Chapter 4

Head and neck intensity modulated radiation therapy leads to an increase of opportunistic oral pathogens

JM Schuurhuis, MA Stokman, MJH Witjes, JA Langendijk, AJ van Winkelhoff, A Vissink, FKL Spijkervet

Edited version of:
Abstract

Objectives: The recent introduction of intensity modulated radiation therapy (IMRT) has led to new possibilities in the treatment of head and neck cancer (HNC). Limited information is available on how this more advanced radiation technique affects the oral microflora. In a prospective study we assessed the effects of various advanced treatments for HNC on the oral microflora, as well as the effects of elimination of oral foci of infection.

Materials and Methods: All consecutive dentate patients >18 years, diagnosed with a primary oral or oropharynx carcinoma and seen for a pre-treatment dental screening between May 2011 and May 2013, were included. Patients were grouped by oncologic treatment: surgery (SURG), IMRT (IMRT) or IMRT+chemotherapy (CHIMRT). Dental screening data, demographic data, subgingival biofilm samples, oral lavages and whole saliva samples were obtained to microbiologically analyze the effects of cancer treatments (1 year follow-up).

Results: This study included 82 patients (29 SURG, 26 IMRT and 27 CHIMRT). The trends in changes in prevalence and proportions of microorganisms were comparable in the IMRT and CHIMRT group. However, relative to the SURG group, increased prevalence of enteric rods, staphylococci and Candida species was observed in the IMRT and CHIMRT groups. In these groups, elimination of oral foci decreased the frequency of detection of pathogens such as P. gingivalis, T. forsythia and S. mutans.

Conclusion: Different treatments in HNC patients result in different changes in the oral microflora. Opportunistic pathogens such as staphylococci, enteric rods and Candida species tend to increase in prevalence after IMRT with or without chemotherapy, but not after surgical intervention.

Introduction

Head and neck cancer (HNC) patients treated with radiotherapy (RT) have a lifelong risk of developing severe oral problems. These patients may suffer from loss of salivary gland function, which predisposes them to secondary problems such as rapidly progressing dental caries and fungal and bacterial infections [1-3]. Radiation-induced hyposalivation and subsequent dental caries are associated with an increased risk for dental extractions and development of osteoradionecrosis (ORN) [4]. To prevent ORN and other oral sequelae after radiotherapy, pre-radiation dental screening is commonly performed to locate and eliminate oral foci of infection, although the efficacy of these interventions is unclear [5].

During the last decade, treatment techniques in HNC have changed substantially, due to the introduction of intensity modulated radiation therapy (IMRT) and concomitant chemoradiation [6]. The differences between 3D conformal radiotherapy (3D-CRT) and IMRT, with or without chemotherapy, have not been studied regarding their effects on oral microflora. For example, the reduced salivary secretion observed after IMRT relative to 3D-CRT may result in a less acidic oral environment and a lower incidence of hyposalivation-induced dental caries [7]. Teeth might be preserved longer after IMRT, since a less acidic environment may be less prone to induce and promote dental caries. As a consequence, longer survival of teeth provides more time for periodontal pathogens to cause periodontal problems. This might explain why recently periodontal pocket progression in irradiated patients was seen [8].

Although IMRT reduces the risk of xerostomia, it is not known whether the effects on the oral microflora are similar or different compared to changes induced by 3D-CRT. Changes related to 3D-CRT have been described for both the short term (<1 year) [9-12] and long term (>1 year) [13-15]. In general, microorganisms associated with oral disease increased in time after RT. This was related to salivary secretion rate and buffering capacity [14]. Only short-term effects (during 6 weeks of RT) of IMRT have been reported for a small sample of patients [16]. The latter study showed that IMRT is more conducive to maintaining the relative stability of the oral ecosystem than 3D-CRT. To the best of our knowledge long term (>1 year) effects of IMRT on oral microflora have not been described so far. Since loss of salivary secretion is less after IMRT than after 3D-CRT, it is worth studying whether this results in less pronounced alterations to the oral flora. Due to ethical considerations, it is not possible to compare 3D-CRT with IMRT prospectively. Therefore, we conducted a prospective study to assess the effects of three advanced HNC treatments—surgery, IMRT and IMRT with chemoradiation—on the oral microbial composition with a follow-up of 1 year. Also, the effects of elimination of oral foci of infection on the oral microbial composition in patients subjected to IMRT or IMRT and chemoradiation were assessed.
Materials and Methods

Patients
All consecutive dentate or partially dentate patients >18 years, diagnosed with a primary oral cavity or oropharynx carcinoma, who were referred to the Department of Oral & Maxillofacial Surgery of the University Medical Center Groningen (UMCG) in the Netherlands for a pre-treatment dental screening between May 2011 and May 2013, were included in this study. To be eligible for this study, post-oncologic treatment was performed within 6 months. Treatment plans of all patients were discussed in the multidisciplinary tumor board of the UMCG. Patients were placed into one of three groups according to their oncologic treatment: 1) intensity modulated radiation therapy (IMRT), 2) IMRT concurrent with chemotherapy (CHIMRT) or 3) surgery (SURG). Patients who had undergone previous surgical removal of a tumor and/or RT and/or chemoradiation to the head and neck region were excluded, as were patients with an unknown primary or parotid gland tumor. The medical ethical committee of the University Medical Center of Groningen approved the study protocol (METC 2012/091).

Surgery group
The surgery group consisted of patients who received oral oncologic surgery (SURG), not followed by IMRT or CHIMRT. Patients eligible for oncologic surgery were operated according to the guidelines of the Dutch Head & Neck Society (NWHTT) [17].

Radiotherapy and chemoradiation groups
The radiotherapy group (IMRT) consisted of patients who were subjected to definitive primary or post-operative IMRT. The chemoradiation group (CHIMRT) consisted of patients who were subjected to definitive primary or post-operative CHIMRT.

IMRT was delivered using megavoltage equipment (6 MV linear accelerator). For all patients, a contrast-enhanced planning CT scan was made in supine treatment position. Patients received a conventional fractionation schedule of 2 Gy daily, five times per week up to 70 Gy on the primary tumor and pathological lymph nodes in 7 weeks or an accelerated schedule with 6 fractions per week. Elective lymph node areas in the neck (both sites) were irradiated with a dose of 54.25 Gy, in fractions of 1.55 Gy. IMRT treatments attempted to spare the parotid glands without compromising the dose to the target volumes. In general, 7-field equidistant, non-opposing beams were applied. The radiation dose was delivered using a simultaneously integrated boost IMRT technique.

Chemotherapy was given concurrently with fractionated IMRT and consisted of Carboplatin on day 1 (300–350 mg/m² in 30 min intravenously) and 5-fluouracil (5-FU) from day 1 to 4 by continuous infusion (600 mg/m²/24 h), consisting of 3 courses given with an interval of 3 weeks. Postoperative chemotherapy consisted of 6x50 mg Cisplatin weekly. When chemotherapy was considered to be infeasible, patients were treated with cetuximab using a loading dose of 400 mg/m² one week prior to radiotherapy and a weekly dose of 250 mg/m² during radiotherapy.

Dental screening
All patients were evaluated before their oncologic treatment as part of routine clinical practice by means of an oral and dental screening, including radiographic examination. This screening is based on the protocol published by Jansma et al. [18]. Oral foci of infection were defined as follows [5]:
- deep caries in which excavation may lead to pulpal exposure;
- active periodontal disease with pockets ≥6mm, furcation ≥grade 1, mobility ≥grade 1, gingival recession ≥6mm and especially a combination of these periodontal problems;
- non-restorable teeth with large restorations, especially those extending beyond the gum line or with root caries, or those with severe erosion or abrasion;
- periapical granuloma and avital teeth;
- impacted, partially impacted or partially erupted teeth not fully covered by bone or showing radiolucency;
- cysts and other radiographic abnormalities.

To quantify periodontal disease, the periodontal inflamed surface area (PISA) was used [19]. Patients were asked about their smoking and drinking habits. Self-reported smoking options were ‘current smoker’, ‘past smoker’, or ‘never smoked’ and self-reported alcohol consumption options were ‘never drink alcohol’ or ‘drink alcohol’.

Additionally, baseline oral lavage, subgingival biofilm samples and unstimulated and stimulated whole saliva samples were taken at the dental screening. All data obtained at baseline and follow-up visits were collected in a predetermined order and recorded using a standardized study form designed for this study.

Sampling methods
At various time points, an oral lavage [20] and subgingival biofilm samples [21] were obtained for microbiological evaluation. The total anaerobic bacterial count as well as detection frequencies and bacterial load of the periodontal pathogens Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, Parvimonas micra, Fusobacterium nucleatum, and Campylobacter rectus were determined in subgingival biofilm samples. Total aerobic bacterial count and detection frequencies and bacterial load of Streptococcus mutans, lactobacilli, Actinomyces species, Gram negative enteric rods and Candida albicans were determined in the oral lavage samples. Microbiological analysis of the samples was performed by the Oral Microbiology Laboratory of the UMCG, according to standard laboratory procedures [20-22]. Unstimulated and stimulated whole saliva samples were taken at the same time points.
Statistical analysis
All data (dental screening and follow-up visits) were recorded using a study form designed for this study. Dental hygienists were instructed by the researcher on how to use the study form. Demographic data were retrieved from the patient files. Data were analyzed using SPSS Statistics 22. Values of p<0.05 were considered significant.

Comparison between the 3 treatment groups at baseline, 6 months and 1 year of follow-up was done using Kruskal-Wallis tests for quantitative data (age, proportions of microorganisms, salivary flow rate) and Chi-square tests for binary data (gender, prevalence of microorganisms). The percentage of each specific microorganism was given as a proportion of the total anaerobic count. For calculation of the means, we used culture positive patients only. Comparing baseline and 1 year data within a group was done using Wilcoxon signed rank test.

Testing for significant differences in prevalence and proportions of microorganisms within and between the treatment groups was done for all cultured microorganisms. Only the statistically significant different values were reported in the results section. Regarding the other factors of influence on oral microflora composition, again only the statistically significant different values were reported in the results section.

Results

Demographics
Eighty-nine patients met the inclusion criteria. Patients who had full mouth dental extractions to eliminate oral foci of infection (n=7) were excluded from analysis. The final study population therefore consisted of 82 patients who were grouped by oncologic treatment modality (Table 1). Follow-up was 1 year for all patients, except for 2 IMRT-patients who were lost to follow up after 6 months due to metastatic disease. No statistically significant differences between the groups were present at baseline.

Subgingival total bacterial counts
The subgingival total bacterial count decreased from 1.9x10^8 cfu/ml at baseline to 0.53x10^8 cfu/ml after 6 months (p<0.001) in the IMRT group, and from 1.9x10^8 cfu/ml at baseline to 1.6x10^8 cfu/ml after 6 months (p=0.014) in the CHIMRT group. The subgingival total bacterial counts decreased from 1.5x10^8 cfu/ml at baseline to 0.46x10^8 cfu/ml after 1 year (p=0.032) in the SURG group, from 1.9x10^8 cfu/ml at baseline to 0.19x10^8 cfu/ml after 1 year (p=0.001) in the IMRT group, and from 1.9x10^8 cfu/ml at baseline to 0.61x10^8 cfu/ml after 1 year (p=0.004) in the CHIMRT group.
Oral lavage total bacterial counts
No significant differences were found between or within groups for total bacterial counts from the oral lavage at the various time points.

Prevalence and proportion of oral microorganisms within the groups
The prevalence and proportion of oral microorganisms in subgingival biofilm samples and oral lavage are shown per treatment group in Figures 1 and 2. The prevalence of periodontal bacterial species in SURG patients tended to decrease at 6 and 12 months, but was only statistically significant for T. forsythia (P=0.046).

In the IMRT-group, the prevalence of several periodontal bacterial species, notably A. actinomycetemcomitans, P. gingivalis, P. intermedia, T. forsythia, P. micra, F. nucleatum and C. rectus, decreased during follow-up with a prominent decrease after baseline and before IMRT (Fig 1B). In contrast, the prevalence of staphylococci increased. In the oral lavage of these patients, the prevalence of S. mutans tended to decrease while an increase in prevalence was observed for enteric rods and Candida species. Similar changes in prevalence were observed in the CHIMRT patients (Fig 1C).

The prevalence of P. micra, F. nucleatum and lactobacilli was high in all treatment groups. A drop in prevalence over time was observed in IMRT and CHIMRT patients for P. gingivalis, P. intermedia, T. forsythia, S. mutans and Actinomyces species.

Differences between the treatment groups at baseline
The prevalence of lactobacilli at baseline was significantly lower in the IMRT group compared to the CHIMRT group and SURG group (p=0.037; Fig. 1), while the prevalence of C. albicans was lower in the SURG group (p=0.043) than in the IMRT and CHIMRT groups (Fig. 1).

Differences between the treatment groups at 6 months follow-up
At 6 months, the prevalence of Actinomyces species (p=0.028) and P. intermedia (p=0.031) was significantly higher in the SURG group than in the IMRT and CHIMRT groups (Fig. 1).

Differences between the treatment groups after 1 year of follow-up
At 1 year follow-up, the prevalence (p=0.001) and proportion (p=0.025) of C. albicans were both significantly higher in IMRT and CHIMRT patients compared to SURG patients; the prevalence of T. forsythia (p=0.026) and P. intermedia (p=0.015) was higher in SURG patients compared to IMRT and CHIMRT patients (Figures 1 and 2).

### Table 1. Demographic data of the 82 included patients per treatment group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>SURG group n=29</th>
<th>IMRT group n=26</th>
<th>CHIMRT group n=27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>Median (IQR)</td>
<td>60 (54-64)</td>
<td>63 (58-69)</td>
<td>58 (50-62)</td>
</tr>
<tr>
<td>Gender</td>
<td>Male / Female</td>
<td>16 / 13</td>
<td>14 / 12</td>
<td>18 / 9</td>
</tr>
<tr>
<td>Tumor site</td>
<td>Oral cavity</td>
<td>29 (100%)</td>
<td>17 (65%)</td>
<td>9 (33%)</td>
</tr>
<tr>
<td></td>
<td>Oropharynx</td>
<td>0 (0%)</td>
<td>9 (35%)</td>
<td>18 (67%)</td>
</tr>
<tr>
<td>TNM-classification</td>
<td>T1N0M0</td>
<td>18</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>T1N1M0</td>
<td>2</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>T1N2bM0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>T2N0M0</td>
<td>5</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>T2N1M0</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>T2N2bM0</td>
<td>0</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>T2N2cM0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>T2N3M0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>T3N0M0</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>T3N2bM0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>T4N0M0</td>
<td>0</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>T4N1M0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>T4N2bM0</td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>T4N2cM0</td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Not reported</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Wound closure</td>
<td>Primary</td>
<td>13 (45%)</td>
<td>5 (19%)</td>
<td>4 (15%)</td>
</tr>
<tr>
<td></td>
<td>Skin graft/flap</td>
<td>16 (55%)</td>
<td>10 (38%)</td>
<td>6 (22%)</td>
</tr>
<tr>
<td>Self-reported smoking</td>
<td>Yes / No / Not reported</td>
<td>11 / 9 / 8 / 1</td>
<td>9 / 9 / 8</td>
<td>7 / 9 / 11</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td>Median (IQR)</td>
<td>23 / 5 / 1</td>
<td>18 / 8 / 0</td>
<td>23 / 4 / 0</td>
</tr>
<tr>
<td>Cumulative IMRT dose</td>
<td>Median (IQR)</td>
<td>66 (66-70)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Primary IMRT</td>
<td>NA</td>
<td>11 (42%)</td>
<td>17 (63%)</td>
<td></td>
</tr>
<tr>
<td>Post-operative IMRT</td>
<td>NA</td>
<td>15 (58%)</td>
<td>10 (37%)</td>
<td></td>
</tr>
<tr>
<td>Chemotherapy type</td>
<td>Carboplatin/ 5-FU</td>
<td>NA</td>
<td>NA</td>
<td>18 (67%)</td>
</tr>
<tr>
<td></td>
<td>Cisplatin</td>
<td>NA</td>
<td>NA</td>
<td>7 (26%)</td>
</tr>
<tr>
<td></td>
<td>Cetuximab</td>
<td>NA</td>
<td>NA</td>
<td>2 (7%)</td>
</tr>
</tbody>
</table>

NA= not applicable
IQR= inter quartile range
SURG= surgery
IMRT= intensity modulated radiation therapy
CHIMRT= intensity modulated radiation therapy with chemotherapy
Figure 1A. Bar chart showing the prevalence of microorganisms cultured from subgingival biofilm samples (A.a. up to Staph) and oral lavages (S.m. up to C.a.) at baseline, after 6 months and after 1 year of follow-up in surgery patients. A.a= Aggregatibacter actinomycetemcomitans; P.g= Porphyromonas gingivalis; P.i= Prevotella intermedia; T.f= Tannerella forsythia; P.m= Parvimonas micra; F.n= Fusobacterium nucleatum; C.r= Campylobacter rectus; S.m= Streptococcus mutans; Lact= lactobacilli; Acti= Actinomyces species; Ente= Enterics and C.a= Candida albicans.

* = significant difference between baseline and 1 year follow-up (p=0.046) for T. forsythia.

Figure 1B. Bar chart showing the prevalence of microorganisms cultured from subgingival biofilm samples (A.a. up to Staph) and oral lavages (S.m. up to C.a.) at baseline, before IMRT, 6 weeks after IMRT, after 6 months and after 1 year of follow-up in IMRT patients. A.a= Aggregatibacter actinomycetemcomitans; P.g= Porphyromonas gingivalis; P.i= Prevotella intermedia; T.f= Tannerella forsythia; P.m= Parvimonas micra; F.n= Fusobacterium nucleatum; C.r= Campylobacter rectus; S.m= Streptococcus mutans; Lact= lactobacilli; Acti= Actinomyces species; Ente= Enterics and C.a= Candida albicans.

* = significant difference between baseline and 1 year follow-up for P. intermedia (p=0.001), T. forsythia (p=0.001), P. micra (p=0.046), staphylococci (p=0.025) and Actinomyces species (p=0.012).
Prevalence of microorganisms in CHIMRT patients

Figure 1C. Bar chart showing the prevalence of microorganisms cultured from subgingival biofilm samples (A.a. up to Staph) and oral lavages (S.m. up to C.a) at baseline, before CHIMRT, after 6 weeks CHIMRT, after 6 months and after 1 year of follow-up in chemoradiation patients. A.a= Aggregatibacter actinomycetemcomitans; P.g= Porphyromonas gingivalis; P.i= Prevotella intermedia; T.f= Tannerella forsythia; P.m= Parvimonas micra; F.n= Fusobacterium nucleatum; C.r= Campylobacter rectus; S.m= Streptococcus mutans; Lact= lactobacilli; Acti= Actinomyces species; Ente= Enterics and C.a= Candida albicans.

* = significant difference between baseline and 1 year follow-up for P. intermedia (p=0.005) and T. forsythia (p=0.020).

Mean % of microorganisms in positive cultured surgery patients

Figure 2A. Bar chart showing the mean percentage of microorganisms in culture positive surgery patients cultured from subgingival biofilm samples (A.a. up to Staph) and oral lavages (S.m. up to C.a) at baseline, after 6 months and after 1 year of follow-up. A.a= Aggregatibacter actinomycetemcomitans; P.g= Porphyromonas gingivalis; P.i= Prevotella intermedia; T.f= Tannerella forsythia; P.m= Parvimonas micra; F.n= Fusobacterium nucleatum; C.r= Campylobacter rectus; S.m= Streptococcus mutans; Lact= lactobacilli; Acti= Actinomyces species; Ente= Enterics and C.a= Candida albicans. Mean proportions for Enteric rods and C. albicans were close to zero; due to scaling they are not visible on this chart.
Figure 2B. Bar chart showing the mean percentage of microorganisms in culture positive IMRT patients cultured from subgingival biofilm samples (A.a. up to Staph) and oral lavages (S.m. up to C.a) at baseline, before IMRT, after 6 weeks IMRT, after 6 months and after 1 year. A.a = Aggregatibacter actinomycetemcomitans; P.g = Porphyromonas gingivalis; P.i = Prevotella intermedia; T.f = Tannerella forsythia; P.m = Parvimonas micra; F.n = Fusobacterium nucleatum; C.r = Campylobacter rectus; S.m = Streptococcus mutans; Lact = lactobacilli; Acti = Actinomyces species; Ente = Enteric rods; C.a = Candida albicans.

* = significant difference between baseline and 1 year follow-up for F. nucleatum (p=0.015). Mean proportions for Enteric rods and C. albicans were close to zero; due to scaling they are hardly visible on this chart.

Figure 2C. Bar chart showing the mean percentage of microorganisms in culture positive CHIMRT patients cultured from subgingival biofilm samples (A.a. up to Staph) and oral lavages (S.m. up to C.a) at baseline, before CHIMRT, after 6 weeks CHIMRT, after 6 months and after 1 year of follow-up. A.a = Aggregatibacter actinomycetemcomitans; P.g = Porphyromonas gingivalis; P.i = Prevotella intermedia; T.f = Tannerella forsythia; P.m = Parvimonas micra; F.n = Fusobacterium nucleatum; C.r = Campylobacter rectus; S.m = Streptococcus mutans; Lact = lactobacilli; Acti = Actinomyces species; Ente = Enteric rods; C.a = Candida albicans. * = significant difference between baseline and 1 year follow-up for C. albicans (p=0.019). Mean proportions for Enteric rods and C. albicans were close to zero; due to scaling they are not visible on this chart.
**Salivary flow rate**

Unstimulated and stimulated salivary flow rate decreased significantly in IMRT and CHIMRT patients (Table 2). Notably, hyposalivation was ascertained at baseline in 2 SURG patients, 4 IMRT patients and 6 CHIMRT patients.

**Relation oral microflora and salivary flow rate**

Patients who tested positive for *C.albicans* at baseline, 6 months and 1 year had a significantly lower unstimulated saliva flow at all three time points than patients who tested negative (p=0.018, p=0.001 and p=0.027, respectively).

**Other factors of influence on oral microflora composition**

**Plaque and bleeding score**

Plaque and bleeding scores did not differ between treatment groups at baseline, 6 months and 1 year. In the total group (n=82), baseline median plaque score was 50% (IQR 23-70%) and median bleeding score was 40% (IQR 20-60%). After one year, median scores declined to 25% for plaque and 10% for bleeding (p<0.001 and p=0.001, respectively). In the SURG group, baseline median plaque score was 50% (IQR 20-68%) and median bleeding score was 50% (IQR 20-75%). After one year, median scores declined to 20% for both plaque and bleeding (p<0.001 and p=0.001, respectively). In the IMRT group, baseline median plaque score was 50% (IQR 20-71%) and median bleeding score 30% (IQR 10-50%). After one year, median scores declined to 30% for plaque and 20% for bleeding (p=0.085 and p=0.840, respectively). In the CHIMRT group, baseline median plaque score was 50% (IQR 25-80%) and median bleeding score 40% (IQR 20-70%). After one year, median scores declined to 20% for both plaque and bleeding (p=0.001 and p=0.001, respectively).

**PISA**

The baseline PISA score was significantly different between treatment groups, with lower values in the IMRT group (mean 341mm²) compared to the SURG and CHIMRT groups (mean 729 and 733mm², respectively). Baseline PISA scores were not associated with baseline prevalence of the cultured microorganisms.

**Smoking**

The number of patients who smoked did not differ between the treatment groups at baseline and after 1 year (Table 1). In the total group (n=82), the number of smokers had decreased from 27 at baseline to 17 after 1 year (p=0.005). Baseline prevalence of *T. forsythia* was significantly higher in smokers than in non-smokers (p=0.022). The number of smokers decreased within all groups comparing baseline and 1 year; in the SURG group from 11 to 9 (p=0.480), in the IMRT group from 8 to 4 (p=0.046) and in the CHIMRT group from 8 to 4 (p=0.083).

### Table 2. Mean and SD of salivary flow rate per treatment group.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Baseline UWS flow ml/min mean (SD)</th>
<th>6 months UWS flow ml/min mean (SD)</th>
<th>1 year UWS flow ml/min mean (SD)</th>
<th>Baseline SWS flow ml/min mean (SD)</th>
<th>6 months SWS flow ml/min mean (SD)</th>
<th>1 year SWS flow ml/min mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SURG group</strong></td>
<td>0.53 (0.46)</td>
<td>0.68 (0.72)</td>
<td>0.76 (0.84)</td>
<td>0.38 (0.33)</td>
<td>0.41 (0.36)</td>
<td>0.66 (0.68)</td>
</tr>
<tr>
<td><strong>IMRT group</strong></td>
<td>0.57 (0.50)</td>
<td>0.20 (0.18)</td>
<td>0.25 (0.39)</td>
<td>0.38 (0.33)</td>
<td>0.22 (0.20)</td>
<td>0.29 (0.32)</td>
</tr>
<tr>
<td><strong>CHIMRT group</strong></td>
<td>0.38 (0.33)</td>
<td>0.22 (0.20)</td>
<td>0.22 (0.33)</td>
<td>0.38 (0.33)</td>
<td>0.22 (0.20)</td>
<td>0.29 (0.32)</td>
</tr>
</tbody>
</table>

Significant differences were found after 6 months of follow-up between the 3 treatment groups for unstimulated (p<0.001) and stimulated salivary flow (p<0.001). After 1 year of follow-up, significant differences were found between the 3 treatment groups for unstimulated (p=0.003) and stimulated salivary flow (p=0.001).
Oral foci
The number of patients with oral foci of infection at dental screening was highest in the SURG group (93%) compared to 73% in the IMRT group and 82% in the CHIMRT group (p=0.0139).

The prevalence of *F. nucleatum* at dental screening was significantly higher amongst patients with oral foci of infection, mainly periodontal disease, compared to patients without oral foci of infection (99% versus 85%, p=0.015). The prevalence of *F. nucleatum* was still significantly higher amongst patients with oral foci of infection at 6 months (p=0.005) but no significant difference was found between patients with and without oral foci of infection after 1 year.

Discussion
The trends in changes in prevalence and proportions of microorganisms were comparable in the IMRT and CHIMRT groups, whereas the SURG group showed a different pattern. In the IMRT and CHIMRT groups, increased prevalence of opportunistic pathogens, such as enteric rods, staphylococci and *Candida* species, was observed. In these groups, elimination of oral foci decreased the frequency of detection of major pathogens such as *P. gingivalis*, *T. forsythia* and *S. mutans*. The oral side effects of chemotherapy are essentially temporary and reversible [28]. Apparently, IMRT/CHIMRT has a similar long-term effect on microbial composition as 3D-CRT.

Comparison of our findings with previous studies
The results of this study showed an increased prevalence of opportunistic pathogens such as enteric rods, staphylococci and *Candida* species, which are associated to underlying disease. In the 1960s, Johanson et al. (1969) showed that illness causes a shift of microbial populations of the throat towards gram-negative bacilli [29]. After 3D-CRT, increased prevalence of both of *C. albicans* [9,11,15,30] and staphylococci [30] have been described. However, to our knowledge, no studies have been shown such effects in IMRT patients. After IMRT it could be expected that different changes in oral microflora would be seen due to the less reduced salivary flow compared to 3D-CRT [31-33]. We found a 56% reduction of unstimulated salivary flow rate after 1 year of follow-up in patients with oropharynx carcinoma treated with 3D-CRT or chemoradiation [34]. Even though we did find less reduced salivary flow after IMRT, our study showed that the prevalence of opportunistic pathogens still increases after IMRT to levels similar to those reported after 3D-CRT [13-15].

The total bacterial counts decreased over time in all groups. It is known that patients with oral squamous cell carcinoma have a significantly larger median number of cfu/ml saliva than healthy controls [35]. Treatment of the cancer probably led to the decrease in total bacterial counts in all treatment groups (SURG, IMRT and CHIMRT). Also, oral hygiene improved substantially in our cohort, which could also explain this effect.

Particularly remarkable is the decline in prevalence over time in all 3 treatment groups for *S. mutans* and in the SURG and CHIMRT group for lactobacilli. These findings are in contrast with literature reporting high numbers and proportions of acidogenic microorganisms after 3D-CRT [13,15,30,36]. However, after IMRT a smaller reduction in salivary flow was reported [7], which might explain the lower numbers of acidogenic microorganism found in our study, as the oral cavity is probably less acidic. Another explanation could be that only few patients in this study had active caries at baseline and/or during follow-up. Patients were subjected to an intensive oral care protocol and their oral hygiene improved substantially during follow-up.

After full-mouth tooth extraction, de Waal et al. reported a reduction of *A. actinomyctetemcomitans*, *P. gingivalis*, *P. intermedia*, *T. forsythia*, *P. micra*, *F. nucleatum*, and *C. rectus* after 4 weeks and 3 months [37]. Similarly, we observed a decrease of the same periodontal pathogens in the IMRT and CHIMRT groups during follow-up with a profound decrease after ‘baseline’ and ‘before RT’. In the period between ‘baseline’ and ‘before RT’, patients’ teeth that were classified as oral foci of infection were removed at least 10 days before the onset of IMRT or CHIMRT. Our results demonstrate the almost immediate effect on oral microflora after the elimination of oral foci of infection (mainly periodontally affected teeth).

We found a significantly higher baseline prevalence of *T. forsythia* in smokers than in non-smokers. This was an expected outcome since *T. forsythia* is associated with smoking [38]. In the SURG group, we found a significant decrease in prevalence of *T. forsythia* over time. Although no significant decrease of the number of smokers in the SURG group was found, 50% of smokers in the SURG group did quit smoking, which may have contributed to the significant decrease in prevalence of *T. forsythia*. Our institution supports HNC patients in their efforts to quit smoking as this improves their survival and treatment outcomes [39].

Implications
Our study reported on the long term effects of IMRT on oral microflora. Few comparable studies are available, primarily because this treatment modality is relatively new. Regarding the prevalence of opportunistic pathogens after IMRT and CHIMRT, the results after IMRT were comparable to those after 3D-CRT. This was unexpected, since IMRT causes a smaller reduction in salivary flow and has presumed positive effects on oral tissues and microflora. Our study showed that despite the less reduced salivary flow, opportunistic pathogens still increase.
Conclusion

Different treatments in HNC patients result in different changes in the oral microbiota. Opportunistic pathogens such as staphylococci, enteric rods and Candida species tend to increase in prevalence after IMRT with or without chemotherapy, but not after surgical intervention.

References


