• Silk Road Medicine Research •

Perioperative changes in lower gut microbiocenosis in patients with colon and rectum cancer

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Abstract: This clinical study was designed to determinate the value of colon microbiota microflora in colorectal cancer patients. We investigate pre- and post-operation stool samples in patients with different tumor localizations, trying to correlate those to postoperative complications. Our findings shows that if opportunistic pathogenic microflora levels are elevated before operations, it can lead to further microbiota disbalance in postoperative period, especially in cases of antibacterial prophylaxis or treatment. Further, this changes increase a chance of receiving some complications after surgery. So, we suggest an analysis of stool microbiota in patients with colorectal cancer to be performed prior and after the operation. Furthermore, a perioperative decontamination therapy should be performed in cases of pathogenic microflora levels elevation. Still, monitoring a perioperative microbiota changes is not a routine in most recommendations and we assume that including this relatively unencumbered patient testing in preoperative survey will help to reduce a number of postoperative complications.

Keywords: colorectal cancer, colon microbiota microflora, dysbacteriosis, colitis, antibacterial prophylaxis or treatment

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Introduction

Technological advances in molecular genetics have significantly altered qualitative and quantitative perceptions of microbiota composition. It is known that an absolute majority (89%) of the microbiota in a healthy human being belongs to one of two phyla-Bacteroidetes and Firmicutes-each of which consists of a multitude of genera. Bacteroidetes is a group of anaerobic, gram-negative bacteria of three classes-the Bacteroidetes, the Flavobacteria, and the Sphingobacteria. Firmicutes are gram-positive bacteria of two primary classes-the Bacilli and Klebsiella. The general related to Firmicutes include Streptococcus, Staphylococcus, Lactobacillus, and Enterococcus. Not well-known but widespread genera are Veillonella, Ruminococcus, Eubacteria, and Peptostreptococcus (Fig. 1). The most prevalent of the Firmicutes are the Clostridia. It has been established that the total quantity of Bifidobacterium in the order Actinomycetales generally does not exceed 5% of the total intestinal flora, and Lactobacillus does not exceed 1%[1].

A significant number of international journals

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are reporting the presence of a vast intestinal microbiota detected using bacteriological methods, together with surgical practice, in patients with colorectal cancer. As a result, some degree of intestinal microbiocenosis disorder was detected in almost all patients because of low counts of Lactobacillus and Bifidobacterium and growth of opportunistic flora; however, most of these studies are very basic[2-5].

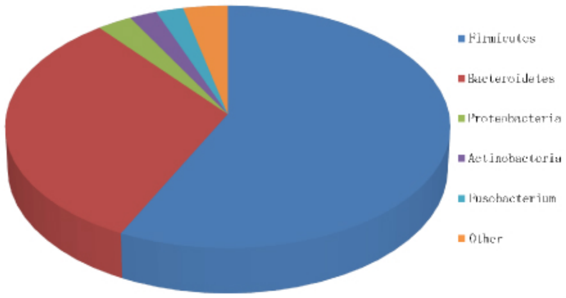

Figure 1　Composition of human intestinal microbiota

Despite advances in surgery and intensive medicine, very low incidence of postoperative complications, and patients following early rehabilitation protocols, the intestinal microflora plays a major role in the pathogenesis of a specific proportion of complications (abdominal sepsis, antibiotic-associated colitis, and incompetence of intestinal anastomosis)[6-8].

Many methods of correcting intestinal dysbiosis have been suggested, from administering eubiotics and probiotics to fecal transplantation; however, no method has become standard practice or is sufficiently effective[9-12].

Currently, most scientific studies are focused on investigating the possible role of microbiota in intestinal carcinogenesis and developing cancer treatments that normalize microbiocenosis. Additionally, the search for microbiological markers that would permit early diagnosis of colon cancer is ongoing[13-14].

In contrast, there are a few publications tracking colonic microbiota in patients with colorectal cancer prior to and after surgery using modern genetic-based methods.

The search for correlations between the condition of the intestinal microbiota and type of complication, tumor localization, and antibiotic prophylaxis is the purpose of this study.

Materials and Methods

A follow-up comparative study was performed. All of the procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Declaration of Helsinki and its later amendments. For all of the patients, informed consent was obtained.

The intestinal microflora of 38 patients with colorectal cancer was examined. Women slightly outnumbered men at 55.5% (n = 21) and 44.5% (n = 17), respectively. Most patients (73%) were either of an elderly or of a senile age (60—89 years old). The study included all patients on whom surgery had been performed for the diagnosis of colon cancer. Patients with distant metastasis were excluded.

Tumors were localized in 40% (n = 15) of cases in the right colon, in 26% (n = 10) of cases in the rectosigmoid transition zone, in 18% (n = 7) of cases in the rectum, and in 16% (n = 6) of cases in the left colon. Tumors in all the patients were verified to be adenocarcinomas (Table 1).

Table 1　The adenocarcinoma tumors′ localization in patients

Tumor localization	n	%
Right colon cancer	15	40
Rectosigmoid colon cancer	10	26
Rectum cancer	7	18
Left colon cancer	6	16
Total	38	100

In 37% (n = 14) of patients, the tumor had narrowed the intestinal lumen (1-cm wide colonoscopy instrument could not pass through the lumen giving a clinical picture of subcompensated intestinal obstruction) and possessed signs of peritumoral infiltrate. Neoadjuvant chemoradiation was performed on four (57%) of seven patients with rectal cancer.

Preoperative preparation of the colon included a low-residue diet for seven days prior to surgery. Patients with rectal tumors received purgative enemas the night before and the morning prior to surgery. Bulk laxatives were not prescribed.

All patients underwent surgery as scheduled.

Standard laparoscopic procedures were performed in 66% (n = 25) of cases, whereas open surgery was performed in 34% (n = 13) of cases. Anterior rectal resection operations with total mesorectumectomy were followed by mandatory reversal of preventive transverse colostomy (Table 2).

Table 2 The type of performed operation

Operation type	Laparoscopic	Laparotomy
Abdominoperitoneal resection	2	1
Frontal rectal resection with total mesorectal excision	7	2
Right hemicolectomy	9	6
Left hemicolectomy	5	1
Hartmann's procedure	2	3
Total	25	13

All patients were administered antibiotic prophylaxis. The following regimen was followed most often-intraoperative: Ampisid (Ampicillinum + Sulbactamum) 1.0 g, postoperative: Ampicillinum 1.5 g TID for three days. Seven patients received additional Metronidazole therapy, 400 mg 2 times a day, primarily due to signs of peritumoral inflammation. Microflora of all the patients were analyzed seven days prior to and ten days after surgery. The sampling, transport, and storage of fecal matter was conducted in strict compliance with methodical recommendations[16].

The sample container was stored until analysis at 2—8℃. The time from sampling to analysis was no greater than 48 h.

We used real-time polymerase chain reaction (PCR) using a selection of KOLONOFLOR reagents, which included a PCR amplification mix for all bacteria (general bacterial mass) and amplification mixes for each type (or group) of bacteria detected (the list of detected microorganisms is given in Table 3.

Results

Analysis of our findings showed changes in quantitative and qualitative microflora composition before and after surgery in all patients (Table 4) (Fig. 2). Primarily, changes were observed as elevated total bacterial mass (Fig. 3), counts of Lactobacillus spp. (Fig. 4) and Bifidobacterium spp. (Fig. 5), significant anaerobic imbalance (Fig. 6), and progressing elevation of opportunistic pathogenic microflora. Prior to surgery, 87% of patients had an elevated total bacterial mass, and 95% of Lactobacillus spp. and 97% of Bifidobacterium spp. were elevated. In 32% of patients, anaerobic imbalance was detected, primarily due to significantly (89%) elevated count of Bacteroides fragilis group (Fig. 7). Prior to surgery, Escherichia coli (Fig. 8) was elevated in 50% of patients, and Enterobacter spp. (Fig. 9) was elevated in 71% of cases. Of the opportunistic pathogenic flora, 63% of patients had elevated enteropathogenic E. coli (Fig. 10), and a third had elevated Fusobacterium spp. and Clostridium perfringens. Clostridium difficile (Fig. 11) was observed in 8% of patients prior to surgery.

Table 3 The list of detected microorganisms and amplification mixes

Sample №	Amplification reagents	
	FAM	HEX
1	Total bacterial mass	
2	Lactobacillus spp.	
3	Bifidobacterium spp.	
4	Escherichia coli	
5	Bacteroides fragilis group	
6	Faecalibacterium prausnitzii	
7	Klebsiella pneumoniae	Klebsiella oxytoca
8	Candida spp.	Staphylococcus aureus
9	Escherichia coli enteropathogenic	
10	Enterococcus spp.	
11	Bacteroides thetaiotaomicron	
12	Clostridium difficile	Clostridium perfringens
13	Proteus spp.	
14	Enterobacter spp. /Citrobacter spp.	
15	Fusobacterium nucleatum	
16	Salmonella spp.	Shigella spp.

Table 4 The changes in microflora composition before and after surgery

Index		Before surgery		After surgery	
		%	n	%	n
Total bacteria mass	↑	87	33	97	29
Lactobacillus spp	↑	95	36	97	29
Bifidobacterium spp	↑	97	37	87	26
Escherichia coli	↑	50	19	70	21
Bacteroides fragilis group	↑	89	34	67	20
Bacteroidesfragilis/Faecalibacterium prausnitzii ratio (anaerobic disbalance)	↑	32	12	63	19
Enterobacter spp	↑	71	27	93	28
Escherichi coli enteropathogenic	↑	63	24	73	22
Fusobacterium spp	+	34	13	17	5
Clostridium perfringens	+	32	12	10	3
Klebsiella oxytoca	↑	18	7	20	6
Klebsiella pneumoniae	↑	13	5	29	11
Clostridium difficile	+	8	3	26	10
Citrobacter spp	↑	13	5	20	6
Enterococcus spp	↑	10	4	23	7
Proteus vulgaris	↑	5	2	7	2
Candida spp	↑	3	1	7	2
Staphylococcus aureus	↑	3	1	10	3

Comment: ↑: increased amounts; +: bacteria presence

In the postoperative period, anaerobic imbalance was noted in 63% of patients. In this regard, Lactobacillus spp., Bifidobacterium spp., and B. fragilis group levels were either elevated or normalized. The increase in the number of patients with elevated E. coli (70%), Enterobacter spp. (93%), and enteropathogenic E. coli (73%) was notable. Almost one-third of patients possessed elevated C. difficile, Klebsiella pneumoniae, Citrobacter spp., and Enterococcus spp. after surgery.

Multi-factor analysis demonstrated that microflora changes were not statistically dependent on the gender or age of patients, scope or method of surgical intervention, or on tumor-induced stenosis and infiltration ($P<0.05$).

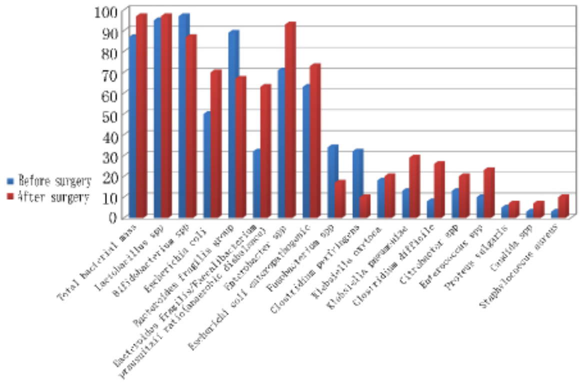

Figure 2　Dynamics of perioperative intestinal microbiota

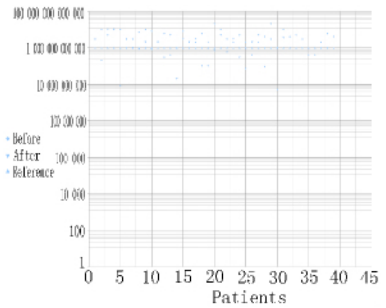

Figure 3　Dynamics of perioperative total bacterial mass

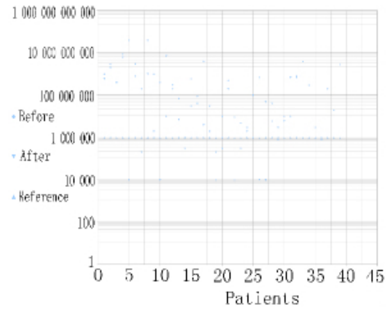

Figure 4　Dynamics of perioperative Lactobacillus spp

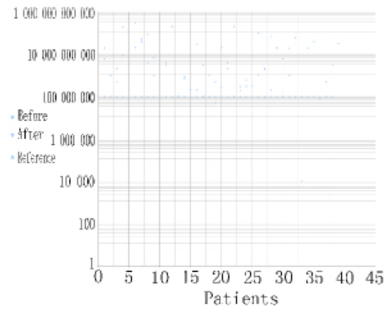

Figure 5　Dynamics of perioperative Bifidobacterium spp

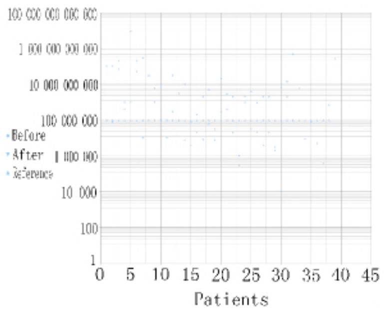

Figure 6　Dynamics of perioperative Escherichia coli

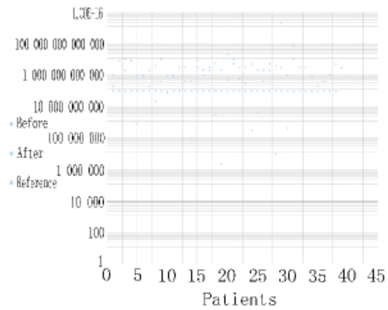

Figure 7　Dynamics of perioperative Bacteroides fragilis group

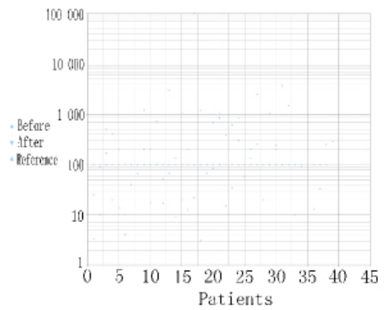

Figure 8　Ratio of Bacteroides fragilis/Faecalibacterium prausnitzii (anaerobic imbalance) in perioperative period

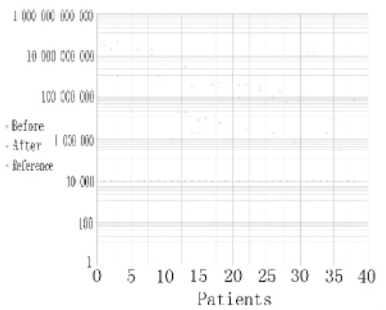

Figure 9　Dynamics of perioperative enteropathogenic Escherichia coli

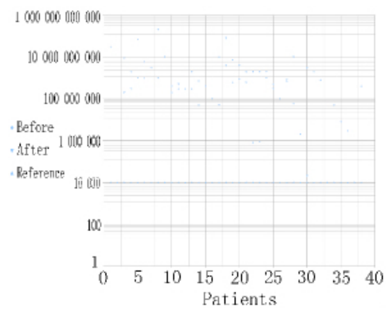

Figure 10　Dynamics of perioperative Enterobacter spp

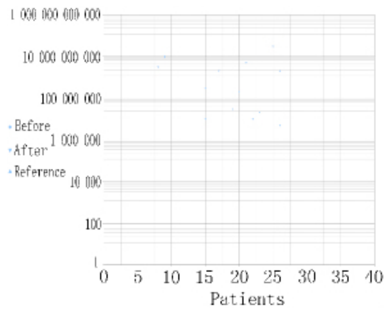

Figure 11　Dynamics of perioperative Clostridium difficile

The elevation of Lactobacillus spp., Bifidobacterium, and B. fragilis group in all patient categories was statistically significant, which somewhat contradicts data presented in the publications provided[2-5]. Significant anaerobic imbalance was most frequently diagnosed during the postoperative period, especially after antibiotic prophylaxis and therapy. C. difficile and C. perfringens were most frequently diagnosed in patients with tumors of the right colon ($P < 0.05$).

Postoperative complications arose in five patients (13.2%): (1) lower pelvic abscess (infected hematoma); (2) postoperative wound inflammation; and (2) dehiscence of anastomosis (6%). However, no deaths occurred. It should be noted that all patients underwent laparoscopic surgery and two received preoperative chemoradiation.

Detailed analysis of the changes in microflora composition in patients with complications revealed that elevated Lactobacillus spp., Bifidobacterium spp., and B. fragilis group levels tended to normalize. In this regard, more so than in the remaining group of patients, this group experienced anaerobic imbalance due to dominance of opportunistic pathogenic microflora, the significance of the specific types thereof may not be evaluated due to a small number of observations (Table 5). A month after the surgery, 34% ($n = 13$) of patients exhibited signs of irritable bowel syndrome, and most of these patients were diagnosed with clostridial infection during the postoperative period. This required decontamination by prescribing enterotropic antibiotics and probiotics, which helped to cure this complications.

Table 5 The changes in microflora composition before and after surgery

Index	1		2		3		4		5	
	before	after	before	after	before	after	before	after	before	after
Total bacteria mass	↑	↑	N	↑	↑	N	N	↓	↑	↑
Lactobacillus spp	↑↑	↑	↑	N	↑	↑↑	↑↑	↑	↑	N
Bifidobacterium spp	↑	↑↑	↑	↑↑	↑↑	↑	↑	N	↑	↑
Escherichia coli	N	↑	↑	↑↑	N	↑	↑	↓	↑	↑
Bacteroides fragilis group	↑	↑↑	↑	↑↑	↑	↓	↑	↓	↑	N
Bacteroides fragilis/Faecalibacterium prausnitzii ratio	N	N	N	↑	N	0	↑	↑↑	↑	↑↑
Enterobacter spp	N	↑	↑	↑↑	↑	↑↑	↑↑	↑	↑	↑
Escherichi coli enteropathogenic	N	↑	↑	↑	N	↑	↑	0	↑	↑
Fusobacterium spp	0	0	↑	0	0	0	↑	0	↑	0
Clostridium perfringens	0	0	↑	0	↑	0	↑	0	0	0
Klebsiella oxytoca	N	↑	N	N	N	N	N	N	N	N
Klebsiella pneumoniae	N	N	N	N	N	N	↑	N	N	N
Clostridium difficile	0	0	0	↑	0	0	0	0	0	↑
Citrobacter spp	N	N	N	N	↑	N	↑	N	N	N
Enterococcus spp	N	↑	N	N	N	N	N	N	N	N
Proteus vulgaris	N	N	N	N	N	N	↑	N	N	N
Candida spp	N	N	N	N	N	N	N	N	N	N
Staphylococcus aureus	N	N	N	N	N	N	↑	N	N	N

Comment: ↑: increased amounts; ↑↑: highly increased amount; 0: lack of bacteria; N: reference

Discussion

This issue is very relevant to clinical practice at present. Many publications provide detailed analysis of colonic microbiota in patients with colorectal cancer and almost immediately suggest a method for treating detected changes using eubiotics and probiotics. Nevertheless, quotidian surgical practice indicates that dysbiosis remains a "dark horse". No single pre-or postoperative colonic microflora analysis exists as an accepted part of standardized care for patients undergoing surgery or any category of patients with colorectal cancer[17-19].

The results of our study somewhat contradict the data found in the literature[20]. The study revealed the presence of lacto- and bifidoflora both before and after surgery, but individual patients tested at low-normal level. Almost all patients were diagnosed with anaerobic imbalance, primarily due to elevated bacteroides and opportunistic pathogenic microflora. Quantitative and qualitative opportunistic pathogenic microflora composition was also different from those described in other publications[20-21]. Analysis of postoperative colonic microbiocenosis is especially interesting. C. difficile and many other pathogenic and opportunistic pathogenic microbes were discovered in every third patient, especially those with tumors in the right colon.

Conclusions

Changes in microflora changes did not depend on patient gender or age, scope or method of surgical intervention, or tumor-induced stenosis and infiltration (p 0.05). Significant anaerobic imbalance was most frequently diagnosed during the postoperative period, especially after antibiotic prophylaxis and therapy in patients with tumors of the right colon (p 0.05). Analysis of microbiota in stool samples of patients with colon cancer should be performed prior to and after surgery. If opportunistic pathogenic microflora are elevated, preoperative decontamination should be performed using enterotropic antibiotics or personalized antibiotic prophylaxis to be followed with mandatory postoperative analysis and outpatient treatment. Specialized medical communities should consider colonic microbiota analysis prior to and after surgery as a part of standard medical care.

The study poses questions that absolutely must be answered. What is the distinction between increased pathogenic and opportunistic pathogenic microbes and infection? Does opportunistic pathogenic microflora require preoperative decontamination? Alternatively, perhaps should preventive antibiotic treatment be personalized, taking into account the changes in microbiota? What is the place and significance of eubiotics and probiotics during the preoperative period considering the findings?

Our study indicates that the role of dysbiosis in complications is unclear. However, it is clear that microflora should be analyzed before the patient is discharged. One-third of patients were diagnosed with elevated pathogenic and opportunistic pathogenic microbes. The quality of life of these patients is significantly diminished, thus dysbiosis should be treated in the outpatient setting.

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实护理责任制，深化护理专业内涵，整体提高护理服务水平。《WHO噪声指南》指出，医院内噪声不超过30 dB(A)。有研究报道，病房内的各种噪声均超过80 dB(A)[3]。为患儿及家长提供一个安静的病区环境，降低噪音带来的危害，不仅是落实优质护理服务的具体举措，同时也是科室护理管理的目标之一。科室在应用"站点式流动治疗车"工作模式后，由实施前的733次/w，降为实施后的368次/w，有效地降低了儿科病房铃声呼叫的频次。

3.2 "站点式流动治疗车"的建立，提高了护理质量和护士的满意度 科室主要收治小儿呼吸系统疾病，其疾病的特点是年龄越小，病情越重，并发症越多，死亡率越高[4]。住院患儿中，婴幼儿占80%，其病情变化快，静脉穿刺难度大，家长要求高，输液的批次又较多，因此护士心理压力大，身心疲惫。护士对管理是否满意直接关系到护士的工作效率和质量。有调查显示，76.96%护士感到工作强度过大，89.09%护士担心工作出错[5]。巨大的心理压力影响护士的健康[6]。响铃次数多，反映出护士在患儿身边时间短，巡视病房时间有限，而且响铃次数多时，护士往往不得不放下正在进行的工作，去应答铃声和处理事件，干扰了正常的工作程序，极易发生护理差错[7]。实施"站点式流动治疗车"将治疗车前移至病区走廊，无需往返于病房、护士站及治疗室之间，缩短了护士来回频繁奔跑的距离，既保存了护士体力又节省了时间，护士对其满意度达100%。护士独立管理患儿，责任成就感增强。彻底改变了以前完成患儿治疗工作滞留护士站的现象，减少了病房铃声次数，患儿安全得到保障[8]。护士将更多的时间用于患儿及家长的交流，既可以及时解决患儿的输液问题，提高输液质量，又可以及时发现患儿的病情变化，降低了隐患的发生，保证了护理质量。

3.3 "站点式流动治疗车"的开展，提高了患儿家长的满意度 开展"站点式流动治疗车"工作模式前，患儿家长满意度不高的原因之一是由于护士有依赖铃声的心理，主动服务意识欠缺，有时因为没有主动巡视、合理统筹安排，导致工作忙乱，患儿输液完毕得不到及时地更换或拔针，患儿家长不停地按呼叫器，使输液高峰期铃声频繁，直接影响患儿的输液安全及安静休养，更增加了患儿家长的不满。其次是护士健康教育能力和护患沟通能力欠缺。"实施站点式流动治疗车"后，定期培训护士沟通能力和业务知识，不仅提高了护士的工作效率，而且提升了护士的主动服务意识及专业服务内涵。在工作中，让护士明白巡视工作本身即是关注患儿也是保护自己[9]。护士主动服务意识增强，为患儿及家长提供及时有效的服务，得到了患儿家长的肯定和好评，提高了满意度。

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