Oral bacterial community dynamics in paediatric patients with malignancies in relation to chemotherapy-related oral mucositis: a prospective study

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Abstract

The role of oral bacteria in the development of chemotherapy-related oral mucositis has not been fully elucidated. This study aimed to investigate oral bacterial community diversity and dynamics in paediatric patients with malignancies in relation to the occurrence of oral mucositis. Patients with malignancies (n = 37) and reference individuals without known systemic disorders (n = 38) were recruited. For patients, oral bacterial samples were taken from mucosal surfaces both at the time of malignancy diagnosis and during chemotherapy. If oral mucositis occurred, samples were taken from the surface of the mucositis lesions. Oral mucosal bacterial samples were also taken from reference individuals. All samples were assessed using a 16S ribosomal RNA gene 454 pyrosequencing method. A lower microbial diversity ($p < 0.01$) and a higher intersubject variability ($p < 0.001$) were found in patients as compared with reference individuals. At the time of malignancy diagnosis (i.e. before chemotherapy) patients that later developed mucositis showed a higher microbial diversity ($p < 0.05$) and a higher intersubject variability ($p < 0.001$) compared with those without mucositis. The change of bacterial composition during chemotherapy was more pronounced in patients who later developed mucositis than those without mucositis ($p < 0.01$). In conclusion, we found a higher microbial diversity at the time of malignancy diagnosis in patients who later develop oral mucositis and that these patients had a more significant modification of the bacterial community by chemotherapy before the occurrence of mucositis. These findings may possibly be of clinical importance in developing better strategies for personalized preventive management.

Keywords: 16S rRNA gene, 454 pyrosequencing, cancer, oral microflora, stomatitis
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Background

All forms of cytostatic therapy give rise to side-effects that have a major impact on the patients' quality of life during anticancer treatment. One of the side-effects is the inflammation of mucosal tissues, mucositis, which can involve the entire alimentary tract. Oral mucositis is one of the most frequently encountered forms and commonly occurs 7–10 days after the administration of cytostatic drugs. Oral mucositis presents as mucosal ulceration, bleeding and severe pain, which may require the use of opiates and parenteral nutrition. The mucositis-inflicted tissue damage also provides a port for the invasion of host endogenous bacteria into the circulation, causing bacteraemia and sepsis [1,2]. The patho-mechanisms of chemotherapy-related oral mucositis have not been fully

elucidated, although considerable progress has been made during the last decade in defining a cascade of destructive and inflammatory events [3]. However, beyond this paradigm, the association between mucositis and the commensal bacterial microflora is so far poorly understood [4,5].

It is now well recognized that the diversity of microorganisms colonizing the oral cavity has been greatly underestimated [6]. Most of the bacterial species cause no harm under healthy conditions. However, in patients with malignancies, the delicate homeostasis between host defence and commensal bacteria could be disturbed by the cancer itself, by the cancer-related secondary immunodeficiency, or by prophylactic antibacterials. The disrupted homeostasis might contribute to the oral mucosal tissue breakdown following chemotherapy. In addition, the chemotherapeutics can be bacteriostatic or bactericidal, thus affecting the oral bacterial community [4,7,8]. However, no clear pattern regarding the changes in the oral bacterial community and occurrence of oral mucositis can be discerned from the literature, most likely due to the limited number of studies published.

The impact of the bacterial community on the mucosal integrity during chemotherapy cannot be fully understood without comprehensive knowledge of the bacterial community composition. The conventional culture-based or biochemical methods can identify anticipated bacterial taxa, but lack the capacity to detect non-cultivable microorganisms and the possibility to address hitherto unknown taxa. However, modern molecular methods for identifying bacterial taxa have made it possible to assess a bacterial community with a reduction in bias experienced in culture-based methods [9], and furthermore, a massively parallel DNA sequencing technique, 454 pyrosequencing, has now greatly increased the capacity to detect bacteria of low abundance [10,11].

In this study, we employed 16S rRNA gene 454 pyrosequencing, in order to determine the diversity and relative abundance of oral mucosal bacterial taxa in paediatric patients with malignancies. The oral bacteria were assessed at the time of malignancy diagnosis prior to chemotherapy, during chemotherapy and at the time of mucositis in an attempt to follow the dynamics of the bacterial community in conjunction with chemotherapy-related oral mucositis.

Materials and Methods

Subjects

This study was designed as a prospective longitudinal cohort study. An ethics permit was granted by the Regional Ethical Review Board, situated at Karolinska Institutet, Stockholm, Sweden.

From November 2008 to December 2010, patients with newly diagnosed malignancies (n = 109) were enrolled from the Paediatric Cancer Ward, Astrid Lindgren Children's Hospital, Karolinska University Hospital, Stockholm, Sweden. The exclusion criteria were as follows: (i) patients under 4 years of age or above 18 years of age, (ii) the treatment protocol did not include cytostatic drugs, and/or (iii) patients without national population registration number. Out of 60 patients that met inclusion criteria, 37 patients agreed to participate in the present study. Age and gender-matched children (n = 38), without any known systemic disorder and who had not been treated with antibacterials 3 months prior to the study, were recruited as reference individuals during their routine dental visit to the Division of Paediatric Dentistry, Department of Dental Medicine, Karolinska Institutet, Sweden. Assent and informed consent were obtained from all the included children and their parents, respectively.

For the patients, data regarding age, gender and diagnosis of malignancies were collected. Data including blood counts of neutrophils, leukocytes and thrombocytes, and levels of haemoglobin at the time of malignancy diagnosis, were extracted from laboratory test reports. Oral health status, including decayed, missing or filled teeth of permanent/deciduous teeth (DMFT/dmft) and gingival bleeding index (GBI) were assessed by the same dentist for all patients to avoid inter-examiner difference. Oral care instructions, including recommendation of a single 2.5 mg/mL benzydamine-based mouth rinse for the period of chemotherapy, were provided to the patients and parents. All patients were followed during the entire cytostatic treatment. One dose of the antibacterial cefotaxime was given intravenously to each patient as prophylaxis before placing a central venous catheter. The individual chemotherapeutic scheme and antibacterial agents used for treating infections were retrieved from medical charts and information regarding the use of the mouth rinse was gathered from the parents. The occurrence of oral mucositis was recorded and the grade of oral mucositis was scored using the World Health Organisation (WHO) system [12], which grades oral toxic effects into five levels: grade 0, no change; grade 1, soreness/erythema; grade 2, erythema, ulcers, can eat solids; grade 3, ulcers, requires liquid diet only; grade 4, alimentation not possible. A WHO grade > 1, which indicates ulcerative mucositis, was considered as occurrence of oral mucositis in the current study to avoid false-positive diagnosis.

For the reference individuals, data including age, gender and oral health status in terms of DMFT/dmft and GBI were recorded. The same professional performed the oral health evaluations for the patients and the reference individuals.

Oral mucosal and mucositis samples

For the patients, oral mucosal samples were collected at two time-points: firstly at the time of malignancy diagnosis (before the administration of cytostatic drugs and use of mouth rinse; however, after the single-dose prophylactic cefotaxime) and secondly during chemotherapy prior to any sign of oral mucositis (range from 5 to 84 days among patients after the initiation of the entire chemotherapy, depending on the chemotherapeutic schemes and hospital appointment). In order to minimize the pain the mucosal samples were collected using two paper strips (2×6 mm^2) (PerioPaper, Oralflow Inc., NY, USA) placed centrally on the lower lip and on the bucca, respectively, for 15 seconds. For patients in whom oral mucositis occurred, mucositis samples were taken using paper strips placed on the top of the lesion and on the surrounding mucosa. For the reference individuals, mucosal samples were taken from the lower lip and bucca with the same procedure as for the patients. All samples were immediately stored at -20°C then transferred to -80°C until analysis.

DNA extraction, 16S rRNA gene amplification and sequencing

The mucosal bacterial samples were analysed using 454 FLX pyrosequencing according to previously described methods [13,14] with minor modifications. After DNA extraction, lip and buccal samples from the same individual at the same time-point were pooled, and samples from mucositis lesions and the surrounding mucosa from the same individual at the same time-point were pooled with an equal volume of each DNA extraction. The primer pairs for DNA amplification were 341f (5′-CCTACGGGNGGCWGCAG) with adaptor B and 805r (5′-GACTACHVGGGTATCTAATCC) with adaptor A and a specific sequence barcode consisting of seven nucleotides. The PCR products were purified, pooled, amplified in PCR mixture in oil emulsions, and sequenced using a two-lane PicoTiterPlate on a Genome Sequencer FLX system (Roche, Switzerland) [15] at the Science for Life Laboratory, Karolinska Institutet.

Sequence processing and taxonomic classification

Sequence processing was carried out with the software AmpliconNoise version 1.25 [16], correcting for errors introduced in PCR and pyrosequencing, as well as removing chimeric sequences. Denoised sequences were aligned and sorted into operational taxonomic units (OTUs) at the 97% similarity level using complete linkage clustering at the Ribosomal Database Project [17].

In order to identify the taxonomical belonging of each OTU cluster, the sequence aligner SINA was performed against the SILVA SSU reference database version 111 [18], in which the algorithm considers up to 40 of the best hits ($\geq$ 70% identity) and assigns taxonomy as the least common ancestor. If no hit was found, the sequence was assigned as 'unclassified'.

After the sequence noise reduction, 350 710 high-quality sequences remained, with 376–4140 reads per sample (mean 2828). These sequences comprised 1613 OTUs clustered at a 97% similarity level. After taxonomic identification, sequences ascertained to be of bacterial origin consisted of 99% reads and 305 genera or the most detailed level of consensus taxonomy. After a manual check of the taxonomic data, the sequences assigned as 'chloroplast' and 'mitochondria', which are likely to have been derived from eukaryotic cell organelles, were excluded from the following statistical analysis.

Statistics

Analysis of the clinical data was carried out using the statistical software package SPSS version 20. For the bacterial sequence data with regard to relative abundance, the dominant taxa were visualized as a heat map using MultiExperiment Viewer [19]. The microbial diversity of each sample was evaluated using the Shannon diversity index (H') based on an equal sub-sampling level. The Shannon index, which takes both taxa richness and the relative abundance into account, ranges from 0 (one species presents) to about 4.5 (species are relatively evenly distributed). The R package vegan (http://CRAN.R-project.org/package=vegan) was used to calculate the Shannon index and significance was tested using the Student's *t*-test. The inter-subject variability between each pair of samples was evaluated on the platform Fast Unifrac [20] using normalized weighted Unifrac distance, which ranges from 0 (100% OTUs shared, two communities are identical) to 1 (0% OTUs shared, two communities are completely distinctive). The Unifrac distance was further interpreted and visualized using the principal coordinate analysis (PCoA) on Fast Unifrac. Comparison of each taxon between subject groups or between time-points was made at the phylum level and the most detailed taxonomic level using the R package edgeR [21,22]. The p values were converted to false discovery rate (*q* value) to correct for multiple testing. For all the statistical methods used, the level of significance was accepted at $\alpha = 0.05$.

Results

Firstly, we compared the clinical parameters of the patients at the time of malignancy diagnosis, which is before chemotherapy, with those of the reference group. There was no significant difference regarding clinical characteristics in terms of age, gender or oral health status between patients and reference individuals (Table S1). The relative abundance of the

dominant bacteria in all mucosal samples is presented in Fig. S1. The diversity of the total bacterial community was lower in the patients at the time of malignancy diagnosis compared with the reference individuals (Fig. 1a). Furthermore, as shown in Fig. 1(b,c), the intragroup Unifrac distance was higher in patients than in the reference individuals, which indicates that the patients were more heterogeneous among each other in terms of their oral bacterial community. The intergroup comparison of Unifrac distance showed a significant difference of the entire bacterial profile between patients and reference individuals; in addition, taxa with different relative abundance between groups were identified (Table S2).

The clinical data and bacterial composition at the time-point of malignancy diagnosis, prior to chemotherapy, were then compared between patients who later developed oral mucositis and those who did not. These two patient groups were similar regarding age, gender and oral health status (Table 1). However, lower levels of neutrophils, thrombocytes and haemoglobin at the time of malignancy diagnosis were found in individuals who later developed oral mucositis, most likely due to the high number of cases with haematological malignancies in this group (18 out of 25). As shown in Fig. 2(a), the total microbial diversity, was significantly higher in patients who later developed mucositis compared with those who did not. Regarding intersubject variability, at the time of malignancy diagnosis, patients who later developed mucositis were more dissimilar to each other than the no mucositis group (Fig. 2b, c). To further identify the taxa that contribute to the differences between groups, edgeR analysis showed that patients who developed mucositis presented with higher levels of the phyla Fusobacteria and Spirochaetes, compared with those who did not develop mucositis (Table 2).

Between the initiation of chemotherapy and the sampling time-point during chemotherapy, no difference was found between patients who later developed mucositis and those who did not regarding the use of antibacterial agents for treating infections (Table S3). The antibacterials used include cephalosporin, penicillin, aminoglycoside, thienamycin, trimethoprim-sulphamethoxazole and imidazole. The glycopeptide, which targets gram-positive bacteria, was not used prior to the mucosal sampling. In addition, there was no difference between the groups regarding the use of cytostatic regimens and benzydamine-based mouth rinse, which both have potential antibacterial effects [23–25] (Table S3). The microbial diversity during chemotherapy did not change significantly compared with that at the time-point for malignancy diagnosis, either for patients who developed mucositis (p 0.111) or for those who did not (p 0.679). In comparing the variability

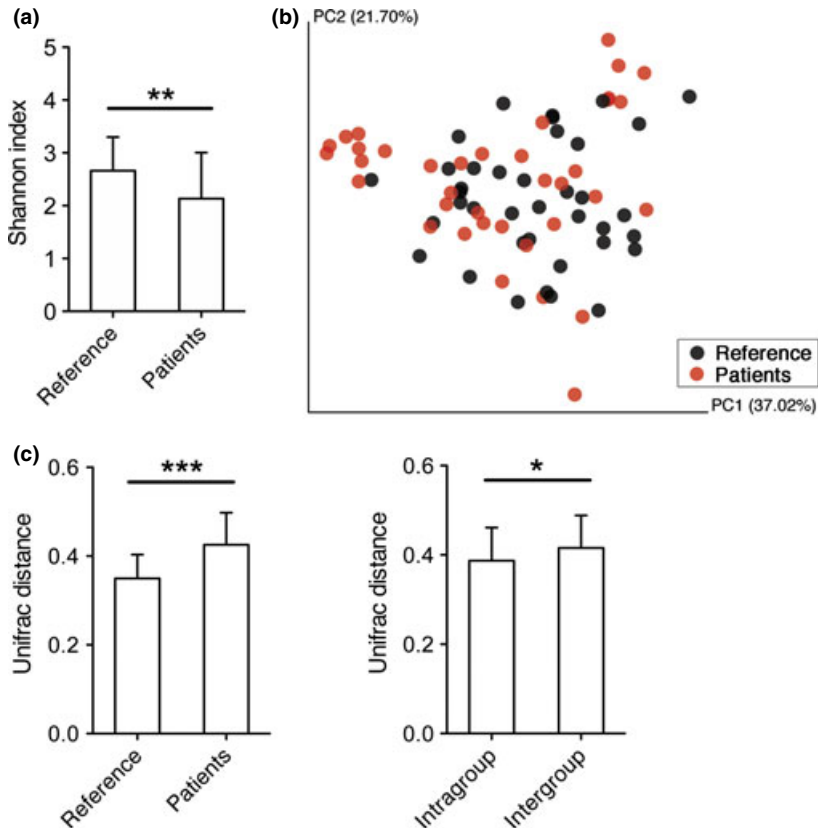

FIG. 1. Comparison of the oral bacterial community between reference individuals and patients at the time of malignancy diagnosis. The data represent 38 reference individuals and 37 patients. (a) Microbial diversity in Shannon index (mean ± SD). (b) Principle coordinate analysis. (c) Unifrac distance (mean ± SD), which was firstly calculated for each subject group as intragroup variability (left), and then the combined data were compared with intergroup variability (right). High values of Unifrac distance indicate dissimilarity. Student's *t*-test was used. PC, principle coordinate. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

between the time-points, the change of bacterial community composition was more evident in patients who later developed mucositis than in those who did not (Fig. 3). In the comparison regarding all bacterial taxa, the relative abundance of the phylum Proteobacteria was decreased in the patients who developed mucositis (Table 3).

The bacterial composition of samples from mucositis lesions is included in Fig. S2, presenting a high variety of microbial diversity (Shannon H', median, 2.08; range, 0.37–3.71) and a high intersample variability (Unifrac distance, mean ± SD, 0.55 ± 0.06). The comparison of each bacterial taxon was made between all mucositis samples and all mucosal samples (Table S4).

TABLE 1. Clinical characteristics of patients at the time of malignancy diagnosis in patients who later developed oral mucositis and those who did not

Variables	All patients $n = 37$ Mean (SD)	Mucositis $n = 25$ Mean (SD)	No mucositis $n = 12$ Mean (SD)	p-Value[a]
Age (year)	10.3 (4.4)	10.3 (4.3)	10.3 (4.8)	0.973
Gender (M/F)	28/9	19/6	9/3	1.000
DMFT/dmft	0.9 (1.8)	0.9 (1.6)	0.9 (2.3)	0.955
GBI (< 25%/> 25%)	33/4	22/3	11/1	1.000
Neutrophil ($\times 10^9$/L)	4.0 (3.3)	2.6 (2.6)	6.7 (3.1)	<0.001
Leukocyte ($\times 10^9$/L)	23.9 (73.1)	30.4 (88.9)	10.2 (3.7)	0.438
Thrombocyte ($\times 10^9$/L)	246 (183)	193 (186)	356 (118)	0.009
Haemoglobin (g/L)	109 (17)	103 (15)	120 (15)	0.003
Diagnosis				
ALL	12	12	0	–
AML	4	4	0	–
Non-Hodgkin lymphoma	5	2	3	–
Hodgkin lymphoma	3	0	3	–
Brain tumour	5	1	4	–
Skeletal sarcoma	3	3	0	–
Rhabdomyosarcoma	3	2	1	–
Renal tumour	1	0	1	–
Carcinoma	1	1	0	–

SD, standard deviation; DMFT/dmft, decayed, missing or filled teeth of permanent/deciduous teeth; GBI, gingival bleeding index; ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia.
[a]Student's *t*-test for continuous data; Fisher's exact test for categorical data.

Discussion

The current study presents novel insights into the oral mucosal bacterial dynamics in relation to chemotherapy-related oral mucositis. We showed that at the time of malignancy diagnosis, which is before chemotherapy, a more heterogeneous bacterial community with higher microbial diversity was found in the patients who later developed oral mucositis compared with no mucositis group. In addition, we found a more pronounced shift of the bacterial composition after the initiation of chemotherapy in patients who later developed oral mucositis compared with those who did not.

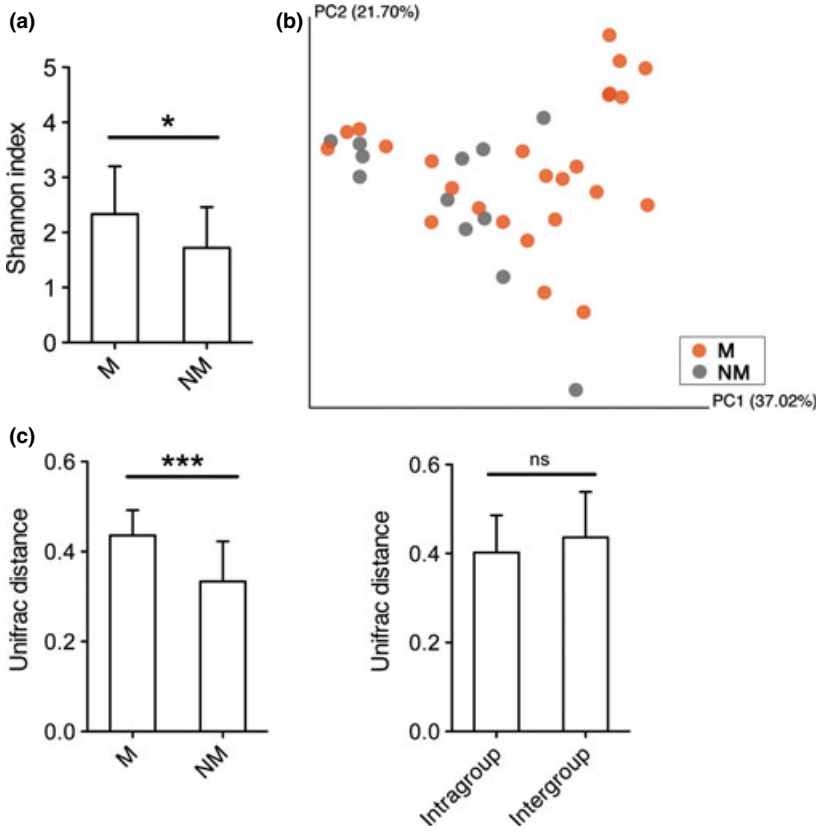

FIG. 2. Comparison of the oral bacterial community at the time of malignancy diagnosis between patients who later developed oral mucositis ($n = 25$) and patients who did not develop oral mucositis ($n = 12$). (a) Microbial diversity in Shannon index (mean ± SD). (b) Principle coordinate analysis. (c) Unifrac distance (mean ± SD), which was firstly calculated for each subject group as intragroup variability (left), and then the combined data were compared with intergroup variability (right). High values of Unifrac distance indicate dissimilarity. Student's *t*-test was used. M, mucositis; NM, no mucositis; PC, principle coordinate; ns, no significance. *$p < 0.05$, ***$p < 0.001$.

The entire group of patients with malignancies exhibited a less diverse and significantly different bacterial community and presented more dissimilarity to one another compared with the reference children. This difference may be attributed to the single-dose prophylactic cefotaxime administrated prior to chemotherapy and potentially as a result of a compromised host immunity and systemically altered inflammatory response caused by the malignancies [26,27]. The inflammatory mediators may impose a selective pressure resulting in bacteria that have a greater ability to escape host phagocytic defence and a higher tolerance against oxidative conditions, features that are typical for human pathogens.

Upon comparison of microbial diversity before chemotherapy, a higher level of diversity was detected in patients who later developed mucositis compared with the group that did not. Increased diversity of oral microbial communities has previously been linked to several oral diseases, including periodontal disease [28] and childhood caries [29]. Notably, the taxon *Capnocytophaga*, which was more abundant in the mucositis group, has been reported to be associated with various tissue infections [30,31]. The accumulation of these

TABLE 2. Relative abundance (%) of taxa with different levels at the time of malignancy diagnosis in patients who later developed oral mucositis and those who did not

	Mucositis $n = 25$		No mucositis $n = 12$			
Taxonomy[a]	Mean	SD	Mean	SD	p-Value	q-Value[b]
Bacteroidetes						
Capnocytophaga	2.9	3.9	0.4	0.3	0.001	0.017
Firmicutes						
Peptostreptococcaceae Incertae Sedis	0.2	0.5	0.0	0.0	<0.001	0.001
Lactococcus	0.9	4.2	<0.1	0.1	<0.001	0.003
Fusobacteria	4.1	6.0	1.0	0.9	0.004	0.027
Spirochaetes	0.1	0.2	<0.1	< 0.1	0.003	0.027

[a]Taxonomies in phylum (boldface) and genus levels.
[b]*q* value was used to determine statistical significance.

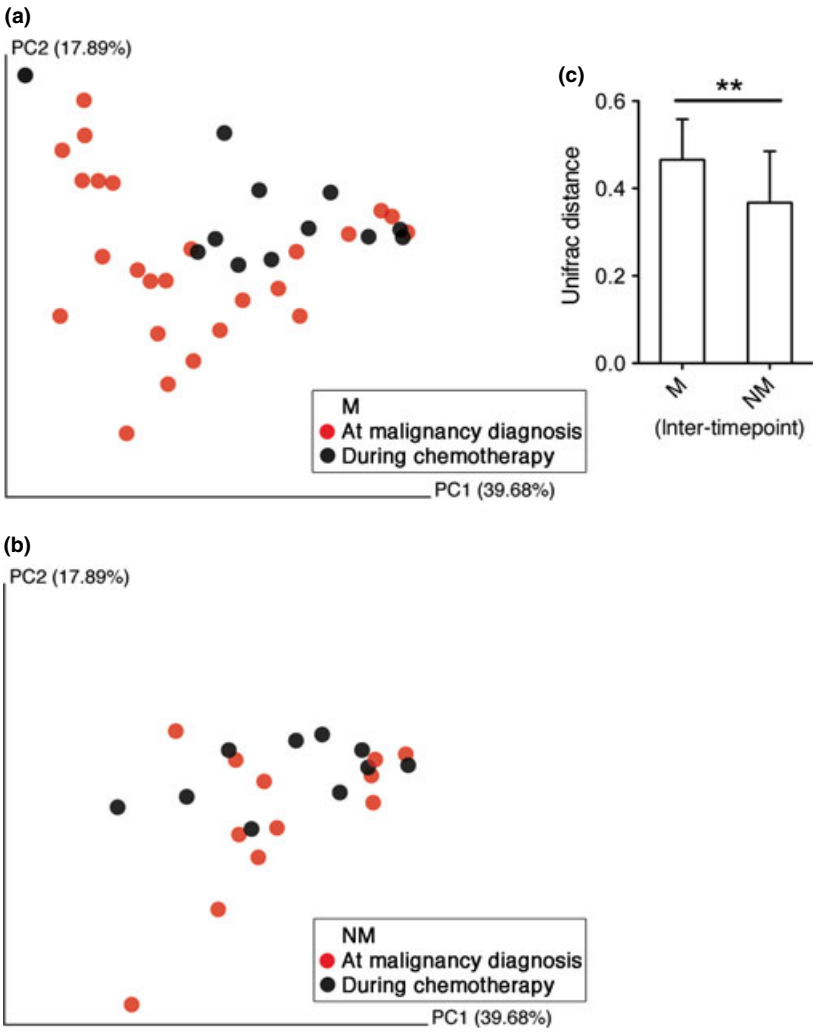

FIG. 3. Comparison of oral bacterial community between the time-point of malignancy diagnosis and during chemotherapy. At malignancy diagnosis, samples were taken from all patients. During chemotherapy samples were taken from 12 out of 25 patients in the group that later developed oral mucositis and 10 out of 12 patients in the group that did not. (a and b) Principle coordinate analysis. (c) Unifrac distance (mean ± SD), which was calculated between the two time-points as inter-time-point variability for each subject group and compared. High values of Unifrac distance indicate dissimilarity. Student's *t*-test was used. M, mucositis; NM, no mucositis; PC, principle coordinate. **p < 0.01.

TABLE 3. Relative abundance (%) of taxa with different levels between the time-point of malignancy diagnosis and during chemotherapy

Taxonomy[a]	At malignancy diagnosis		During chemotherapy		p-Value	q-Value[c]
	Mean	SD	Mean	SD		
Mucositis (n = 25)[b]						
Firmicutes						
Staphylococcus	0.1	0.1	6.7	20.9	<0.001	<0.001
Proteobacteria	21.9	18.1	8.3	7.0	0.001	0.015
Derxia	3.9	8.4	0.4	0.5	0.001	0.027
No mucositis (n = 12)[b]						
Proteobacteria						
Xanthomonas	0.0	0.0	0.2	0.7	<0.001	0.003

[a]Taxonomies in phylum (boldface) and genus levels.
[b]During chemotherapy samples were taken from 12 patients in the mucositis group and 10 patients in the no mucositis group.
[c]q value was used to determine statistical significance.

bacteria on the mucosal surface may possibly contribute to the progression of mucositis and potentially to bacteraemia. The bacterial community composition of the mucositis group was found to be more heterogeneous than that of the no mucositis group, which is consistent with the view that no single bacterial taxon can be expected to be entirely accountable for affecting the outcome of mucositis.

During chemotherapy, the bacterial community profile was altered in all patients. This is in line with studies on the microbiota profile of the intestine [32,33] and the oral cavity [4,8] from chemotherapy-treated individuals or animal model. The higher Unifrac distance in patients who developed mucositis is largely driven by the changes of Proteobacteria abundance during chemotherapy, which might be a consequence of antibacterials used to treat systemic infections and additionally a direct antimicrobial effect of cytostatic drugs [23,24]. It also appeared that the no mucositis group was less modified by the chemotherapeutic treatment, which might indicate a beneficial effect of a higher microbial stability. Interestingly, our results show that the complexity of oral bacteria in terms of Shannon diversity does not change after the initiation of chemotherapy in either patient group, which is contrary to a previous study using Sanger-based sequencing [34]. These opposing results are most likely a reflection of the different methods applied. In order to determine diversity it is important to be able to detect low abundant taxa and the 454 pyrosequencing technique has a higher sensitivity in this respect.

The genus *Streptococcus*, which has been reported to increase following chemotherapy [35], showed an elevated mean value in patients who later developed mucositis in our study; however, it was not statistically confirmed. The level of *Staphylococci* was found to be increased during treatment in the group that later developed mucositis; however, this was due to a surprisingly elevated level in one patient. The previously suggested mucositis-associated species, including *Enterococcus* [8], *Escherichia* [4], *Porphyromonas* [7] and *Pseudomonas* [4], were not statistically confirmed in terms of relative abundance in the present study, which may in part be attributed to methodological differences. It is noticed that because of the limited number of patients from each malignant diagnosis in this study, it is not possible to clarify the question of whether the individual bacterial profile among patients receiving identical treatment is related to oral mucositis. Large cohort studies that are controlled for the chemotherapy scheme are thus needed in the future.

The breakdown of the mucosal barrier provides a port for the invasion of endogenous bacteria [30,31,36–39]. In this study, we identified a distinctive bacterial composition from the mucositis lesions compared with all the mucosal samples from the lip and bucca. Within the dominant bacteria from the lesions, an increased abundance of *Lactobacillus*, *Mycoplasma* and *Peptostreptococcus* was identified. Both *Mycoplasma* and *Peptostreptococcus* are recognized as significant pathogenic microorganisms due to their resistance to common antibacterial agents. While bacteria belonging to the genus *Lactobacillus* are well known for contributing to deep tooth decay in the oral cavity, their facultative anaerobic characteristics and tolerance to oxidative conditions may also account for the growth in the lesions of mucosal tissues. Overall, the increase of these potential pathogens in mucosal ulcers might be the effect of the ulcers representing a different niche in which virulent pathogens can compete successfully with the resident bacteria, in addition to the effect of the empirical antimicrobial therapy administered.

Importantly, although beyond the scope of this study, Candida and viruses have long been indicated to play a role in oral mucositis and subsequent systemic infection [8,40]. Therefore, studies with a focus on their relation to the commensal bacteria, including bacteriophages, will be required to clarify the role of the entire oral microbiota in the pathogenesis of mucositis.

Conclusions

Powered by high-throughput 454 pyrosequencing, the current study indicates that at the time of malignancy diagnosis, patients who later developed oral mucositis showed higher oral mucosal microbial diversity and were more heterogeneous among one another compared with those who did not develop mucositis. A more pronounced modification of the bacterial community by chemotherapy was detected in patients who later developed oral mucositis, indicating that oral microbial stability might be beneficial. These findings might contribute to the development of better prophylactic treatments and improved intervention protocols for oral mucositis, tailored to the individual patient.

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Transparency Declaration

The authors declare no conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Relative abundance of the dominant taxa (≥ 0.5%) in oral mucosal samples from all subjects.

Figure S2. Relative abundance of the dominant taxa (≥ 0.5%) in samples from oral mucositis lesions.

Table S1. Clinical characteristics of reference individuals and patients at the time of malignancy diagnosis.

Table S2. Relative abundance (%) of taxa that exhibited different levels between reference individuals and patients at the time of malignancy diagnosis.

Table S3. Antibacterials, cytostatic drugs and mouth rinse administered between the first (at malignancy diagnosis) and second (during chemotherapy) mucosal samples.

Table S4. Relative abundance (%) of taxa with different levels between all mucosal samples from lip and bucca and samples from mucositis lesions.

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