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Modifications of the Microflora of the Oral Cavity Arising During Immunosuppressive Chemotherapy

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Infections are a major cause of mortality among immunosuppressed cancer patients. The oral cavity is a possible reservoir for those microorganisms, both commensal and acquired, whose virulence is exacerbated in the immunosuppressed patient. The mouth consists of multiple habitats offering ecological niches to a variety of organisms. The object of this article is to review the literature devoted to quantitative and qualitative variations in the flora of the oral cavity during immunosuppressive treatment of cancer patients. Examination of these different studies reveals modifications of the commensal flora, as well as an increase in Gram negative rods, in staphylococci and in yeasts. These data confirm the necessity for constant surveillance of the oral cavity during chemotherapy.

Key words: microbiology, drug therapy, immunodepression, mouth

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INTRODUCTION

Infections are a major cause of mortality among immunosuppressed cancer patients. A variety of phenomena favour the development of serious infections. An example is the immunosuppression resulting from neutropenia related to the pathology or to the chemotherapy used to treat it. Similarly, microorganisms whose virulence is exacerbated among immunosuppressed patients may be either casually acquired, or selected by the pathology or ensuing therapy. The oral cavity is one of the possible reservoirs for these commensal and acquired microorganisms. It comprises multiple habitats which offer a number of ecological niches to a variety of organisms. The colonisation of habitats in the oral cavity is regulated by interbacterial competition, nutrient supply, and the physicochemical environment (pH, temperature, etc.). Specific and non-specific components of the immune system found in the saliva and gingival fluid also play a primordial role. The gingival fluid contains the humoral factors found in serum (notably those relating to the immune system), together with cells of the immune system, particularly polynuclear neutrophils. It first encounters the subgingival plaque, and then the supragingival plaque, before its elements become mingled with the saliva. The buccal flora changes with age. The appearance of the teeth, their loss, and hormonal changes occurring during adolescence are some of the major causes of modification to the buccal ecosystem. Thus, when treatment of a cancer patient begins, the microbial content of the oral cavity is a function of individual factors: the flora acquired before the development of the disease (this being, related to individual family, social and ethnic influences), factors related to the pathology, such as type and progression of the pathology, and previous treatments undergone by the patients, such as antibiotic therapy, mouth washing, etc. These different parameters complicate our understanding of the dynamics of the buccal flora during therapeutic immunosuppression. Several studies have attempted to analyse these different elements, but many questions remain. The object of this article is to review the literature devoted to quantitative and qualitative variations in the bacterial and fungal flora of the oral cavity during immunosuppressive chemotherapy of cancer patients.

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PROBLEMS ENCOUNTERED DURING ANY ANALYSIS OF THE BUCCAL FLORA

Most studies have involved adult populations. The differences between populations, pathologies, treatments, and times of sampling, render comparison difficult. Several problems are inherent to any study of the flora during treatment.

Choice of target flora

An exhaustive study is extremely complicated, as the oral bacterial flora is abundant and highly diverse. There are 300–400 detectable species, and no doubt many other species remain to be discovered. Comparison of different studies is further complicated by the fact that some studies relate to the aerobic flora and others to the facultative or anaerobic flora.

A search for quantitative and qualitative variation provides some interesting indications. It remains to correlate such variation with physiological or biological modifications, or to the appearance of new pathologies, and to draw clear inferences regarding therapeutic indications. All studies involving detection by culture also encounter the problem of detection threshold. A species may be present in the sample, but in too few numbers to appear on culture plates, creating a "false negative". The appearance of a new bacterial species at a certain stage in treatment is not necessarily a sign of acquisition, but may be a sign of an increase in the numbers of this organism at the sampling site. In cases of genuine acquisition, the reservoir may be another site in the same individual, but may also be from an outside source. The possibility for nosocomial colonisation must always be considered.

A search may be made for specific organisms, either because these are known to cause infection among cancer patients, or because they are considered to represent the "normal" flora. In the first case, techniques with a lower detection threshold, such as those employing immunochemical or genomic tools, may be useful, but can be used to detect only a limited number of species.

Does the choice of site influence the results?

There are many sites to choose from: the cheek mucosa, dorsum of the tongue, floor of the mouth, palate, subgingival or supragingival dental plaque, tonsils, and saliva. Must all of these be sampled, or is there one, or more, representative site? The diversity of sites used in the various studies makes comparison difficult. Few studies have compared the flora at different sites. A swab from the gingival margin of all of the teeth, or a swab from the mucosa beneath the dentures in patients without natural teeth, may give a sample representative of the flora of all the other sites in the buccal cavity [1]. In the latter study, the samples were taken from adults presenting a malignant haematological pathology, with fever, who had undergone anti-cancer therapy during the 28 days preceding the infection. The difficulty in taking samples (e.g. from young children) may also influence the choice of site.

Despite the difficulties encountered in comparing studies on the subject, several common themes nonetheless emerge, relating to the quantitative and qualitative aspects of the buccal flora.

QUANTITATIVE ASPECTS OF THE BUCCAL FLORA

Several studies show a decrease in total bacterial numbers during chemotherapeutic treatments, frequently correlated with antibiotic therapy (Table 1). Bergmann [2] noticed an increase in the number of microorganisms recovered by culture during chemotherapy, before the use of antibiotics. According to him, this increase is related to reduced saliva production by the population studied (and the ensuing reduced elimination of microorganisms). This observation has been confirmed by another study [3]. Conversely, no reduction in salivary output during immunosuppressive therapy, or only a transient reduction, may be noted [4]. These variations are probably due to differences in population, pathology and treatment.

QUALITATIVE ASPECTS OF THE BUCCAL FLORA

Published studies have generally dealt either with commensal microorganisms of the oral cavity, or with microorganisms actually or supposedly acquired during treatment, particularly enterobacteria and the genera *Pseudomonas* and *Candida*.

Commensal organisms of the oral cavity

Streptococci. A drop of about 75% [5] in the numbers of streptococci inhabiting the oral cavity is regularly observed: 78% after 7 days of antibiotic therapy, followed by a marked increase in streptococci observed 4.5 days after the cessation of antibiotic therapy [2]. During episodes of fever, a 77% decrease in the prevalence of the "normal" flora (streptococci and actinomycetes) is observed after 7 days of antibiotic administration [1]. *Streptococcus mutans*, a species which initiates caries development, has been particularly well studied. Reynolds *et al.* [6] noticed an increased proportion in adults, at mid-chemotherapy, in the supragingival plaque (1–2.2% of the cultivable flora), and a decrease in the subgingival flora (0.6–0.1%). A decrease (88%) in the

Table 1. *Quantitative modifications of the total cultivable flora (TCF), aerobic or anaerobic, of the oral cavity, during immunosuppressive therapy*

Ref.	Year	Pathology	Sites	Results TCF
Fainstein *et al.* [5]	1981	AML (ALL)	Pharynx* (gargling)	>75% reduction with respect to initial flora, of which >65% during antibiotic therapy No modification in total count
Minah *et al.* [15]	1986	non-ALL AL	Supragingival* Subgingival* Wash*	75% reduction mid-treatment 30% reduction mid-treatment 53% reduction mid-treatment
Wahlin [4]	1988	AML-ALL	Stimulated saliva†	No change in aerobic flora
de Medeiros *et al.* [35]	1995	ALL	Supragingival*	80% reduction at days 7 and 14, increase to 40% of initial level at day 21.

*Anaerobic flora; †aerobic flora.

AL, acute leukaemia; ALL, acute lymphoid leukaemia; AML, acute myeloid leukaemia.

total numbers of *S. mutans* in the saliva, during immunosuppressive treatment of children, has been observed [7]. Their quantity returned to normal during the maintenance phase, and decreased in the event of general infection. These modifications may be due to the antibacterial action of daunorubicin and antibiotics [7]. Although alpha-haemolytic streptococci, particularly *Streptococcus mitis* and *Streptococcus sanguis*, have been isolated several times from cases of septicaemia [8–13], they have never been the object of a specific study.

Enterococci. Forty-five per cent of leukaemia patients may be carriers of enterococci, compared with 24% of a control population [14]. The largest numbers are isolated from patients undergoing antibiotic therapy who have spent at least 5–10 days in hospital. In another study, enterococci were isolated from 17% of patients with malign haematological tumours presenting an infectious episode [1].

Gram-negative rods. Several studies have shown a clear increase in the number and proportion of Gram-negative rods (GNR) in the oral cavity of immunosuppressed cancer patients. Minah *et al.* [15] observed an increase in GNR throughout the entire oral cavity, the supragingival plaque showing the greatest increase. This study, like most others, was specifically concerned with those species frequently encountered in general microbiology, i.e. the enterobacteria and the genus *Pseudomonas*. The GNR most frequently found in the oral cavity of healthy individuals generally belong to the genera *Bacteroides, Capnocytophaga, Cardiobacterium, Centipeda, Eikenella, Fusobacterium, Haemophilus, Leptotrichia, Prevotella* and *Selenomonas* [16] and are rarely the subject of specific studies. Other genera, usually associated with periodontal lesions, may also be isolated from the healthy individual, even during youth: *Actinobacillus, Campylobacter, Porphyromonas, Treponema*, etc. [17]. Although *Capnocytophaga* is regularly associated with septicaemia, bacteraemia and other local/regional and general infections [18, 19], no studies have, to our knowledge, specifically sought it in immunosuppressed cancer patients. *Capnocytophaga* may show multiple antibiotic resistance, and represent a real threat in cases of septicaemia [20]. *Fusobacterium nucleatum* [19], and *Leptotrichia buccalis* [21] have also been isolated from cases of septicaemia. Reynolds *et al.* [6] noticed an increase in the proportion of *Fusobacterium nucleatum* in the anaerobic flora of adults in mid-chemotherapy. The proportion of this species increased from 4.2 to 11.4% in the supragingival plaque, and from 7.2 to 12.5% in the subgingival flora. Little change has been noted in the black-pigmented *Bacteroidaceae*, whose proportion remains low. However, the latter may form a majority in periodontal abscesses in myelosuppressed patients [22].

Generally speaking, an increase in GNR, particularly *Klebsiella* and *Pseudomonas* [15, 23, 24] is frequently related to infection, particularly septicaemia and pulmonary infections.

Staphylococci. Almost all studies have observed an increase in the prevalence and proportion of staphylococci during chemotherapy [1, 5, 14–16, 25, 26]. They are found in all sites of the oral cavity. Wahlin and Holm [14] isolated them from all adult and child subjects. Reynolds *et al.* [6] demonstrated their presence in all patients, at mid-chemotherapy, in the supragingival and subgingival plaque, whereas no patients carried them before treatment. These authors noted a positive correlation between the loss of gingivo-dental attachment (degradation of tissue supporting the tooth) and a marked increase in both the staphylococci in the supragingival plaque and the total quantity of yeasts in the supragingival and subgingival plaque. Similarly, a correlation between the proportion of staphylococci and the total quantity of yeasts in the supragingival plaque of acute myeloid leukaemia patients was observed at mid-chemotherapy [25]. Staphylococci have also been isolated from periodontal abscesses in patients with myeloid leukaemia [22].

Non-commensal microorganisms

Enterobacteria and the genus Pseudomonas. The prevalence and proportion of the GNR generally increase during chemotherapy [15]. Enterobacteria (particularly *Klebsiella* and *Enterobacter*) and the *Pseudomonaceae* are the most commonly described GNR [1, 2, 5, 6, 14, 15, 24, 25, 27–31]. They are regularly isolated from cases of bacteraemia or septicaemia. Although the source is non-oral in most cases, a case of septicaemia caused by these bacteria from an oral source has been described [29]. Although scarce, if not absent from the oral cavity before treatment [5, 6], they increase sharply during treatment. Reynolds *et al.* [6] report that enteric GNR represent 16.6% of the anaerobic supragingival flora, and 14.4% of the anaerobic subgingival flora. The presence of *Pseudomonas aeruginosa* in the supragingival plaque, before or at mid-chemotherapy, has been positively correlated with the total numbers of yeasts in dental plaque [25]. Studying *Enterobacteriaceae* during bone marrow transplantation, Galili *et al.* [31] noted a positive correlation between the percentage of positive oral cultures and the patients' age. A higher prevalence was shown in patients older than 16 years. In the latter study, leukaemia patients who received either total body irradiation or total lymph node irradiation showed more positive cultures than lymphoma patients who did not receive any irradiation.

Fungi. The presence of yeasts in the oral cavity of patients during chemotherapy has been abundantly described. They are isolated from the saliva of 80% of leukaemia patients during chemotherapy [2, 14], and may form part of the predominant flora of the oral cavity [2]. The increase in prevalence of *Candida* in febrile patients after 4 days of antibiotic therapy is significant [1]. An increase in prevalence of *Candida*, in number and in proportion of the flora, has also been described [3]. Reports of lesions associated with their presence or with the increase of their concentration in the saliva [2, 4, 14, 32] has heightened the interest in these potential pathogens. Oral inflammations due to *Candida* are essentially encountered in cases of leukaemia [33]. Apart from local lesions, yeasts can extend their colonisation to the oesophagus, and cause septicaemia. *Candida albicans* and *Candida tropicalis* are the species most frequently encountered [34].

CONCLUSIONS AND PROPOSAL FOR SURVEILLANCE OF THE ORAL MICROFLORA

Quantitative and qualitative modifications in the flora of the oral cavity have been observed which may be due to

local and/or general treatments. These variations show an imbalance in the ecosystem. Some species, which are found rarely or in small numbers in the healthy individual, may suddenly appear or increase in number and in proportion, occasionally becoming dominant. This sharp increase and preponderance may be at the origin of local or general infectious manifestations. The microorganisms may benefit from the weakening of the immune system, and may enter the body at a variety of sites, such as through mucosal lesions or the gingival crevice. These observations underline the importance of constant surveillance of the oral cavity during chemotherapy treatments. Further studies are needed to evaluate the link between modifications of the oral microflora and the potential infectious risk. Furthermore, observations of the oral microbiota should be compared with those made in other areas, such as the faecal flora and haemoculture, and correlated with the presence of oral lesions. In our opinion, changes should be looked for in both anaerobic and aerobic floras. No particular site has been demonstrated to be representative of all the oral habitats. An ideal surveillance procedure would include sampling from several dental sites, one or more mucosal sites, and the saliva. In practical terms, this cannot be performed. Dental plaque contains a complex and profuse flora, and is under salivary and serum influence (mediated by the gingival fluid). Pooling of dental plaque samples from different sites might give interesting information. We suggest assessment of the following three parameters: (i) Presence and percentage of members of the normal flora: streptococci, especially alpha-haemolytic streptococci, *Actinomyces* species, and *Lactobacillus* species. Their absence or presence at low percentages in the cultivable flora would signify a disturbance of the oral ecosystem; (ii) Complexity of this flora: the dental plaque is usually polymorphic. A low number of cultivable species may also be the sign of an ecological disturbance. (iii) Presence and percentage of target microorganisms that are known to cause infections in immunocompromised subjects, e.g. *Capnocytophaga* spp., enterobacteria, *Pseudomonas* spp., staphylococci, and fungi (this is not an exhaustive list). In the case of dramatic disturbances in the microflora, we suggest performing antibiograms of the predominating microorganisms and of any unusual microorganism detected. Antibiogram results might be used either to select a more appropriate antibiotic regimen for each individual patient or to adapt the antimicrobial therapy in case of infection due to these organisms.

1. Bergmann OJ, Kilian M, Ellegaard J. Potentially pathogenic microorganisms in the oral cavity during febrile episodes in immunocompromised patients with haematologic malignancies. *Scand J Infect Dis* 1989, **21**, 43–51.
2. Bergmann OJ. Alterations in oral microflora and pathogenesis of acute oral infections during remission-induction therapy in patients with acute myeloid leukaemia. *Scand J Infect Dis* 1991, **23**, 355–366.
3. Main BE, Calman KC, Ferguson MM, *et al.* The effect of cytotoxic therapy on saliva and oral flora. *Oral Surg Oral Med Oral Pathol* 1984, **58**, 545–548.
4. Wahlin YB. Salivary secretion rate, yeast cells, and oral candidiasis in patients with acute leukemia. *Oral Surg Oral Med Oral Pathol* 1991, **71**, 689–695.
5. Fainstein V, Rodriguez V, Turck M, Hermann G, Rosenbaum B, Bodey GP. Patterns of oropharyngeal and fecal flora in patients with acute leukemia. *J Infect Dis* 1981, **144**, 10–18.
6. Reynolds M, Minah G, Peterson D, *et al.* Periodontal disease and oral microbial successions during myelosuppressive cancer chemotherapy. *J Clin Periodont* 1989, **16**, 185–189.
7. O'Sullivan EA, Duggal MS, Bailey CC, Curzon MEJ, Hart P. Changes in the oral microflora during cytotoxic chemotherapy in children being treated for acute leukemia. *Oral Surg Oral Med Oral Pathol* 1993, **76**, 161–168.
8. Cohen J, Worsley AM, Goldman JM, Donnelly JP, Catovsky D, Galton DAG. Septicaemia caused by viridans streptococci in neutropenic patients with leukaemia. *Lancet* 1983, **2**, 1452–1454.
9. Ringdén O, Heimdahl A, Lönnqvist B, Malmborg AS, Wilczek H. Decreased incidence of viridans streptococcal septicaemia in allogeneic bone marrow transplant recipients after the introduction of acyclovir. *Lancet* 1984, **1**, 744.
10. Bostrom B, Weisdorf D. Mucositis and alpha-streptococcal sepsis in bone marrow transplant recipients. *Lancet* 1984, **1**, 1120–1121.
11. Steiner M, Villablanca J, Kersey J, *et al.* Alpha-streptococcal shock in bone marrow transplantation patients. *Blood* 1988, **72**(Suppl. 1), 409a.
12. Heimdahl A, Mattsson T, Dahllöf G, Lönnqvist B, Ringdén O. The oral cavity as a port of entry for early infections in patients treated with bone marrow transplantation. *Oral Surg Oral Med Oral Pathol* 1989, **68**, 711–716.
13. Schimpff SC. Surveillance cultures. *NCI Monogr* 1990, **9**, 37–42.
14. Wahlin YB, Holm AK. Changes in the oral microflora in patients with acute leukemia and related disorders during the period of induction therapy. *Oral Surg Oral Med Oral Pathol* 1988, **65**, 411–417.
15. Minah GE, Rednor JL, Peterson DE, Overholser D, DePaola LG, Suzuki JB. Oral succession of Gram-negative bacilli in myelosuppressed cancer patients. *J Clin Microbiol* 1986, **24**, 210–213.
16. Slots J, Taubman MA. *Contemporary Oral Microbiology and Immunology* St. Louis, Mosby Year Book, 1992.
17. Sixou J-L, Robert J-C, Mouton C. Les pathogènes parodontaux chez l'enfant sain. *J Parodontol* 1993, **12**, 129–136 (in French).
18. Parenti DM, Snydman DR. *Capnocytophaga* species: infections in nonimmunocompromised and immunocompromised hosts. *J Infect Dis* 1985, **151**, 140–147.
19. Baquero F, Fernandez J, Dronda F, *et al.* Capnophilic and anaerobic bacteremia in neutropenic patients: an oral source. *Rev Infect Dis* 1990, **12**, S152–S160.
20. Gomez-Garces JL, Alos JI, Sanchez J, Cogollos R. Bacteremia by multidrug-resistant *Capnocytophaga sputigena*. *J Clin Microbiol* 1994, **32**, 1067–1069.
21. Reig M, Baquero F, Garcia-Campello M, Loza E. *Leptotrichia buccalis* bacteremia in neutropenic children. *J Clin Microbiol* 1985, **22**, 320–321.
22. Slots J, Rams TE. New views on periodontal microbiota in special patient categories. *J Clin Periodont* 1991, **18**, 411–420.
23. Schimpff SC, Moody M, Young VM. Relationship of colonization with *Pseudomonas aeruginosa* to development of *Pseudomonas* bacteremia in cancer patient. *Antimicrob Ag chemother* 1971, 240–244.
24. Galili D, Donitza A, Garfunkel A, Sela MN. Gram-negative enteric bacteria in the oral cavity of leukemia patients. *Oral Surg Oral Med Oral Pathol* 1992, **74**, 459–462.
25. Peterson DE, Monah GE, Reynolds MA, *et al.* Effect of granulocytopenia on oral microbial relationships in patients with acute leukemia. *Oral Surg Oral Med Oral Pathol* 1990, **70**, 720–723.
26. Jobbins J, Bagg J, Parsons K, Finlay I, Addy M, Newcombe RG. Oral carriage of yeasts, coliforms and staphylococci in patients with advanced malignant disease. *J Oral Pathol Med* 1992, **21**, 305–308.
27. Johansson WG, Pierce AK, Sanford JP. Changing pharyngeal bacterial flora of hospitalized patients. Emergence of Gram-negative bacilli. *New Engl J Med* 1969, **281**, 1137–1140.
28. Newman RN, Schimpff SC, Young VM, Wiernik PH. Lessons learned from surveillance cultures in patients with acute nonlymphocytic leukemia. Usefulness for epidemiologic, preventive and therapeutic research. *Am J Med* 1981, **70**, 423–431.

29. Greenberg MS, Cohen G, McKitrick JC, Cassileth PA. The oral flora as a source of septicemia in patients with acute leukemia. *Oral Surg Oral Med Oral Pathol* 1982, **53**, 32–36.
30. Dreizen S, Keating MJ, McCredie KB, Bodey GP. Oral infections associated with chemotherapy. *Postgrad Med* 1982, **71**, 133–146.
31. Galili D, Tagger N, Sela MN, Garfunkel AA. Surveillance of oral cultures for *Enterobacteriaceae* during bone marrow transplantation. *Oral Oncol, Eur J Cancer* 1995, **31B**, 58–62.
32. Williams MC, Martin MV. A longitudinal study of the effects on the oral mucosa of treatment for acute childhood leukemia. *Int J Paediat Dent* 1992, **2**, 73–79.
33. Scully C, MacFarlane TW. Orofacial manifestations of childhood malignancy: clinical and microbiological findings during remission. *J Dent Child* 1983, **50**, 121–125.
34. Wingard JR. Infectious and noninfectious systemic consequences. *NCI Monogr* 1990, **9**, 21–26.
35. Medeiros O de, Sixou JL, Jolivet-Gougeon A, Le Gall E, Bonnaure-Mallet M. Microorganisms in the dental plaque of children with acute lymphoblastic leukemia. *J Dent Res* 1995, **74** (Special Issue), 942.

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