedures, frequently involving total or subtotal colectomy with ileo-colic or ileo-rectal anastomosis (24-26). While segmental colectomy is associated with superior functional outcomes compared to total or subtotal colectomy, the anastomosis between a dilated proximal colon and a normal-sized distal colon or rectum poses challenges and carries a heightened risk of postoperative leakage. Nevertheless, such procedures can be successfully executed in carefully selected cases under the expertise of experienced colorectal surgeons (16,27-30).

Emergency treatment of complicated colon cancer has been a preoccupation of our department for many years. While two-stage procedures are associated with lower morbidity and mortality, there are undeniable

advantages of single stage procedures but with careful case selection. As previous literature advocates for higher rates of resections with primary anastomosis, it is hard to know how much we can increase the rate of single stage interventions. It is difficult to choose the right management in the emergency surgery of left colon cancer. Literature data, personal experience and department experience can have a role in the decision making process.

Our retrospective analysis of the first time period, 2001-2010, revealed a rate of single stage procedures of 11.2% for Group 1. At the time this retrospective analysis was conducted, in 2011, literature findings suggested the possibility of much higher rates of resections with primary anastomosis, either by performing subtotal and total colectomies or segmental colectomies with intra-operative bowel wash-out. This attitude needs to be associated with high colorectal surgical experience and careful patient selection (31-35).

Encouraged by the literature of the time and the increasing experience of our surgical staff, we concluded that a bolder attitude could be taken towards complicated left colon cancer management resulting in an increase of the single stage procedure rate, provided of course that we observe a very careful patient selection.

Analysing the next period of time, we observed a notable escalation in the proportion of patients with complicated left colon cancer who underwent single-stage surgery within Group 2 (2011-2016) in contrast to Group 1 (2001-2010), with rates of 33.8% versus 11.4%, respectively. This observed trend aligns with the current literature. Moreover, there was a statistically significant reduction in postoperative morbidity and mortality among patients undergoing primary anastomosis in Group 2 in comparison to those in Group 1. We attribute this decline in morbidity and mortality rates to a more discerning selection process for cases undergoing single-stage procedures. Additionally, an increased expertise of the surgical team warrants consideration as a contributing factor.

An alternative perspective involved examining postoperative morbidity and mortality rates among patients undergoing single-stage procedures compared to those undergoing two-stage procedures. Notably, single-stage procedure cases within Group 1 displayed significantly elevated levels of morbidity and mortality when compared with two stage procedures. Although in Group 2, morbidity and mortality rates for single-stage procedures decreased relative to Group 1, they remained markedly higher when compared to the corresponding rates for the two-stage procedures in Group 2, despite meticulous case selection and increased surgical team experience.

Also, morbidity and mortality rates for single stage procedures in Group 2, corresponding to the 2011-2016 period, were higher than the data retrieved from current literature which presented rates of anastomotic leak as high as 9.1% compared to 22.7% in our study. Furthermore, our rates of global morbidity and mortality were also higher than literature findings (36-40).

The conclusion at the time was that a revision of our attitude needed to be considered with a more conservative approach towards complicated left colon cancer.

Consequently, this prompted a more cautious approach in the management of complicated left-sided colon cancer for the following period, 2017-2023. As a result, within Group 3, there was a reduction in the proportion of patients undergoing single-stage procedures with 20.8% compared to 33.8% in Group 2. While the postoperative morbidity and mortality rates remained higher than those for two-stage procedures within Group 3, they were significantly lower than those observed for single-stage procedures within Group 2. This proves that besides high experience in colorectal surgery of the operative team, careful case selection is the key to lowering postoperative morbidity and mortality.

Current literature shows a decreasing trend in the rate of single stage interventions for obstructive left colon cancer in favour of

colonic stents as bridge to surgery but still present a 48% rate of colon resections with primary anastomosis, a significantly higher rate than our study. However, the morbidity and mortality rates registered in our research are comparable to literature findings (41-45).

Conclusions

For complicated left colon cancer, our study recorded an initial increase in the rate of colonic resections and primary anastomosis encouraged by the high rate of single stage procedures observed in the literature findings at the time. But, the high rates of morbidity and mortality registered in our research led to a more cautious strategy in the following period with decreased percentage of single stage procedures and subsequently lower rates of morbidity and mortality.

Two stage procedures seem to be a better strategy in emergency setting for complicated left colon cancer, as resections with primary anastomosis, even with selected cases and an experienced surgical team, are still associated with higher postoperative morbidity and mortality. Further studies are needed to settle the controversy, perhaps taking into consideration the alternative of colonic stents as bridge to curative surgery.

Conflict of Interest and Funding

There is no conflict of interest to disclose concerning this paper.

Ethical Statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the University of Medicine and Pharmacy of Craiova (registration number: 68 / 28th of April, 2021).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

Data available on request.

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