Factors influencing oral health in patients during cancer treatment; with emphasis on the relationship between the oral microbiome and oral mucositis
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Chapter 1

General Introduction
Healthy oral ecosystem

The human body consists of $10^{13}$ mammalian cells and $10^{14}$ microorganisms (mostly bacteria, fungi and archaea) are living on or in the human body. The whole human microbiome makes up 1-3% of the total body weight of an individual. Every part of the body harbors a specific microbial community that varies widely within one individual and among different individuals (1). Humans have a symbiotic relationship with the resident microflora (2). The human body provides colonization opportunities, nutrients and optimal growth conditions for the microflora, while the resident microflora actively promotes the development of the normal physiology of the host and contributes to maintaining health (3). Furthermore, the resident microflora prevents the colonization or overgrowth of opportunistic and pathogenic microorganisms (4). The immune system of the host plays an important role in maintaining the homeostasis. Also the oral cavity is an ecosystem where homeostasis is maintained due to the interactions between the host and the residing microbes (2).

The homeostasis in the oral cavity can change because of several reasons. In case of a disease like cancer, there are metabolic changes and the immune system of the host is compromised. Furthermore, cancer treatment may significantly influence oral homeostasis. When the homeostasis in the oral cavity is disturbed this can lead to the colonization of exogenous microorganisms and also opportunistic pathogens in the resident flora can grow out (2). Following chemo- and radiotherapy to the head and neck area, a shift in the oral microbiome takes place (5-8).

Radiotherapy to the head and neck area

In the Netherlands, the yearly incidence of tumors in the head and neck area is about 3.000 (Dutch Cancer Registration). Radiotherapy is often part of the treatment plan, either alone or in combination with surgery and/or chemotherapy. The choice of treatment is mainly determined by the diagnosis and the stage of the disease. Radiotherapy is often required in advanced stages of the disease (9, 10). Radiotherapy is aimed at eradicating malignant cells as it uses high dosed radiation to damage DNA of rapidly dividing cells. Besides the tumor, the salivary glands, the jaws and the oropharyngeal mucosa are often in the field of radiation and as a consequence of the therapy they are negatively affected. Curative radiotherapy is delivered at a total dose of 50-70 Gy. Delivery schemes usually consist of a 2 Gy dose per day, five days per week over a period of five to seven weeks in total (11).
Hematopoietic stem cell transplant (HSCT)

HSCT is a treatment for hematologic malignancies like all variants of leukemia, lymphomas, myelodysplastic syndrome, myeloproliferative disorders and multiple myeloma. The incidence of blood, bone marrow or lymphoid cancer types is about 8.000 in the Netherlands (Dutch Cancer Registration). Originally a treatment of last resort, HSCT is now being used early in the course of many diseases like congenital disorders and autoimmune diseases as well (12, 13). Hematopoietic stem cells for transplantation purposes can be of autologous or allogeneic origin. In case of a HSCT with stem cells from autologous origin, the patients receive their own stem cells. The advantages of this type of HSCT are that there is no donor needed, only a minimal risk for graft failure, and there is no risk for graft-versus-host-disease (GVHD, see the next paragraph). Furthermore, the transplant-related mortality rate is relatively low after autologous HSCT. Disadvantages of autologous HSCT include a small risk for transplanting malignant cells and there is no benefit from the graft-versus-tumor effect. The graft-versus-tumor effect means that the immune cells of the donor attack the cancer cells of the patient.

In case of a HSCT from allogeneic origin, a donor is needed. The advantages of this transplant type are that there is no risk of transplanting cancerous cells and there is a graft-versus-tumor effect; which is the main treatment effect. A large disadvantage is that the foreign immune cells can also attack the host’s own cells and GVHD may develop. In case of GVHD, the cells of the donor attack the cells of the patient. The incidence of acute GVHD after HSCT, in which the skin, the liver and the intestine are most often affected (14) is largely unknown (15). About 40-70% of the patients experience some form of chronic GVHD, despite immunosuppressive therapy (14).

Before the actual infusion of hematopoietic stem cells, patients receive a conditioning treatment (16). The conditioning chemo- and radiotherapy regimens for HSCT can be divided in myeloablative conditioning and reduced intensity conditioning regimens (RIC). Myeloablative conditioning is aimed at eradicating malignant cells and inducing immunosuppression to enable engraftment. It comprises of one or more chemotherapeutic agents possibly in combination with total body irradiation. RIC regimens are less cytotoxic and primarily aimed at immunosuppression (12).
Oral complications after treatment for cancer

Chemotherapy and radiotherapy to the head and neck area greatly disrupt the homeostasis in the oral cavity. After radiotherapy many biological changes take place, including a decrease in the amount of saliva (17), changes in the protein composition of saliva (18, 19), a decrease in the salivary buffer capacity (20) and a decrease in the vascularity of the mucosa and periodontal tissues (11). After chemo- and radiotherapy the composition of the oral microflora changes. More Gram-negative species and aciduric and acidogenic bacteria have been reported in the oral cavity after treatment (5, 7, 8, 21). The fungal load increases during treatment and the fungal species that is isolated most often is *Candida albicans* (8, 22).

Several clinical oral complications occur during and after treatment. Xerostomia, the subjective feeling of a dry mouth, is a highly frequent complication of head and neck radiotherapy. Xerostomia is often related to a decreased salivary flow (hyposalivation), although this is not necessarily the case. Almost all patients that undergo radiotherapy complain of a dry mouth (23). An abnormal or impaired sense of taste (dysgeusia) occurs in 56%-76% of patients after chemo- and radiotherapy (24).

An extraction of a tooth or another traumatic event to the jawbone can lead to a non healing area of exposed bone (osteoradionecrosis). This occurs in a minority (5-7%) of the patients that were treated with radiotherapy (25). However, when it develops, osteoradionecrosis can have a major impact on the structural integrity of the jaws. A limited opening of the jaw (trismus) occurs mostly in patients with head and neck cancer that undergo combined chemo- and radiotherapy (26).

Viral reactivation of Herpes Simplex virus type I (HSV-1) occurs frequently after stress, hormonal changes or exposure to UV-light in patients that are carriers of the virus. Reactivation usually appears as a painful (peri)oral ulceration (27). HSV-1 infection often occurs on the keratinized mucosa, while mucositis after HSCT usually involves the non-keratinized mucosa. However, HSV-1 may appear on the non-keratinized mucosa as well (28), and clinically it may be difficult to distinguish between the two conditions. Between 43% and 49% of patients develop an HSV-1 lesion somewhere in the oral cavity during treatment (22).

After chemotherapy, patients have a higher plaque index than healthy controls and have more inflammation of the periodontal tissues (29). Furthermore, a part of the patients develop more carious lesions. That may be the case after chemotherapy, but particularly radiotherapy involving the salivary glands increases caries risk. The average of decayed, missing and filled teeth
General Introduction

DMFT after radiotherapy doubles on average (29).

*Candida* species are part of the normal commensal oral microflora in a large part of the population (30). A disrupted homeostasis in the oral cavity is a risk factor for the outgrowth of *Candida* species and may result in local infection (31). Oral fungal infections occur in 39% of patients during chemo- or radiotherapy (22). *Candida* species are potentially linked to mucositis risk (32) because *Candida* species can grow invasively (33), the fungal load increases after chemo- and radiotherapy (22) and the clinical appearance of an oral fungal infection can resemble mucositis.

**Oral mucositis**

The best studied side effect of chemo- and radiotherapy is oral mucositis. Oral mucositis is an inflammatory process of the oral mucosa ranging from mild erythematous lesions to extensive ulcerations. Mucositis occurs not only in the oral cavity, but in the whole orodigestive tract (34).

Oral mucositis is one of the most common, serious and painful side effects of chemo- and radiotherapy (35). It typically occurs on the non-keratinized mucosa; the inner surfaces of the cheeks and lips, the floor of mouth, the lateral and ventral surfaces of the tongue and the soft palate. Between 40%-100% of the patients suffer from some form of oral mucositis during treatment (11, 36, 37).

Oral mucositis is accompanied by severe pain, difficulties with speech and nutrition, a higher risk for local and systemic infections, and consequently more medication use, a prolonged stay in the hospital after HSCT, and a decrease in the quality of life (36). In head and neck cancer patients, severe mucositis can lead to so much pain and difficulties with eating that the cancer therapy sometimes needs to be interrupted. That negatively influences the treatment outcome (38).

The pathobiology of mucositis consists of five interdependent stages: initiation, the primary damage response, amplification of damage responses, ulceration and finally the healing stage of the ulcerations (39). Radiotherapy and chemotherapeutic agents result in DNA and non-DNA based damage to the mucosal tissues and NF-κB is upregulated. NF-κB is considered to play a central role in mucositis, since it activates various inflammatory pathways that are involved in mucositis (40). The NF-κB dependent and independent pathways result in cell death and consequently in ulceration of the mucosa. Finally healing of the ulcerations takes place (39).

The healing of wounds consists of several phases that overlap in time: after inflammation the phases of cell proliferation and re-epithelialization, and remodeling of the tissue follow in time (41). The phases of cell proliferation and
re-epithelialization consist of the migration, proliferation and differentiation of the epithelial cells adjacent to the wound. At first, the shape of the epithelial cells changes as they flatten. Then the cells stretch and migrate into the wounded area and at the same time the cells start to proliferate resulting in thickening of the newly established epithelial barrier. Finally the cells mature and differentiate and the wound is healed (41, 42).

Recently the importance of changes in tissue structure, of inflammation and of the role of the microbiome in the pathobiology of mucositis have gathered more attention. There are studies that investigated the relationship between mucositis and genetic variance of the host. However, since only a limited number of studies have been performed and the results are not confirmed yet, no definitive conclusions can be drawn about specific candidate genes that are linked to mucositis risk (40).

The classic risk factors for developing oral mucositis are summarized in Table 1. They can be divided in treatment-related and patient-related risk factors (11, 36, 40, 43). The higher the dose and the longer exposure to radiation, the higher the risk for developing oral mucositis. For instance, if the cumulative radiation dose exceeds 30 Gy, virtually all patients develop oral mucositis (37). In myeloablative HSCT the conditioning regimen determines mucositis severity (44). Oral mucositis may be less severe in reduced-intensity conditioning regimens for HSCT (45). Patient-related risk factors have also been identified, however they are not all well supported by data (43).

<table>
<thead>
<tr>
<th>Table 1 Classic risk factors for developing oral mucositis</th>
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<tbody>
<tr>
<td><strong>Treatment-related</strong></td>
</tr>
<tr>
<td><strong>radiotherapy</strong></td>
</tr>
<tr>
<td>Radiation dose, fractionation scheme, size of ionizing irra-</td>
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<tr>
<td>diation, type of ionizing irradiation, concomitant chemoo-</td>
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<td>radiation and radiotherapy</td>
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<td><strong>Treatment-related</strong></td>
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<tr>
<td><strong>chemotherapy</strong></td>
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<tr>
<td>Type of agent, dose of agent, administration scheme</td>
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<tr>
<td><strong>Patient-related</strong></td>
</tr>
<tr>
<td>Age, body mass index, gender, hyposalivation, renal and</td>
</tr>
<tr>
<td>hepatic function, poor oral health</td>
</tr>
</tbody>
</table>

The ulcerative stage of mucositis offers a portal of entry for microorganisms to the bloodstream and patients are at risk for developing infectious complications from oral microorganisms such as *Streptococcus mitis* and *Streptococcus oralis* (46, 47). Furthermore, *Candida* species that reside in the oral cavity such as *C. albicans*, but also non-albicans species like *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei* and *C. kefyr* are found in systemic infections in
HSCT patients (48, 49). Infection with non-albicans \textit{Candida} species is more likely to spread systemically than \textit{C. albicans} (22).

\textbf{Interaction between periodontitis and oral mucositis}

Periodontitis is an inflammatory disease of the gums and other tooth supporting tissues that is caused by the subgingival biofilm. In periodontitis patients, the homeostasis in the oral cavity is disturbed by the accumulation of dental plaque (2). Similar to mucositis, periodontal infection leads to the activation of NF-κB and consequently to an increase of several pro-inflammatory cytokines (50). NF-κB plays a central role in mucositis (40). Therefore it is hypothesized that periodontal disease contributes to oral mucositis, and vice versa. The inflammation of the periodontium in combination with the inflammatory response induced by radio- and chemotherapy can lead to an exacerbated response in the form of oral mucositis (43). However, to date, there is only circumstantial evidence for this hypothesis.

Interesting is the modulating role of the oral microbiome in relationship to periodontitis. Periodontitis is traditionally associated with a group of bacteria from the so-called red complex: \textit{Porphyromonas gingivalis}, \textit{Tannerella forsythia} and \textit{Treponema denticola}. These bacteria are associated with increased pocket depth and clinical signs of periodontitis (51). From this group, especially \textit{P. gingivalis} has been the subject of extensive research. \textit{P. gingivalis} is a black pigmented, anaerobic, Gram-negative bacterium. It is able to invade periodontal tissues and to evade host defense mechanisms. \textit{P. gingivalis} possesses the virulence factors lipopolysaccharide, capsular polysaccharide, gingipains and fimbriae that cause deregulation of the innate immune and inflammatory responses (52).

Nowadays \textit{P. gingivalis} is considered to be a ‘keystone pathogen’ in periodontal disease (53). \textit{P. gingivalis} does not act as a ‘dominant pathogen’, meaning that it does not need to be present in large, dominant numbers to exert its effect. At low levels of colonization, \textit{P. gingivalis} is able to change the immune response of the host in such a way that it favors growth and development of the whole biofilm. This leads to a destructive change in the normally homeostatic relationship between the host and the resident flora. The microflora comes in dysbiotic state with the host leading to periodontal disease (Figure 1). Additionally, \textit{P. gingivalis} may directly modulate the resident microflora by its virulence factors (53).

Since \textit{P. gingivalis} possesses many virulence factors and is considered a keystone pathogen in soft tissue damage, it is possible that \textit{P. gingivalis}, in
collaboration with other members of the resident oral flora, plays a key role in the development and prolongation of oral mucositis.

**Figure 1** *Porphyromonas gingivalis*-induced dysbiosis and periodontal disease
Hajishengallis et al., 2012 (53)

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**Aims and outline of this thesis**

The general aim of this thesis was to study consequences of chemo- and radiotherapy for oral health. The following questions were addressed:

- Does radiotherapy to the head and neck area alter the composition of saliva?
- What is the link between oral bacteria, viruses and yeasts; and oral and systemic complications after HSCT?
- Are members of the oral microbiome, and especially *P. gingivalis*, able to influence the course of oral ulcerative mucositis by inhibiting wound healing?

A common side effect of radiotherapy to the head and neck area is a lowered salivary flow. In addition, it may be possible that radiotherapy alters the protein composition of saliva. Other studies describe differences in targeted salivary proteins after radiotherapy only. But, there are still no studies that use open-end large scale proteomic techniques looking at a wide range of salivary proteins after
radiotherapy to the head and neck area. In chapter 2 the results of a pilot study were described in which we compared the salivary proteins in parotid saliva of irradiated head and neck cancer patients and compared these to control subjects using SELDI-TOF-MS.

Studies on the relationship between oral microorganisms and oral ulcerative mucositis after HSCT commonly used traditional culturing techniques and patients were not prospectively followed over time. Therefore a prospective clinical study was performed in 49 adult HSCT patients during their stay in the hospital prior to and immediately following HSCT. Using real-time PCR, we analyzed the relationship between oral ulcerations and Herpes Simplex virus type-I, Epstein Barr virus and cytomegalovirus (chapter 3), bacteria that are traditionally associated with periodontitis and several Candida species (chapter 4).

In periodontitis certain bacteria, including P. gingivalis, cause inflammation of the periodontium. An inflamed periodontium can act as a portal of entry for oral microorganisms that may cause a bacteremia in neutropenic HSCT recipients. Thus, gingivitis and periodontitis may be risk factors for developing systemic infections after HSCT. In chapter 5 the link between clinical signs of an inflamed periodontium and the development of bacteremia in HSCT patients was studied.

The link between the oral microbiome and mucositis seems to be present, however the mechanism remains unclear. We hypothesized that several oral microorganisms influence the ulcerative stage of mucositis by hindering the healing of established lesions. Therefore the influence of the oral bacteria that we identified as predictors of oral ulcerations in chapter 4 on the healing of a wound in oral epithelial cells using an in vitro wound healing model in chapter 6 was studied.

The anaerobic, Gram-negative bacterium P. gingivalis turned out to be the most potent inhibitor of wound healing in chapter 6. Several major virulence factors that P. gingivalis possesses may be responsible for this effect. In chapter 7 we studied the influence of lipopolysaccharide, the capsular polysaccharide, the gingipains and the major fimbriae of P. gingivalis on wound healing in vitro.

In chapter 8 an overview is provided of the current literature on the role of the oral microbiome in oral ulcerative mucositis. We hypothesize that these bacteria may have a larger role in oral mucositis than thought previously. In chapter 9 a summary of the main results is given. Furthermore, a general discussion and directions for future research are provided.
References


