Systematic review: the role of the gut microbiota in chemotherapy- or radiation-induced gastrointestinal mucositis – current evidence and potential clinical applications

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SUMMARY

Background
Gastrointestinal mucositis is defined as inflammation and/or ulcers of the gastrointestinal tract occurring as a complication of chemotherapy and radiation therapy, and affects about 50% of all cancer patients.

Aim
To assess the role of gut microbiota in the pathogenesis of gastrointestinal mucositis and the potential for manipulations of the microbiota to prevent and to treat mucositis.

Methods

Results
The gut microbiota plays a major role in the maintenance of intestinal homeostasis and integrity. Patients receiving cytotoxic and radiation therapy exhibit marked changes in intestinal microbiota, with most frequently, decrease in Bifidobacterium, Clostridium cluster XIVa, Faecalibacterium prausnitzii, and increase in Enterobacteriaceae and Bacteroides. These modifications may contribute to the development of mucositis, particularly diarrhea and bacteremia. The prevention of cancer therapy-induced mucositis by probiotics has been investigated in randomised clinical trials with some promising results. Three of six trials reported a significantly decreased incidence of diarrhoea. One trial reported a decrease in infectious complications.

Conclusions
The gut microbiota may play a major role in the pathogenesis of mucositis through the modification of intestinal barrier function, innate immunity and intestinal repair mechanisms. Better knowledge of these effects may lead to new therapeutic approaches and to the identification of predictive markers of mucositis.

Aliment Pharmacol Ther
INTRODUCTION
Gastrointestinal mucositis is inflammation and/or ulcers of the gastrointestinal tract occurring as a consequence of chemotherapy and radiotherapy. Oral mucositis is also a frequent complication of cancer therapies, but will not be discussed in this review. Symptoms of gastrointestinal mucositis can include diarrhoea, abdominal pain, bleeding, fatigue, malnutrition, dehydration, electrolyte imbalance and infections, with potentially fatal complications. In addition, gastrointestinal mucositis can result in dosing delays and reductions, contributing to suboptimal treatment.

The incidence is difficult to define. Terms ‘Enterocolitis’, ‘colitis’, ‘anal mucositis’ are listed in the Common Terminology Criteria for Adverse Event (version 4.0). However, patients do not routinely have imaging or endoscopy to diagnose the mucosal inflammation. The incidence of chemotherapy-induced diarrhoea has been reported as high as 50–80% of patients, but this is highly dependent on the chemotherapy regimen.2 The risk of Grade 3 or 4 diarrhoea (defined as severe diarrhoea that require hospitalisation or that have life-threatening consequences, according to the National Cancer Institute Common Toxicity Criteria of Adverse Events (NCI CTCAE) classification) is about 1–3% in patients receiving standard chemotherapy for non-Hodgkin lymphoma or breast cancer, but is higher in patients treated for a colorectal cancer, with about 10% risk with Folfox (5-Fluorouracil and oxaliplatin) or Folfiri (5-Fluorouracil and irinotecan) regimens, and about 20% in patients receiving a triple Folfirixi regimen (5-Fluorouracil, oxaliplatin and irinotecan)3, 4. It has been estimated that pelvic or abdominal radiation cause gastrointestinal mucositis in about 50% of patients, with a higher incidence in patients treated with concurrent chemotherapy.2 In the large EORTC 22921 trial investigating pre-operative and post-operative therapies for rectal cancer, the incidence of grade 2 or more diarrhoea was 17% in patients receiving pre-operative radiation therapy (45 Gy in 25 fractions) and 34% in patients receiving concurrent infusional 5FU.5

Glutamine, antibiotics, granulocyte–macrophage colony-stimulating factor and sucralfate have all failed to demonstrate a benefit. Amifostine is an organic thiophosphate, acting as a free radical scavenger. It is the only radioprotector approved for use in the clinic with clinical data supporting its benefits mainly in the prevention of mucositis in head and neck, lung and pelvic cancers, with reductions in risk of mucositis ranging from 50% to 70% in meta-analyses.1, 6, 7

A better understanding of the pathogenesis of chemotherapy- or radiation-induced mucositis is required to develop and implement optimal preventive and curative approaches for patient care. This review aims at highlighting the influence of the intestinal microbiota on the pathogenesis and the treatment of gastrointestinal mucositis, and discussing potential implications for clinical practice.

METHODS

The pathogenesis of chemotherapy- or pelvic radiation-induced mucositis: emerging role of gut microbiota
Microbiota as a cause of inflammation. Rapid developments in DNA technology have greatly facilitated investigation of the intestinal microbiota and there is growing information about its changes in intestinal inflammation. These can be either causative or consequential and differentiating the two can be very difficult. To demonstrate the impact of the gut microbiota on intestinal inflammation, one study assessed the role of the colonic microbiota on intestinal inflammation induced by 2,4,6-trinitrobenzenesulfonic acid (TNBS) in rats. Animals with a colonic segment excluded from faecal transit were colonised with pre-selected bacteria to test the effects of different species on intestinal inflammation and damage. Rats colonised with anaerobes showed significantly higher eicosanoid release than rats colonised with aerobes only. Moreover, mucosal lesions were mostly observed in rats with anaerobes suggesting that colonic anaerobes may play a role on intestinal inflammation.8

Impact of inflammation on composition and function of the intestinal microbiota. Many studies have investigated the microbiota modifications in different models of
intestinal inflammation. The gut microbiota becomes profoundly modified in the dextran sodium sulphate (DSS)-induced colitis model in mice. Principal Component Analysis of Terminal Restriction Fragment Length Polymorphism (T-RFLP) patterns revealed that the distribution of the microbial communities was distinct between DSS-treated and control mice. Using quantitative PCR, *Lactobacillus* were reduced in the DSS group, while *Akkermansia*, *Desulfovibrio* and Enterobacteriaceae were increased. Another study evaluated the dominant model of mice that develop colitis with high resemblance in DSS-induced colitis mice. Decreases in gut microbiota in faecal samples using quantitative PCR Ruminococcus, Methanobrevibacter, Bacteroides and Bifidobacterium were reported in DSS-treated mice. Decreases in *Lactobacillus*, *Ruminococcus* Methanobrevibacter, Bacteroides and Bifidobacterium were reported in DSS-treated mice after 2 days of treatment. A study using 454 pyrosequencing of bacterial 16S rRNA reported that DSS-induced colitis was associated with an increased abundance of unclassified Clostridiales, Bacteroidaceae, *Akkermansia*, *Mucispirillum* and Enterobacteriaceae and decrease in unclassified Bacteroidiales and Rikenellaceae.

It should be noted that DSS induces rapid depletion of the colonic adherent mucus layer, bringing bacteria into contact with the epithelial surface before any infiltration of inflammatory cells has occurred. The mucus layer plays an important role in the mucosa-associated microbiota population. Mucus glycans can interact with bacteria and be used as adhesion substrate for bacterial adhesins. TM-IEC C1galt-/- mice carry an inducible deficiency of core 1-derived O-glycans in epithelial cells, a key enzyme in the O-glycosylation of MUC-2. These mice are more susceptible to DSS-induced colitis. The TM-IEC C1galt-/- mice with defective mucin glycosylation show an increased abundance of the phylum Bacteroidetes and a reduced abundance of Firmicutes.

Although the human intestinal microbiota can vary markedly between individuals, the functional gene profiles tend to be quite similar in health. A metatranscriptomic study revealed that DSS-induced colitis is accompanied by major shifts in the function of the intestinal microbiota. In DSS-treated mice, there was an increased abundance of gene transcripts associated with regulation and cell signalling, carbohydrate metabolism and respiration. In addition, Akkermansia transcripts were detected in DSS-treated mice. These transcripts are associated with mucin degradation, including glycosyl hydrolases and beta-N-acetylhexosaminidase. Based on whole metagenome shotgun sequence analysis, another study investigated the gut microbiome functions in TRUC mice (*T-bet*+/−/Rag2−/−) ulcerative colitis mice, a model of mice that develop colitis with high resemblance to human ulcerative colitis, with studies during active colitis and treatment-induced remission. Metabolic pathways associated with remission included carbohydrate metabolism and biosynthesis of secondary metabolites. In contrast, metabolic pathways associated with active colitis included cell motility, signal transduction, xenobiotic metabolism/biodegradation and lipid metabolism. FUT2 (fucosyltransferase2) encodes an α (1,2)fucosyltransferase that catalyses addition of terminal α (1,2) fucose residues on mucins and other molecules in mucosal epithelium. A metagenomic study with humanised FUT2−/− mice, a model of Crohn’s disease, revealed that intestinal inflammation was associated with increased metabolic functions including carbohydrate and lipid metabolism, cofactors and vitamins metabolism, glycan biosynthesis and metabolism and was accompanied by a decrease in genes related to amino-acid metabolism.

A study in humans analysed the microbiota of intestinal biopsies and stool samples from 231 inflammatory bowel diseases (IBD) and healthy subjects and again showed that microbial function was more consistently altered than composition, with 12% of analysed pathways changed compared with 2% of genera. There were major shifts of gene function, with increased abundance of genes related to carbohydrate transport and nutrient uptake, and decreased abundance of genes related to amino-acid biosynthesis and carbohydrate metabolism.

Altogether, these studies demonstrate that microbiota disruption may be either the cause or consequence of intestinal inflammation, and that major changes in gene function may occur as a consequence of inflammation. These findings may be relevant to the pathogenesis of mucositis.

**Pathogenesis of mucositis.** A five-step model, entailing complex signalling pathways, has been proposed for the development of mucositis. This model includes (i) an initiation phase with the formation of reactive oxygen species, (ii) a primary damage response phase with inflammation and apoptosis, (iii) a signal amplification phase, leading to more inflammation and apoptosis, (iv) a phase of ulcer formation, leading to discontinuity of the epithelial barrier promoting bacterial translocation, and (v) a healing phase, with cell proliferation once chemotherapy or radiotherapy has ceased. These overlapping steps are thought to be largely driven by the activation of Nuclear Factor-κB, subsequently promoting key pro-inflammatory cytokines.

There is evidence that radiation therapy or chemotherapy can induce mucositis through various pathways.
The timing of histological lesions, peak tissue levels of NF-κB and pro-inflammatory cytokines are different according to the chemotherapy agent (irinotecan, methotrexate or 5-fluorouracil). A study in patients with prostate cancer treated by 7 weeks of radiation therapy revealed that rectal histopathological inflammation reached a maximum 2 weeks after the start of treatment, while symptoms of gastrointestinal toxicity continued to increase until week 6. There may also be further modulation of the tissue response according to the local tissue environment, hormonal and genetic factors, or the underlying pathology. Other patient-related risk factors such as comorbidities, malnutrition and smoking history can contribute important risk and may influence the tissue response.

**Involvement of the microbiota in integrity of the mucosal barrier.** Commensal bacteria play an important role in intestinal homoeostasis, and have some protective effect on the intestinal integrity (Figure 1). Their interaction with Toll-like receptors (TLRs), and subsequent NFκB signalling pathway activation, ensure the development of an innate immune response. This signalling contributes to the control of the control of the intestinal homoeostasis, maintaining the barrier function and promoting wound repair and tissue regeneration. Activation of these pathways supports mucosal repair and protects the gut against injury.

Chemotherapy and radiation therapy can increase intestinal permeability, which results in part from intestinal crypt apoptosis and villous atrophy. Commensal bacteria regulate intestinal barrier function, notably by modulating the expression and distribution of tight junction proteins. Thus, microbiota disruption could play an important role in the alterations of intestinal permeability. The epithelial mucus layer is another protective factor, which contributes to the intestinal integrity and is regulated by gut bacteria. As an example, in a rat model, a probiotic supplement, VSL3, can stimulate MUC2 gene expression and secretion in the colon.

It has been demonstrated a few decades ago that germ-free animals are less sensitive to total body irradiation-induced enteritis than animals with microbiota. More recently, a study demonstrated that the small bowel of germ-free mice was markedly resistant to lethal radiation enteritis. Furthermore, in small intestine villi, the microbiota increased the sensitivity of mesenchymal endothelial cells to total body irradiation-induced apoptosis, as compared with germ-free mice. Fasting-induced adipose factor (Fiaf), also known as angiopoietin-like protein 4, is normally secreted epithelial cells in small intestine villi and support endothelial survival. Fiaf secretion is down-regulated by the intestinal microbiota. This regulation could play a role in determining intestinal radiosensitivity. These studies strongly support a major role of the microbiota in the development of gastrointestinal mucositis.

**Intestinal microbiota modifications during and following cancer treatment**

**Gut microbiota alterations in pre-clinical models.** Animal studies have considerable limitations. The mouse and human microbiota are quite similar at the phylum level, with Firmicutes and Bacteroidetes dominating, but the majority of the gut microbial populations are unique, with at least 85% of the sequences representing genera in mice not detected in humans. Moreover, there are many differences between animal models of inflamma-
tory diseases and human diseases, in terms of morphological lesions, microbial colonisation and clinical manifestations. Other limitations of animal models include differences in enzyme activity, concentrations of putrefactive products and immunological activation by the faeces content. However, animal models of intestinal inflammation are essential to provide some insights into the direct effect of the pathobiology of the inflammatory diseases. Even if the animal model should never be viewed as a faithful equivalent of the human intestinal microbiota, these models allow detailed study of the inflammatory process and the complex interactions occurring between the host and the intestinal microbiota.

Several studies using animal models have been published, investigating the modification in intestinal microbiota following two commonly used chemotherapeutic agents: 5-fluorouracil (5-FU) and irinotecan. These studies are summarised in Table 1. These studies used standard microbiological culture and real-time PCR.

Following a single intraperitoneal dose of 5-FU treatment in rats, Enterococcus spp., Lactobacillus spp. and Streptococcus spp. decreased in the colon using standard microbiological culture techniques. In faecal samples, using real-time PCR, significant increases (P < 0.05) were found in Clostridium spp. and Staphylococcus spp. at 24 h. Using the same drug given for 6 days in rats, another study reported an increase in the total number of facultative anaerobes in the colon (18–60%, P < 0.05 compared to untreated) using standard microbiological culture techniques.

Following a single intraperitoneal dose of irinotecan treatment in rats, an increase trend in Escherichia spp., Clostridium spp., Enterococcus spp., Serratia spp. and Staphylococcus spp. in colon has been reported using standard microbiological culture techniques. In faecal samples, they found an increase in Proteus spp., Clostridium spp., and Peptostreptococcus spp. was reported associated with a decrease in Bacillus spp., and Bifidobacterium spp. Moreover, irinotecan treatment has been associated with an increase in bacteria that produce β-glucuronidase, contributing to mucositis-related diarrhoea. In a recent study, using PCR-denaturing Gradient Gel Electrophoresis and real-time PCR, the total bacteria number decreased following irinotecan injections for 3 days in rats, particularly Clostridium clusters IV and XIVa. On day 7, numbers of total bacteria and Bacteroides group were restored, whereas Lactobacillus group and Bifidobacterium spp. remains significantly lower. Moreover, Clostridium cluster XI and Enterobacteriaceae remains higher than before treatment. Following a clinically relevant combination of irinotecan and 5-FU, Clostridium cluster XI (cluster which contains C. difficile spp.) and XIVa (cluster which contains Butyrate-producing Clostridium) and Enterobacteriaceae spp. increased, whereas Clostridium cluster IV (cluster which contains Butyrate-producing Clostridium spp.) decreased.

These animal studies showed a drastic shift, following 5-FU and/or irinotecan treatment, from commensal bacteria (i.e. Bifidobacterium spp. and Lactobacillus spp.) to Escherichia spp., Clostridium spp. and Enterococcus spp. These bacteria are frequently isolated in cancer patients. However, these studies used only culture-based and low-resolution molecular techniques, focused on the most dominant microbial community members, resulting in an incomplete view of the disruption of the intestinal microbiota following cancer treatment.

The effect of radiation on gut microbiota has also been investigated. A study analysed the effects of radiation on intestinal microbiota in the murine ileum of male C57/Bl6 mice. With a culture-dependent method, irradiation reduced the intestinal microbiota bacterial composition in the tissue samples from the irradiated small intestine compared to sham animals with a rapid and significant decrease in aerobic, anaerobic Enterobacteriaceae and Lactobacillus groups (P < 0.05). However, after 24 h, the differences were not significant between irradiated and sham animals.

**Gut microbiota alterations in clinical studies.** Several clinical studies have reported modifications in faecal microbiota following cancer treatment. These studies are summarised in Table 2. In a paediatric population treated with chemotherapy, a marked reduction in the number of anaerobic bacteria (i.e. Bacteroides, Clostridium cluster XIVa, Faecalibacterium prausnitzii and Bifidobacterium) and streptococcus spp. were reported, whereas the number of Enterococcus spp. drastically increased. The authors concluded that the disturbed balance in the intestinal microbiota increases the risk of Gram-positive aerobic bacteraemia. Gram-positive bacteria are frequently isolated from blood culture in bacteraemic cancer patients, and are highly susceptible to acquiring resistance to anti-bacterial agents. In another study including ambulant patients receiving different chemotherapy regimens, patients’ intestinal microbiota were severely disrupted following chemotherapy, characterised by decreased Bacteroides, bifidobacteria, and Clostridium cluster IV and XIVa.
In a recent study, 454 high-throughput pyrosequencing was performed in faecal samples of adult patients undergoing conditioning chemotherapy for allogeneic haematopoietic stem cell transplantation (allo-H SCT). This new generation DNA sequencing technology allowed detection of low abundance taxa and description of rarefactions measures to provide a more complete understanding of the chemotherapy-induced alterations in the intestinal microbiota. Following a cycle of chemotherapy, there was a significant reduction

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Stringer et al.</td>
<td>81 female Dark Agouti rats</td>
<td>Single 150 mg/kg intraperitoneal dose of 5-FU</td>
<td>Real-time PCR</td>
<td>Faecal samples</td>
<td>Increase in <em>Clostridium</em> spp. and in <em>Staphylococcus</em> spp. at 24 h (<em>P</em> &lt; 0.05), increase trend in <em>E. coli</em> at 48 h, decrease trend in <em>Bacteroides</em> and <em>Lactobacillus</em> at 12 h.</td>
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<td>Von Bültzingslöwen et al.</td>
<td>75 female Lewis rats</td>
<td>A 6-day repeated 50 mg/kg dose of 5-FU</td>
<td>Standard microbiological culture techniques</td>
<td>Small intestine and Large intestine</td>
<td>Total number of anaerobic bacteria and facultative anaerobes unchanged in the small intestine, but shift in the type of facultatives that predominated, from Gram-positive cocci (71 to 10%, <em>P</em> &lt; 0.01 compared to untreated) to Gram-negative rods 5–70%, <em>P</em> &lt; 0.01 compared to untreated). No increase in the number of anaerobes in the large intestine, whereas the Gram-negative facultatives increased (48–98%, <em>P</em> &lt; 0.05 compared to untreated) and the Gram-positive facultatives decreased (44 to 2%, <em>P</em> &lt; 0.001 compared to untreated), leading to an increase in the total number of facultative anaerobes in the colon (18–60%, <em>P</em> &lt; 0.05 compared to untreated).</td>
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<tr>
<td>Stringer et al.</td>
<td>81 female Dark Agouti rats</td>
<td>A single 200 mg/kg intraperitoneal dose of Irinotecan</td>
<td>Standard microbiological culture techniques</td>
<td>Colon and Faeces</td>
<td>Increase in <em>Escherichia</em> spp. between 6 and 24 h, increase in <em>Clostridium</em> spp. at 2 h, increase in <em>Enterococcus</em> spp. at 6 h, increase in <em>Serratia</em> spp. at 1 h, increase in <em>Staphylococcus</em> spp. at 1 h. After Bonferroni correction, differences were not statistically significant. Increase in <em>Proteus</em> spp. at 24 h, increase in <em>Clostridium</em> spp. at 2 h, increase in <em>Peptostreptococcus</em> spp. at 1 h, decrease in <em>Bacillus</em> spp. at 12 h, decrease in <em>Bifidobacterium</em> spp. at 1 h. After Bonferroni correction, differences were not statistically significant.</td>
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<td>Stringer et al.</td>
<td>81 female Dark Agouti rats</td>
<td>A single 200 mg/kg intraperitoneal dose of Irinotecan</td>
<td>Real-time PCR</td>
<td>Faeces</td>
<td>Increase in <em>E. coli</em> from 24 to 48 h, increase in <em>Staphylococcus</em> spp. from 2 to 12 h, decrease in <em>Lactobacillus</em> spp. from 12 to 48 h (<em>P</em> &lt; 0.05).</td>
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<td>Lin et al.</td>
<td>30 tumour-bearing rats</td>
<td>3-day 125 mg/kg dose of Irinotecan</td>
<td>PCR-denaturing Gradient Gel Electrophoresis and real-time PCR</td>
<td>Faeces</td>
<td>By day 3, decrease in the total bacteria number (decrease by ~1 log), particularly <em>Clostridium</em> clusters IV and XIVa (decreased by 1-3 logs). By day 7, numbers of total bacteria and Bacteroides group were restored, whereas <em>Lactobacillus</em> group and <em>Bifidobacterium</em> spp remains significantly lower. <em>Clostridium</em> cluster XI (increased by ~0.5 log) and <em>Enterobacteriaceae</em> (increased by ~1.5 logs) remains higher than Day 0. When associated with 5-FU: (irinotecan 50 mg/kg and 5-FU 50 mg/kg on day 1 and 2 and on day 2 and 9, respectively); at Day 11, <em>Clostridium</em> cluster XI increased (~2 logs), <em>Clostridium</em> clusters XIVa and Enterobacteriacea increased (~0.5 log). <em>Clostridium</em> cluster IV decreased (~0.5 log).</td>
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<tr>
<td>Johnson et al.</td>
<td>Male C57Bl/6J mice</td>
<td>Exposed ileum was subjected to a single dose of high dose radiation of 19 Gy</td>
<td>Culture-dependent method</td>
<td>Tissue samples from the irradiated small intestine</td>
<td>After 2 h significant decrease in aerobic, anaerobic <em>Enterobacteriacea</em>, and <em>Lactobacillus</em> groups (<em>P</em> &lt; 0.05). However, after 24 h, the differences were not significant between irradiated and sham animals.</td>
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### Table 2 | Intestinal microbiota modifications during cancer treatment in clinical studies

<table>
<thead>
<tr>
<th>Study</th>
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<tr>
<td>van Vliet et al.</td>
<td>45</td>
<td>Nine paediatric patients with acute myeloid leukaemia</td>
<td>Cytarabine, daunorubicine, etoposide, amsacrine, mitoxantrone with prophylactic antibiotic treatment</td>
<td>Faeces</td>
<td>Following chemotherapy, decrease in the total number of bacteria (100-fold lower than in healthy control samples). Decrease in anaerobic bacteria (10 000-fold decrease): Bacteroides species, Clostridium cluster XIVa, Faecalibacterium prausnitzii and Bifidobacterium species – decreased 3000–6000-fold compared with the healthy individuals, resulting in a 70–20 000-fold decrease in the total number of anaerobic bacteria ($P &lt; 0.004$). A recovery was found for both Clostridium XIVa and F. prausnitzii, whereas Bacteroides and Bifidobacterium were still 10–300-fold lower compared with the healthy individuals at the end of the treatment. Increase in enterococci in cancer patients compared to healthy individuals ($P &lt; 0.038$). 100–1000-fold decrease in streptococci in cancer patients compared with healthy individuals ($P &lt; 0.016$).</td>
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<td>Zwielehner et al.</td>
<td>46</td>
<td>17 ambulant patients</td>
<td>Different chemotherapy treatment protocols with or without concomitant antibiotics</td>
<td>Faeces</td>
<td>Decrease in the total number of bacteria in cancer patients compared to healthy individuals ($P &lt; 0.05$). Decreased abundance of the microbiota following chemotherapy ($P = 0.037$). Increase in Bacteroides (26–28%) and Clostridium cluster IV (16–18%), decrease in bifidobacteria (1.4% to 0.5%) and Clostridium cluster XIVa (22% to 19%) following chemotherapy.</td>
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<tr>
<td>Montassier et al.</td>
<td>47</td>
<td>Eight patients with Non-Hodgkin lymphoma</td>
<td>Carmustine, etoposide, aracytine and melphalan</td>
<td>Faeces</td>
<td>Following chemotherapy, decreased overall diversity with decreased evenness as measured by the Shannon diversity index ($P &lt; 0.001$) and richness as measured by phylogenetic diversity ($P &lt; 0.001$). At phylum level: decrease in Firmicutes (78% to 22%, $P = 0.008$), increase in Bacteroidetes (18–50%, $P = 0.008$) after chemotherapy relative to before. At genus level: increase in Bacteroides (13–30%, $P = 0.008$), an increase in Escherichia (0.6–7% $P = 0.008$) after chemotherapy relative to before. Decrease in Blautia (6% to 0%, $P = 0.008$), Faecalibacterium (17% to 8%, $P = 0.04$) and Roseburia (7% to 0%, $P = 0.008$) after chemotherapy, decrease in Bifidobacterium (2% to 0.1%, $P = 0.04$) after chemotherapy relative to before.</td>
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<tr>
<td>Stringer et al.</td>
<td>48</td>
<td>16 cancer patients</td>
<td>Different chemotherapy</td>
<td>Faeces</td>
<td>Decrease in Lactobacillus spp., Bacteroides spp., Bifidobacterium spp., Enterococcus spp.</td>
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</table>
in bacterial communities’ diversity. At the genus level, there was a very marked increase in Bacteroides and Escherichia and a profound decrease in Blautia, Faecalibacterium and Roseburia. Thus, patients undergoing conditioning chemotherapy exhibit modifications of the gut microbiota characterised by a significant establishment of Escherichia, the most frequently isolated pathogen from blood culture in bacteraemic cancer patients.47

Another study investigated the intestinal microbiota alterations associated with chemotherapy-induced diarrhoea. With quantitative real-time PCR, they showed that patients with diarrhoea exhibit differences in faecal microbiota bacterial composition compared with healthy controls, with a trend towards decrease in Lactobacillus spp., Bacteroides spp., Bifidobacterium spp., Enterococcus spp. and a trend towards increase in Escherichia coli and Staphylococcus spp. Cancer patients with diarrhoea also exhibit a trend towards decreasing methanogenic archaea compared to healthy individuals.48 Gut microbiota modifications have also been investigated in adult patients receiving pelvic radiation therapy for abdominal malignancies. Patients with acute post-radiotherapy diarrhoea had a significant reduction in microbial diversity in faecal samples, compared to healthy volunteers and patients having received pelvic radiation but without diarrhoea. Furthermore, the cluster analysis of the faecal microbial profiles collected before radiotherapy demonstrated significant different between patients who developed diarrhoea and those who did not. Patients with diarrhoea exhibited increased Actinobacteria (0% vs. 17%, percentage of sequences), increase in Bacilli (1% vs. 17%), decrease in Clostridia (45% vs. 34%).

### Table 2 | (Continued)

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<td>and 2 healthy individuals</td>
<td>treatment protocols with or without concomitant antibiotics</td>
<td>Quantitative real-time PCR</td>
<td>Faeces</td>
<td>Healthy controls and patients with no diarrhoea maintained their bacterial profiles with a similarity ~ 60% over a 7-week period. Patients with diarrhoea exhibited a significantly modified bacterial profile ($P &lt; 0.05$) after the beginning of the radiotherapy (49% of similarity), at the end of the radiotherapy (29% similarity) and 2 weeks after (35% similarity). In patients with diarrhoea compared to no diarrhoea patients, increase in Actinobacteria (0% vs. 17%, percentage of sequences), increase in Bacilli (1% vs. 17%), decrease in Clostridia (45% vs. 34%).</td>
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<tr>
<td>Manichanh et al.49</td>
<td>10 patients with abdominal tumours</td>
<td>5 weeks of pelvic radiotherapy</td>
<td>DNA fingerprinting and cloning-sequencing techniques</td>
<td>Faeces</td>
<td>Healthy controls and patients with no diarrhoea maintained their bacterial profiles with a similarity ~ 60% over a 7-week period. Patients with diarrhoea exhibited a significantly modified bacterial profile ($P &lt; 0.05$) after the beginning of the radiotherapy (49% of similarity), at the end of the radiotherapy (29% similarity) and 2 weeks after (35% similarity). In patients with diarrhoea compared to no diarrhoea patients, increase in Actinobacteria (0% vs. 17%, percentage of sequences), increase in Bacilli (1% vs. 17%), decrease in Clostridia (45% vs. 34%).</td>
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<tr>
<td>Nam et al.50</td>
<td>Nine gynaecological cancer patients</td>
<td>Five times a week during a 5-week period</td>
<td>454 high-throughput pyrosequencing</td>
<td>Faeces</td>
<td>Phylum Firmicutes decreased by 10% ($P = 0.09$) and phylum Fusobacterium increased by 3% ($P = 0.05$) during radiation. At family level, Eubacteriaceae was significantly decreased ($P = 0.03$), Fusobacteriaceae and Streptococcaceae were significantly increased ($P &lt; 0.05$).</td>
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patients following pelvic radiotherapy showed that Actinobacteria were commoner and Fusobacteria less common in cancer patients compared to healthy individuals. At the family level, Clostridiaceae and Eubacteriaceae were commoner, whereas Prevotellaceae and Oscillospiraceae and Fusobacteriaceae were less common in cancer patients compared to healthy individuals. Moreover, following radiotherapy, phylum Firmicutes decreased whereas phylum Fusobacterium increased. Following radiotherapy, Eubacteriaceae decreased whereas Fusobacteriaceae and Streptococcaceae increased.50

These clinical studies indicate that patients receiving chemotherapy or radiotherapy exhibit very marked changes in their intestinal microbiota. Although some of these changes may be secondary, either to the underlying

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<tr>
<th>Study</th>
<th>Patients (N)</th>
<th>Primary endpoint</th>
<th>Clinical setting</th>
<th>Investigated probiotics</th>
<th>Commentary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salminen, Clin Radiol64</td>
<td>24</td>
<td>Reduction on the incidence of diarrhoea</td>
<td>Women treated by pelvic internal and external radiation therapy</td>
<td>Lactobacillus acidophilus in fermented milk</td>
<td>Less diarrhoea in the experimental group (90% vs. 30%, both during and 6 weeks after treatment, (P &lt; 0.01)).</td>
</tr>
<tr>
<td>Urbancsek, Eur J Gastroenterol Hepatol65</td>
<td>205</td>
<td>Frequency of anti-diarrhoeal medications</td>
<td>Patients treated with EBRT in abdominal region</td>
<td>Antibiophilus vs. placebo</td>
<td>No statistical difference on the primary endpoint (35% in the experimental group vs. 48%, (P = 0.06)).</td>
</tr>
<tr>
<td>Delia, WJG68</td>
<td>490</td>
<td>Reduction in the incidence and the severity of radiation-induced diarrhoea</td>
<td>Adjuvant EBRT for abdominal or pelvic cancer</td>
<td>VSL#3 vs. placebo</td>
<td>Less all-grades radiation-induced diarrhoea (58% vs. 38%, (P &lt; 0.001), less Grade 3/4 diarrhoea (29% vs. 6%, (P &lt; 0.001))</td>
</tr>
<tr>
<td>Giralt, Int J Radiation Oncology Biol Phys66</td>
<td>85</td>
<td>Reduction in the incidence and the severity of radiation-induced diarrhoea</td>
<td>EBRT for cervical or endometrial cancer</td>
<td>Lactobacillus casei DN-114001 vs. placebo</td>
<td>No significant difference regarding the primary endpoint (68% in the experimental group vs. 58%, (P = 0.57))</td>
</tr>
<tr>
<td>Chitapanarux, Radiation Oncology67</td>
<td>63</td>
<td>Reduction in the incidence and the severity of diarrhoea</td>
<td>Pelvic EBRT concomitant with systemic cisplatin</td>
<td>Lactobacillus acidophilus + Bifidobacterium bifidum vs. placebo</td>
<td>Reduced grade 2/3 diarrhoea in the experimental group (45% vs. 9%, (P = 0.002))</td>
</tr>
<tr>
<td>Wada, Support Care Cancer57</td>
<td>42</td>
<td>Effect on infectious complications</td>
<td>Children treated with chemotherapy</td>
<td>Bifidobacterium breve strain Yakult vs. placebo</td>
<td>Reduction in the frequency of fever and in the need for antibiotics (mean number of days with fever 1.06 vs. 3.00, (P = 0.02))</td>
</tr>
<tr>
<td>Osterlund, Br J Cancer58</td>
<td>150</td>
<td>Reduction in the incidence of severe diarrhoea</td>
<td>Colorectal cancer patients treated with adjuvant 5FU</td>
<td>Lactobacillus rhamnosus vs. no probiotic</td>
<td>Less grade 3/4 diarrhoea (37% vs. 22%, (P = 0.027))</td>
</tr>
</tbody>
</table>

EBRT, external beam radiation therapy.

Table 3 | Clinical trials investigating the effects of probiotics on the prevention of chemotherapy- or pelvic radiation-induced mucositis
microbiota. Synbiotics are combinations of prebiotics that have beneficial effects for the host, through actions on the composition and/or the activity of the intestinal microbiota. Synbiotics are combinations of prebiotics and probiotics.

Probiotics have been evaluated in the prevention of chemotherapy-induced diarrhoea. In pre-clinical models, some promising results have been reported. In rats treated with a single intraperitoneal dose of irinotecan, VSL#3 significantly prevented moderate or severe diarrhoea, weight loss, prevented irinotecan-induced increase in goblet cells and apoptosis of intestinal crypt cells. However, results from pre-clinical studies are largely inconsistent. The effects of probiotics vary according to the model, the strains, the dosing and the treatment plan. As an example to illustrate these difficulties, oral ingestions of Streptococcus thermophilus led to the attenuation of intestinal damage in nontumour-bearing rats treated with methotrexate. However, a subsequent study investigating the effects of S. thermophilus in tumour-bearing rats treated with methotrexate did not demonstrate any beneficial effect.

There is no evidence of the beneficial effect of probiotics on the prevention of pelvic radiation-induced mucositis.

Evidence from animal models. A pre-clinical study in mice evaluated the efficacy of L. rhamnosus supplementation in the prevention of intestinal damage following a 12 Gy whole body irradiation. Probiotic treatment improved the survival of animals and decreased radiation-induced weight loss. Probiotics significantly improved crypt survival by reducing apoptosis. This protective effect was dependent of MyD88, TLR-2 and COX-2. In non-irradiated mice, L. rhamnosus supplementation led to redistribution of COX-2-positive mesenchymal stem cells, from the villi to the crypt region. These results provide new insight into potential radioprotective effects provided by probiotics. A better understanding of the mechanism by which probiotics protect against pelvic radiation-induced mucositis will allow optimisation of current treatment strategies (e.g. optimal strains) and the development of novel therapeutic agents.

Evidence from human trials. Pelvic radiation-induced diarrhoea may result from different mechanisms, notably altered intestinal motility, lactose intolerance, bile-salt malabsorption, bacterial overgrowth and disruption of gut microbiota. Five randomised trials investigating the impact of probiotics on the prevention of pelvic radiation-induced diarrhoea have been published with inconsistent findings. These results highlight the importance of identifying the ideal type and dose of bacterial strains, in high-quality clinical trials. One trial investigated a symbiotic strategy, testing a combination of Lactobacillus acidophilus and lactulose as substrate for the bacteria. Two trials did not meet their primary endpoint (reduction in diarrhoea). Three studies reported a significant
decrease in the incidence of diarrhoea in patients treated with the probiotics *Lactobacillus acidophilus*, VSL#3, or a combination of *Lactobacillus acidophilus* with *Bifidobacterium bifidum*, compared with placebo (Table 3).

Microbiota as a predictive factor for chemotherapy side effects

Intensive chemotherapy regimens used in haematological malignancies to condition patients for allo-HSCT are associated with frequent bacteraemia. In a recent study, faecal samples were collected in 94 patients undergoing allo-HSCT. Intestinal domination was defined as occupation of at least 30% of the microbiota by a single bacterial taxon. In the studied population, the most frequent dominating organisms were *Enterococcus*, *Streptococcus*, and various Proteobacteria. Patients with enterococcal domination had a 9-fold increased risk of vancomycin-resistant *Enterococcus* bacteraemia relative to patients without enterococcal domination, and patients with domination by Proteobacteria had a 5-fold increased risk of bacteraemia with Gram-negative bacilli relative to patients without Proteobacteria domination of the intestinal microbiota. Thus, determination of the faecal microbiota composition during allo-HSCT procedure could predict patients at the highest risk for bacteraemia. Further, intestinal colonisation of a specific microbial species may inhibit vancomycin-resistant *Enterococcus* colonisation. Furthermore, gut bacteria belonging to the *Barnesiella* genus restrict enterococcal colonisation of the intestinal tract and subsequent vancomycin-resistant *Enterococcus* bacteraemia in patients undergoing allo-HSCT. Altogether, these results support a potential role for microbiota analyses to help predict chemotherapy-induced toxicity.

CONCLUSION

Basic and clinical data suggest that the intestinal microbiota may play an important role in the pathogenesis of chemo- or radiation-induced mucositis. Chemotherapy and irradiation induce major changes in the composition of the gut microbiota, these disruptions could also participate in the development of mucositis. Further metagenomic studies, investigating microbiota gene functions, are required to better understand the impact of microbiota disruption during cancer treatments. Strategies can then be developed to prevent or treat these potentially life-threatening complications, using manipulations of the intestinal microbiota. Gut microbiota identification could also be used as a predictive marker for the risk of mucositis, and could guide preventive approaches. Better knowledge of the gut microbiota by modern identification techniques, such as metagenomic approaches, should help to develop personalised investigational strategies that can be tested in future clinical trials.

AUTHORSHIP

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REFERENCES

32. van Vliet MJ, Harmens HJ, de Bont ES, Tissing WJ. The role of intestinal microbiota in the development and severity of chemotherapy-induced mucositis. *PLOS Pathog* 2010; **6**: e1000879.


