Persisting changes of intestinal microbiota after bowel lavage and colonoscopy

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Objective An adequate bowel preparation is essential for a successful colonoscopy, but to date, only scarce information exists on the impact of the bowel cleansing on the gut microbiota, in particular, 1 month after the procedure.

Patients and methods Through 16S rDNA Ion Torrent profiling of fecal samples of 10 patients, we evaluated changes that occurred in the gut microbiota composition immediately after a 4 liter polyethylene glycol-based (SELG Esse) bowel lavage and 1 month thereafter. We studied the gut microbiota at the phylum, class, and family level.

Results At the phyla level, we found a significant decrease in *Firmicutes* abundance and an increase in *Proteobacteria* abundance immediately after the colon cleansing and 1 month after the colonoscopy, whereas, at the class level, a significant increase in γ-*Proteobacteria* immediately after the colonoscopy was observed. Interestingly, 1 month after the endoscopic examination, this bacterial class was decreased 2.5-fold compared with samples before colonoscopy, as well as α-*Proteobacteria.* At the family level, a significant reduction in *Lactobacillaceae* and an increase in *Enterobacteriaceae* abundance were observed immediately after the colonoscopy, whereas 1 month after the bowel cleansing, these families were significantly lower compared with samples collected before the colonoscopy. Moreover, the abundance of *Rikenellaceae* and *Eubacteriaceae* has been observed to be significantly higher compared with samples collected before the bowel lavage. Finally, *Streptococcaceae* were increased 4.0-fold 1 month after the bowel lavage compared with fecal samples collected before the colonoscopy.

Conclusion We provide clear evidence that, in normal individuals, a high-volume polyethylene glycol bowel cleansing preparation has a long-lasting effect on the gut microbiota composition and homeostasis, in particular, with a decrease in the *Lactobacillaceae* abundance, a population of protective bacteria. Further studies are required to assess whether these changes have any metabolic, immunological, or clinical consequence. Eur J Gastroenterol Hepatol 28:532–537

Introduction

Colonoscopy is the preferred method to evaluate the colon in most adult patients with large-bowel symptoms, iron-deficiency anemia, abnormal results on radiographic studies of the colon, positive results on colorectal cancer (CRC) screening tests, postpolypectomy and postcancer resection surveillance, and diagnosis and surveillance in inflammatory bowel disease (IBD) [1]. Irrespective of indication, the success of colonoscopy is closely linked to the adequacy of preprocedure bowel cleansing. Unfortunately, up to 20–25% of all colonoscopies are reported to have an inadequate bowel preparation [2,3]. The existing literature, therefore, has mainly focused on ways to improve the adequacy of colon cleansing, but to date, scarce information exists on the effects of bowel lavage on the colonic microbiota [4]. In particular, how the intestinal bacteria composition is affected by colonic lavage and how it may influence the human health have so far been poorly characterized. The human gut is colonized by 100 trillion microorganisms and at least 1000 different species coexist in the colon [5,6]. The gut microbiota is fundamental to promoting normal mammalian physiology including angiogenesis, metabolism, digestion, and immune system development [7–10], and several studies have highlighted the role of intestinal dysbiosis in many pathologies, such as IBD and allergies [11,12]. Disturbances such as antibiotic therapy are known to have a significant effect on the intestinal bacteria composition, which is markedly altered after the administration of antimicrobial agents [4,5]. It has been observed that not all bacterial taxa are affected in the same way by such disturbances; indeed, some bacterial families and genera are more resistant than others, conferring potential pathogenic bacteria, normally suppressed by the commensal microbiota, the ability to proliferate, being harmful for the host organism [13]. Previous studies have reported no alteration of the gut microbiota composition after bowel cleansing, indicating that colonic lavage did not alter the bacterial diversity even when the total microbial load was halved [14–16]. Moreover, it has been reported that bowel cleansing might lead to temporary changes in the mucus layer and microbial changes in the mucosal tissue after bowel preparation, where an increased amount of *Proteobacteria* has been detected [15]. However, the

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bacterial changes observed in the aforementioned studies are often not coherent and, above all, very patient-specific; thus, it is very difficult to establish an objective and significant trend that can characterize the effect of bowel lavage on the gut microbiota. Nowadays, colonic hydrotherapy is promoted regularly in popular magazines and in the global e-commerce marketplace as this therapy seems to eliminate several symptoms caused by toxic overload [16]. However, it has been shown that introducing a large amount of water trans-anally breaks up solid fecal packaging, leading to the dissemination of toxins produced by certain intestinal microorganisms and contained in fecal boluses [16]. Moreover, it increases the contact surface between the colonic mucosa and bacterial toxins and allows bacteria to enter the systemic circulation [17]. The aim of this study was to evaluate the impact of a standard colonic lavage on the gut microbiota composition of healthy individuals and the impact on the microbial recovery rate 1 month after the colonoscopy to establish whether the bowel cleansing, besides the immediate effects, may have some long-lasting effects on the human microflora.

Patients and methods

Study population

The population analyzed in this study included a group of seven men and three women participating in the regional CRC screening program of Regione Lombardia because of a positive fecal occult blood test. Their mean height was 169.7 cm, their mean weight was 74.3 kg, and their mean BMI was 24.6. The mean age of the individuals was 55.5 years (range 40–68). None of them was vegetarian, four were taking no drugs, two were taking proton pump inhibitors for gastroesophageal reflux disease, one was taking cardioaspirin 100 mg, one was taking metformin and amlodipine, and one was taking candesartan cilexetil. According to the requirements of the Ethical committee, no drug was discontinued during the study. All the participants had a completely negative colonoscopy and were consecutively recruited on this basis.

No participant had been taking antibiotics during the month preceding sampling, nor had developed an infection recently (within the last 3 months). All participants followed a Mediterranean diet during the study period, avoiding in particular the consumption of probiotics and yogurts. Moreover, red meat, alcohol, and fatty foods were excluded from the diet until 1 month after the colonoscopy.

The study was carried out according to ICH guidelines for Good Clinical Practice. All procedures followed were in accordance with the Helsinki of Declaration of 1975, as revised in 2000 and 2008. The study was approved by the Scientific Committee and Scientific Direction of Bolognini Hospital, Seriate (Italy). All participants provided informed consent to have a fecal sample collected three times, respectively, 1 week before, the same day as the intestinal preparation just before the colonoscopy, and 1 month after the procedure. Samples collected at home were immediately frozen in domestic freezers at –20°C before delivery to the Endoscopy Centre within 2 h for storage at –70°C. Samples collected on the day of colonoscopy were immediately frozen at –70°C. After the examination, all participants enrolled in the study followed a recommended balanced diet before collection of the last fecal sample. According to recent endoscopic guidelines [18] all participants were prepared with a standard high-volume (4 liter) polyethylene glycol-electrolyte lavage solution (SELG Esse), four 70 g sachets to be diluted in 1000 ml of tap water each and to be drunk on the afternoon preceding the examination day within 4 h, at a rate of 250 ml/15 min. Patients were instructed to ingest only clear liquids the day before colonoscopy and to eat a low-residue diet during the day before the colon preparation. Colonoscopy was performed under conscious sedation with midazolam (0.03–0.08 mg/kg) intravenously plus meperidine (0.3–0.8 mg/kg) intravenously. The cecum was reached in all patients and the adequacy of the bowel cleansing was confirmed by a Boston Bowel Preparation Scale score of at least 2 (i.e. minor amount of residual staining, small fragments of stool, and/or opaque liquid, but mucosa of the colon segment could be visualized clearly) [19].

DNA extraction

Total DNA was extracted from fecal samples using the QIAamp DNA Stool Mini Kit following the manufacturer's instructions (Qiagen, Milano, Italy). The protocol included the specific binding of DNA to the QIAmp silica-gel membrane while contaminants pass through.

16S rRNA gene amplification

Partial 16S rRNA gene sequences were amplified from extracted DNA using the 16S Metagenomics Kit (Life Technologies, Bologna, Italy), which is designed for rapid analysis of polybacterial samples using Ion Torrent sequencing technology. The kit includes two primer sets that selectively amplify the corresponding hypervariable regions of the 16S region in bacteria: primer set V2-4-8 and primer set V3-6, 7–9. The PCR conditions used were 10 min at 95°C, 30 cycles of 30 s at 95°C, 30 s at 58°C, and 20 s at 72°C, followed by 7 min at 72°C. Amplification was carried out using a SimpliAmp thermal cycler (Life Technologies). The integrity of the PCR amplicons was analyzed by electrophoresis on a 2% agarose gel.

Ion torrent PGM sequencing of 16S rRNA gene-based amplicons

The PCR products derived from amplification of specific 16S rRNA gene hypervariable regions were purified by a purification step involving the Agencourt AMPure XP DNA purification beads (Beckman Coulter Genomics, Bernried, Germany) to remove primer dimers. The DNA concentration of the amplified sequence library was estimated using the Qubit system (Life Technologies). From the concentration and the average size of each amplicon, the amount of DNA fragments per microliter was calculated and libraries were created using the Ion Plus fragment Library kit (Life Technologies). Barcodes were also added to each sample using the Ion Xpress Barcode Adapters 1–16 kit (Life Technologies). Emulsion PCR was carried out using the Ion OneTouch TM 400 Template Kit

(Life Technologies) according to the manufacturer's instructions. Sequencing of the amplicon libraries was carried out on a 318 chip using the Ion Torrent Personal Genome Machine (PGM) system and the Ion PGM Hi-Q kit (Life Technologies) according to the supplier's instructions. After sequencing, the individual sequence reads were filtered by the PGM software to remove low-quality and polyclonal sequences. Sequences matching the PGM 3′ adaptor were also automatically trimmed. 16 rRNA sequences were then analyzed using Ion Reporter Software (Life Technologies, Carlsbad, California, USA), which comprises a suite of bioinformatics tools that streamline and simplify analysis of semiconductor-based sequencing data. The 16S rRNA workflow module in Ion Reporter Software could classify individual reads combining a Basic Local Alignment Search Tool (BLAST) alignment to the curated Greengenes database, which contains more than 400 000 records, with a BLAST alignment to the premium curated MicroSEQ ID database, a high-quality library of full-length 16S rRNA sequences. In the first step, reads were aligned to the MicroSEQ ID library, with any unaligned reads subject to a second alignment to the Greengenes database to achieve rapid and exhaustive bacterial identification. The final output of Ion Reporter Software was the identification and abundance of microorganisms at the phyla, class, and family level.

Statistical analysis

Statistical analyses were carried out with R or Sigma Plot 11.0 using a one-way analysis of variance (ANOVA). *P*-values below 0.05 were considered statistically significant.

The test ANOVA was chosen as it is commonly used to compare the means between three or more groups. Furthermore, the minimum possible sample size for an ANOVA *F*-test would have been one unit greater than the number of groups, and thus, it is applicable to our study, where there were three groups with 10 patients for each group (groups = 3; sample size = 10).

Results

Changes in the gut microbiota composition after the bowel lavage

Results are given in a global structure as on calculating the Shannon index between the patients at each time interval (before the bowel lavage, the day of colonoscopy, and 1 month after the bowel cleansing), no differences in the diversity of gut microbiota composition were observed between different patients (data not shown).

When the gut microbiota were described at the phylum level, significant differences in its composition were observed (Fig. 1). In particular, there was a significant decrease in *Firmicutes* abundance ($P < 0.05$) and a significant increase in *Proteobacteria* abundance ($P < 0.05$). At the class level, the bowel lavage also affected the abundance of γ-*Proteobacteria* and *Coriobacteria*, which showed a significant increase at the time of colonoscopy ($P < 0.05$), and the prevalence of *Clostridia*, which was significantly reduced after the bowel cleansing ($P < 0.05$) (Fig. 2). Several significant differences have also been observed at the family level (Fig. 3); in particular, immediately after the colon cleansing, a decrease in *Lactobacillaceae* ($P < 0.05$) and an increase in *Enterobacteriaceae* ($P < 0.05$) were observed, whereas there was a parallel reduction in the abundance of *Porphyromonodaceae* and *Veillonellaceae* ($P < 0.05$) (Fig. 4).

Specific changes in the gut microbiota composition 1 month after the bowel lavage

At the phylum level, 1 month after the colonoscopy, the microbiota recovered to resemble the composition observed in samples collected before the colonoscopy; indeed, an increase in *Firmicutes* abundance and a reduction in *Proteobacteria* abundance occurred 1 month after the bowel lavage (Fig. 1). At the class level, 1 month after the endoscopic procedure, *Clostridia* and *Coriobacteria* levels reverted to resemble those observed before the colonoscopy, whereas γ-*Proteobacteria* were decreased 2.5-fold compared with samples collected before the lavage (Fig. 2). Moreover, a significant decrease in α-*Proteobacteria* was detected 1 month after the colonoscopy ($P < 0.05$, 3.0-fold changes) (Fig. 2). At the family level, the gut microbiota did not completely revert to the composition observed before the colonoscopy (Fig. 3) as a significant reduction in *Lactobacillaceae* and *Enterobacteriaceae* abundance and an increase in *Rikenellaceae* and *Eubacteriaceae* abundance were observed ($P < 0.05$) (Fig. 5). Furthermore, *Streptococcaceae* increased 4.0-fold 1 month after the bowel lavage ($P < 0.05$) (Fig. 5).

Biodiversity before and after the bowel cleansing

To evaluate the bacterial diversity in the intestinal microbiota before the colonoscopy, after the bowel lavage, and 1 month after the intestinal cleansing, we calculated the Shannon index for each group analyzed, which were, respectively, 2.7, 1.9, and 3.2. Our data indicated that the bacterial biodiversity decreased immediately after the bowel cleansing and increased 1 month after the clinical procedure compared with samples collected before the lavage.

Discussion

Conflicting data on the real impact of bowel cleansing on the gut microbiota composition have been reported [20, 21]. There are several challenges that must be considered during the study of gut microbiota such as undistorted and representative samples from the human gut [4]. In the present study, we observed some significant differences in the intestinal bacteria composition after colonic lavage. A reduction in the relative abundance among the different bacterial phyla was detected after the bowel cleansing; in particular, the lavage procedure led to a significant increase in *Proteobacteria* abundance and a decrease in *Firmicutes* abundance. This intestinal dysbiosis because of an altered microbiota has been linked to diarrhea, and more interestingly, a recent study highlighted the association between the increase in *Proteobacteria* and the onset of moderate to severe diarrhea in children from low-income countries [21], providing a first evidence of the potential negative impact of bowel lavage on the gut microbiota. At the family level, we observed an increased

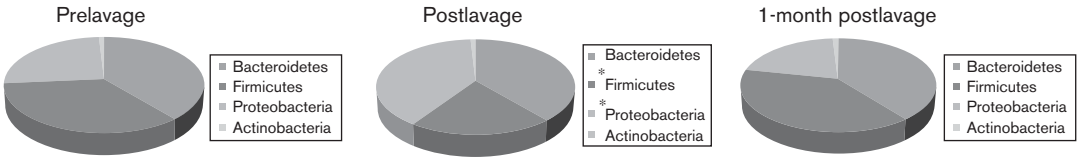

Fig. 1. Effect of bowel lavage on the microbial composition at the phylum level. Statistical significance is indicated with an asterisk (*) ($P < 0.05$).

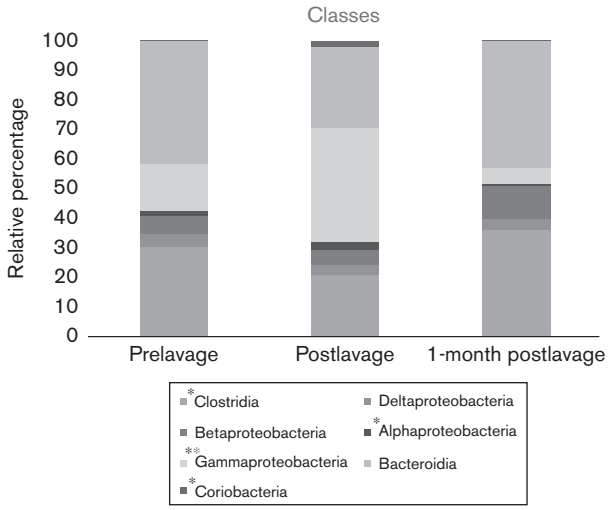

Fig. 2. Effect of bowel lavage on the microbial composition at the class level. Statistical significance between samples collected before the bowel lavage and samples collected on the day of colonoscopy is indicated with a dark gray asterisk (*) ($P < 0.05$), whereas statistical significance between samples collected before the bowel lavage and samples collected 1 month after the colonoscopy is indicated with a light gray asterisk (*) ($P < 0.05$).

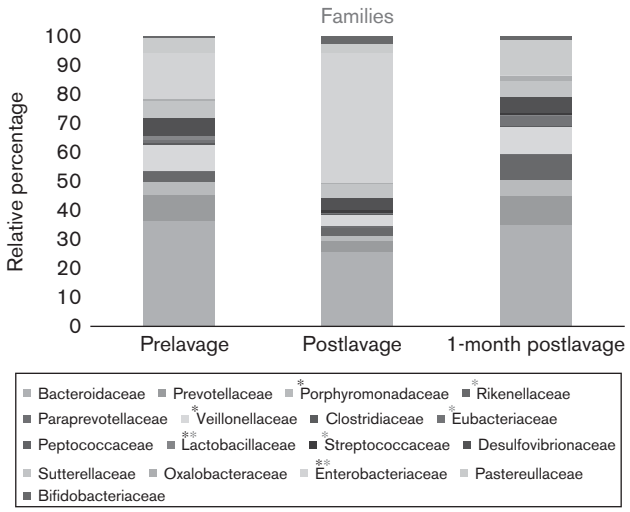

Fig. 3. Effect of bowel lavage on the microbial composition at the family level. Statistical significance between samples collected before the bowel lavage and samples collected on the day of colonoscopy is indicated with a dark gray asterisk (*) ($P < 0.05$), whereas statistical significance between samples collected before the bowel lavage and samples collected 1 month after the colonoscopy is indicated with a light gray asterisk (*) ($P < 0.05$).

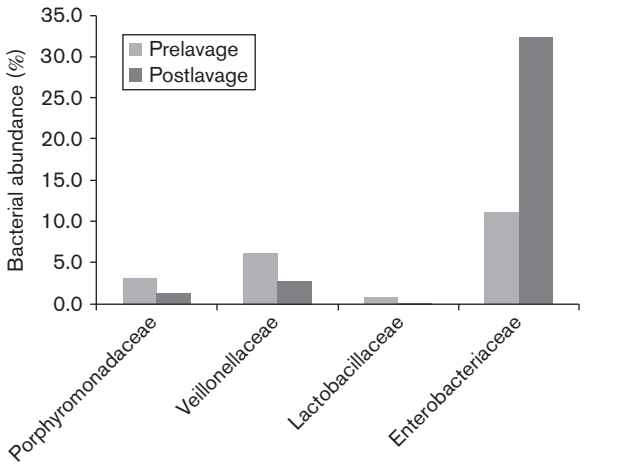

Fig. 4. Significant changes at the family level before the bowel lavage (light gray bar) and on the day of colonoscopy (dark gray bar). ($P < 0.05$).

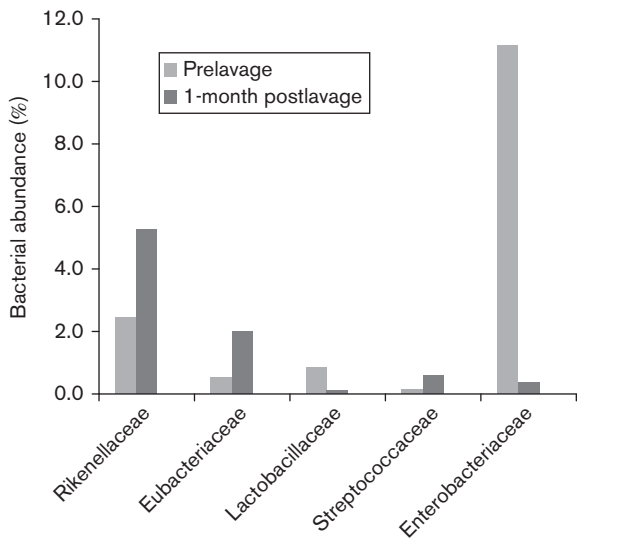

Fig. 5. Significant changes at the family level before the bowel lavage (light gray bar) and 1 month after the colonoscopy (dark gray bar). ($P < 0.05$).

frequency of *Enterobacteriaceae* immediately after the bowel cleansing. The *Enterobacteriaceae* include a number of nosocomial pathogens with considerable antibiotic resistance, which may proliferate and act as pathogens when not counteracted by the physiological gut microbiota, but also act as a clinically relevant antibiotic-resistance reservoir in the intestinal environment [5]. Interestingly, 1 month after the bowel cleansing, we observed that *Enterobacteriaceae* were markedly reduced compared with samples collected before the colonoscopy, which led this bacterial family to be significantly less abundant in the gut. A similar reduction in enterobacterial colonization was observed by Adlerberth *et al.* [22], who highlighted how in the last few decades, the gut microbiota

of several European infants had a low frequency of *Enterobacteriaceae* because of the modern Western and hygienic lifestyle. In all samples analyzed 1 month after the colonoscopy, we also found that the *Streptococcaceae* were significantly higher compared with the samples collected before the bowel cleansing. The *Streptococcaceae* family is positively associated with the production of fecal proteases, which are involved in several physiological mechanisms, such as cell-cycle progression, cell proliferation and cell death, coagulation, tissue remodeling, immune response, and DNA replication [23–25]. Several studies have reported the link between high levels of fecal proteases and the development of IBD in individuals genetically predisposed to developing the pathology [26–29]. Furthermore, high levels of fecal proteases have been observed to increase intestinal inflammation, enhancing enteric permeability. Proteases, indeed, can disrupt mucosal barriers, modulating the host immune response and providing a metabolic advantage for bacteria. Furthermore, the high prevalence of proteases in several pathogenic bacteria strengthens the hypothesis that these enzymes may play a pivotal role in pathological processes harmful to human health [29].

Immediately after the bowel cleansing, the gut microbiota have been observed to be less rich in *Lactobacillaceae*, and this decrease in bacteria was maintained even 1 month after the colonoscopy. Lactobacilli are the main bacterial genera among the *Lactobacillaceae* family, and loss of lactobacilli may negatively affect the establishment of immune tolerance and the development of the immune system [30]. In contrast to pathogenic bacteria, some commensal lactobacilli induce transient, noninflammatory responses, stimulating the polarization of immune T cells toward regulatory T (Treg) cells [31,32]. Moreover, lactobacilli provide a barrier for colonization of pathogens, are involved in the fermentation of nondigestible fibers, salvage of energy as short-chain fatty acids (SCFA), and synthesis of vitamin K [33]. Consequently, the loss or the reduction of lactobacilli may lead to severe pathological consequences to the organism not only in the intestinal environment but also at the systemic level. Interestingly, the reduction of lactobacilli has already been observed in the gut microbiota of celiac patients, who presented a low amount of several *lactobacillus* species and a decreased concentration of intestinal SCFA [34]. SCFA represent the main fuel for colonocytes and are essential for electrolyte and water absorption by colonic mucosa, above all during diarrhea [34]. It is evident that a marked reduction of these metabolic compounds may have adverse impacts on human health, although the increase in *Eubacteriaceae* abundance observed in this study could offset the hypothetical reduction of SCFA because of the low abundance of *Lactobacillaceae*. *Eubacteriaceae*, indeed, are a bacterial family belonging to *Firmicutes* and involved in the production of SCFA and in the degradation of dietary fibers. However, the impact of bowel cleansing on the gut microbiota could be related to the kind of electrolyte solution used for the colon preparation. Jalanka and colleagues, indeed, reported that the microbiota recovers to resemble the baseline composition in 2 weeks when the lavage solution was consumed in two 1 liter doses, but when the single 2 liter dose was used for the bowel cleansing, the gut microbiota differed significantly from the baseline samples, up to 1 month after the medical procedure [4]. This study therefore provides another rationale for recommending a split dose in addition to its yielding better preparation. We chose to investigate the effect of a 4 liter bowel cleansing as high-volume bowel lavages can induce better colon cleansing than low-volume preparations [35]. We did not use a split dose only because, at the time that the study was carried out, the split dose was not included in the protocol of the CRC screening program. In conclusion, our study found a deep impact of colonic lavage on the intestinal microbiota composition until at least 1 month after the clinical procedure as the microbiota did not completely revert to resemble the composition observed before colonoscopy. Some bacterial families reverted to resemble the baseline composition 1 month after the colonoscopy, whereas some changes remained a long time after the bowel lavage had been performed.

One limitation of the present study is the relatively small number of patients enrolled. Nevertheless, we consider the present work an important preliminary study aiming to assess whether changes in the microbiota composition after the bowel lavage persisted after 1 month. Of course, further studies are needed to better understand the effect of bowel cleansing on a greater number of individuals, monitoring not only the microbiota changes after the colonic lavage but also the health status of all patients enrolled in the study at different time points after the endoscopic examination.

From the microbiota-restoring point of view, the administration of a probiotic-rebalancing therapy immediately after the colonoscopy may be useful to recover the bacterial intestinal balance, increasing the amount of beneficial bacteria, such as lactobacilli, which may protect the host from the action of pathogenic microorganisms. However, our results did provide an insight into the general effect of bowel lavage on the intestinal microbiota, indicating that the routine practice of bowel lavage significantly may alter and distort the intestinal bacteria homeostasis.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

References

1 Rex DK, Schoenfeld PS, Cohen J, Pike IM, Adler DG, Fennerty MB, *et al*. Quality indicators for colonoscopy. *Am J Gastroenterol* 2015; 110:72–90.
2 Harewood GC, Sharma VK, de Garmo P. Impact of colonoscopy preparation quality on detection of suspected colonic neoplasia. *Gastrointest Endosc* 2003; 58:76–79.
3 Jalanka J, Salonen A, Salojärvi J, Ritari J, Immonen O, Marciani L, *et al*. Effects of bowel cleansing on the intestinal microbiota. *Gut* 2015; 64:1562–1568.
4 Hill DA, Hoffmann C, Abt MC, Du Y, Kobuley D, Kirn TJ, *et al*. Metagenomic analyses reveal antibiotic-induced temporal and spatial changes in intestinal microbiota with associated alterations in immune cell homeostasis. *Mucosal Immunol* 2010; 3:148–158.
5 Pace NR, Olsen GJ, Woese CR. Ribosomal RNA phylogeny and the primary lines of evolutionary descent. *Cell* 1986; 45:325–326.

6 Stappenbeck TS, Hooper LV, Gordon JI. Developmental regulation of intestinal angiogenesis by indigenous microbes via Paneth cells. *Proc Natl Acad Sci USA* 2002; 99:15451–15455.
7 Bäckhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, *et al*. The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci USA* 2004; 101:15718–15723.
8 Hooper LV, Midtvedt T, Gordon JI. How host-microbial interactions shape the nutrient environment of the mammalian intestine. *Annu Rev Nutr* 2002; 22:283–307.
9 Cebra JJ. Influences of microbiota on intestinal immune system development. *Am J Clin Nutr* 1999; 69:1046S–1051S.
10 Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci USA* 2007; 104:13780–13785.
11 Björkstén B, Sepp E, Julge K, Voor T, Mikelsaar M. Allergy development and the intestinal microflora during the first year of life. *J Allergy Clin Immunol* 2001; 108:516–520.
12 Kamada N, Chen GY, Inohara N, Núñez G. Control of pathogens and pathobionts by the gut microbiota. *Nat Immunol* 2013; 14:685–690.
13 Harrell L, Wang Y, Antonopoulos D, Young V, Lichtenstein L, Huang Y, *et al*. Standard colonic lavage alters the natural state of mucosal-associated microbiota in the human colon. *PLoS One* 2012; 7:e32545.
14 O'Brien CL, Allison GE, Grimpen F, Pavli P. Impact of colonoscopy bowel preparation on intestinal microbiota. *PLoS One* 2013; 8:e62815.
15 Gorkiewicz G, Thallinger GG, Trajanoski S, Lackner S, Stocker G, Hinterleitner T, *et al*. Alterations in the colonic microbiota in response to osmotic diarrhea. *PLoS One* 2013; 8:e55817.
16 Seow-Choen F. The physiology of colonic hydrotherapy. *Colorectal Dis* 2009; 11:686–688.
17 Johnson DA, Barkun AN, Cohen LB, Dominitz JA, Kaltenbach T, Martel M, *et al*. US Multi-Society Task Force on Colorectal Cancer. Optimizing adequacy of bowel cleansing for colonoscopy: recommendations from the US multi-society task force on colorectal cancer. *Gastroenterology* 2014; 147:903–924.
18 Lai EJ, Calderwood AH, Doros G, Fix OK, Jacobson BC. The Boston bowel preparation scale: a valid and reliable instrument for colonoscopy-oriented research. *Gastrointest Endosc* 2009; 69 (Pt 2):620–625.
19 Zoetendal EG, Akkermans AD, De Vos WM. Temperature gradient gel electrophoresis analysis of 16S rRNA from human fecal samples reveals stable and host-specific communities of active bacteria. *Appl Environ Microbiol* 1998; 64:3854–3859.
20 Rajilic-Stojanovic M, Heilig HG, Tims S, Zoetendal EG, de Vos WM. Long-term monitoring of the human intestinal microbiota composition. *Environ Microbiol* 2012.
21 Pop M, Walker AW, Paulson J, Lindsay B, Antonio M, Hossain MA, *et al*. Diarrhea in young children from low-income countries leads to large-scale alterations in intestinal microbiota composition. *Genome Biol* 2014; 15:R76.
22 Adlerberth I, Lindberg E, Aberg N, Hesselmar B, Saalman R, Strannegård IL, Wold AE. Reduced enterobacterial and increased staphylococcal colonization of the infantile bowel: an effect of hygienic lifestyle? *Pediatr Res* 2006; 59:96–101.
23 Carroll IM, Ringel-Kulka T, Ferrier L, Wu MC, Siddle JP, Bueno L, Ringel Y. Fecal protease activity is associated with compositional alterations in the intestinal microbiota. *PLoS One* 2013; 8:e78017.
24 Turk B. Targeting proteases: successes, failures and future prospects. *Nat Rev Drug Discov* 2006; 5:785–799.
25 Dunlop SP, Hebden J, Campbell E, Naesdal J, Olbe L, Perkins AC, Spiller RC. Abnormal intestinal permeability in subgroups of diarrhea-predominant irritable bowel syndromes. *Am J Gastroenterol* 2006; 101:1288–1294.
26 Gecse K, Róka R, Ferrier L, Leveque M, Eutamene H, Cartier C, *et al*. Increased faecal serine protease activity in diarrhoeic IBS patients: a colonic lumenal factor impairing colonic permeability and sensitivity. *Gut* 2008; 57:591–599.
27 Shulman RJ, Eakin MN, Czyzewski DI, Jarrett M, Ou CN. Increased gastrointestinal permeability and gut inflammation in children with functional abdominal pain and irritable bowel syndrome. *J Pediatr* 2008; 153:646–650.
28 Carroll IM, Maharshak N. Enteric bacterial proteases in inflammatory bowel disease-pathophysiology and clinical implications. *World J Gastroenterol* 2013; 19:7531–7543.
29 Ubeda C, Taur Y, Jenq RR, Equinda MJ, Son T, Samstein M, *et al*. Vancomycin-resistant Enterococcus domination of intestinal microbiota is enabled by antibiotic treatment in mice and precedes bloodstream invasion in humans. *J Clin Invest* 2010; 120:4332–4341.
30 McHeyzer-Williams M. Local sentries for class switching. *Nat Immunol* 2007; 8:230–232.
31 Fritz JH, Le Bourhis L, Magalhaes JG, Philpott DJ. Innate immune recognition at the epithelial barrier drives adaptive immunity: APCs take the back seat. *Trends Immunol* 2008; 29:41–49.
32 van Baarlen P, Troost FJ, van Hemert S, van der Meer C, de Vos WM, de Groot PJ, *et al*. Differential NF-kappaB pathways induction by *Lactobacillus plantarum* in the duodenum of healthy humans correlating with immune tolerance. *Proc Natl Acad Sci USA* 2009; 106:2371–2376.
33 Di Cagno R, De Angelis M, De Pasquale I, Ndagijimana M, Vernocchi P, Ricciuti P, *et al*. Duodenal and faecal microbiota of celiac children: molecular, phenotype and metabolome characterization. *BMC Microbiol* 2011; 11:219.
34 Schneider SM, Girard-Pipau F, Filippi J, Hebuterne X, Moyse D, Hinojosa GC, *et al*. Effects of *Saccharomyces boulardii* on fecal short-chain fatty acids and microflora in patients on long-term total enteral nutrition. *World J Gastroenterol* 2005; 11:6165–6169.
35 Hassan C, Bretthauer M, Kaminski MF, Polkowski M, Rembacken B, Saunders B, *et al*. European Society of Gastrointestinal Endoscopy. Bowel preparation for colonoscopy: European Society of Gastrointestinal Endoscopy (ESGE) guideline. *Endoscopy* 2013; 45:142–150.