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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Citation for published version (APA):
Chapter 3

Viral loads and antiviral resistance of herpesviruses and oral ulcerations in hematopoietic stem cell transplant recipients

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Bone Marrow Transplant. 2012 Sep;47(9):1222-8.
Abstract

Background
Ulcerative oral mucositis and infection are frequent complications in hematopoietic stem cell transplant (HSCT) recipients. The aim of this study was to investigate the relationship between oral ulcerations and HSV-1, EBV and CMV excretion and the presence of aciclovir-resistant HSV-1 strains in HSCT recipients.

Methods
This prospective observational study included 49 adult patients who underwent allogeneic HSCT. In total, 26 patients received myeloablative and 23 received non-myeloablative conditioning. Ulcerations on non-keratinized and keratinized oral mucosa were scored and oral rinsing samples were taken twice weekly. Viral loads were determined by real-time PCR. Samples from patients remaining HSV-1 positive despite antiviral treatment were studied for resistance to antivirals.

Results
Having an HSV-1 or EBV DNA positive sample was a significant predictor for ulceration of keratinized mucosa. HSV-1 was a significant predictor for ulcerations on non-keratinized mucosa as well. Persistent HSV-1 infection occurred in 12 of 28 patients treated with antiviral medication and aciclovir-resistant HSV-1 was found in 5 persistent infections.

Conclusion
In conclusion, HSV-1 is a predictor of ulcerations on non-keratinized as well as keratinized oral mucosa following HSCT. The role of EBV deserves further study. Persistent HSV-1 replication despite antiviral treatment is common and is due to resistance in 18% of treated patients.
Introduction

Ulcerative oral mucositis is a frequent and serious complication in patients receiving a hematopoietic stem cell transplant (HSCT). Previous studies reported that between 76 and 89% of patients undergoing myeloablative HSCT suffered from oral mucositis (1-5); reduced-intensity regimens may decrease the prevalence as well as the severity of oral mucositis (2). Patients reported that oral mucositis was the single most debilitating side effect of HSCT conditioning (6). Furthermore, it is associated with lower survival (7).

Following chemotherapy or HSCT, reactivation of latent HSV-1 occurs frequently (8-10). In such patients, HSV-1-induced ulcers may develop at the typical non-keratinized predilection sites for oral mucositis induced by chemoradiation (for example, floor of mouth, buccal and labial mucosa, lateral side and tip of the tongue, and soft palate) and may aggravate mucositis, or be confused with this condition (8). In addition, HSV-1-induced ulcerations may develop at keratinized oral mucosa and periorally, where mucositis as a result of chemoradiation is very uncommon. These sites include the hard palate, the dorsum of the tongue, the gingiva and the vermillion lip. However, HSV-1 reactivation may also result in asymptomatic shedding (8, 11). It is unknown whether the viral load in an oral sample can distinguish between asymptomatic and symptomatic HSV-1 reactivation. Also, it is unknown whether prolonged HSV-1 excretion in HSCT recipients despite antiviral treatment is caused by the profound immunosuppressed status of HSCT recipients, or due to resistance of the virus to the antiviral medication. The prevalence of antiviral resistance has been shown to be very low in immunocompetent subjects, whereas in immunocompromised patients with HSV-1 infections, resistance levels up to 27% have been described (12-14).

A possible role for other herpesviruses such as CMV (15) and EBV (16) in oral ulceration has been suggested but their role has at present only been supported by a very limited number of studies (9). Shedding of EBV in the saliva of healthy carriers occurs frequently (17), whereas oral shedding of CMV, apart from congenital or primary infection, is seen mostly in immunocompromised patients (18).

To our knowledge, no studies have been performed that prospectively sampled HSCT patients, regardless of the presence of oral ulcers, and quantitatively analyzed the presence of HSV-1, EBV and CMV DNA. Also, no data are available on the differential role of herpesvirus excretion on ulcerations at oral sites that may be affected by chemoradiation-induced oral mucositis (non-keratinized mucosa) and at sites atypical for chemoradiation-induced mucositis (keratinized mucosa). Therefore, the aim of this study in adult HSCT recipients was to prospectively explore the relationship between the presence and localization
of oral ulcerations and the duration and amount of HSV-1, EBV and CMV DNA shedding in the oral cavity. Furthermore, persistence of HSV-1 despite antiviral treatment and the occurrence of antiviral resistance in HSV-1 were studied.

**Patients and methods**

*Patients*

Data were collected from 49 adult patients who underwent HSCT for hematological malignancies at the Leiden University Medical Center between November 2006 and June 2009. The medical ethical committee of the Leiden University Medical Center approved this study. All patients gave their informed consent. Oral assessment data were noted on standardized forms, whereas other data, including HSV, CMV and EBV serostatus were retrieved from patient charts and from the laboratory information system.

*Transplantation protocol*

T-cell - depleted transplantation was performed either according to a reduced intensity conditioning protocol or a myeloablative conditioning (MAC) regimen as described previously (19-21). Prophylaxis for GVHD was only administered to recipients of grafts from matched unrelated donors in the MAC regimen (CY 3 mg/kg intravenously starting on day -1). During granulocytopenia, all patients received oral selective digestive tract decontamination, antifungal prophylaxis and, in case of MAC, systemic streptococcal prophylaxis. All patients received a basic oral care regimen aimed at preventing accumulation of dental plaque and keeping oral tissues moist and free of debris. Antiseptic washings were not part of basic oral care. Patients did not receive antiviral prophylaxis. In case of an oral lesion suspect of HSV-1 infection, sampling was performed using a sterile cotton swab and patients were treated with i.v. aciclovir or oral valaciclovir at the discretion of the treating physician.

*Oral assessment*

Oral assessment was performed twice weekly starting before or shortly after HSCT conditioning until hospital discharge by one trained investigator. Any type of oral ulceration was recorded in all patients. Mucositis was scored according to the WHO criteria (22). For the WHO score, ulcerations were evaluated at non-keratinized oral mucosal sites only. Ulcerations on keratinized oral mucosa and the vermilion lips were assessed and noted separately.

*Sampling*

Oral rinsing samples were taken at each oral assessment. Patients were asked...
to rinse their mouth for 30 s with 10 mL of a 0.9% saline solution. Samples were frozen in -20 °C within 3 h after collection and stored at -80 °C until analysis.

**Viral load determination**

DNA was isolated from a 200 mL aliquot of the samples with the MagNA Pure LC Total Nucleic Acid Isolation Kit using a MagNA Pure LC Instrument (Roche Diagnostics, Almere, The Netherlands). Viral loads were determined by real-time PCR on a CFX96 optical reaction module (Bio-Rad, Veenendaal, The Netherlands). Real-time PCRs for β-globin, CMV, EBV, Phocine Herpesvirus and HSV-1 were performed as previously described (23-25). Phocine Herpesvirus served as an internal control for DNA extraction, and PCR inhibition and the b-globin PCR was performed as a control for cell concentration in the samples.

**Resistance analysis**

Samples from patients that remained positive, despite antiviral treatment for at least 5 days, were studied for the development of resistance by comparing pre-treatment samples with subsequent samples during treatment as long as samples remained positive. In addition to the oral rinsing samples, samples routinely submitted to the clinical microbiology laboratory for diagnosis of HSV-1 were included in the resistance analysis. Resistance was determined by sequencing the *viral thymidine* kinase (TK) gene, and in case of TK mutations, by additional sequencing of the viral *DNA-polymerase* gene (25). Phenotypical susceptibility testing was performed on viral isolates containing mutations of unknown significance, as described elsewhere (25).

**Statistical analysis**

Possible predictors of ulcerations at non-keratinized and keratinized mucosa were analyzed separately in all patients. Positive samples with viral loads below 250 copies/mL were set at 125 copies/mL to correct for imprecise quantification at very low loads. To adjust for skewness of the data, 10log- transformed viral and β-globin loads were used in the analysis. To correct for cell count in an oral sample, the β-globin load was included as a predictor in the model. WHO mucositis scores were recoded into a binary variable: mucositis WHO grade 0 - 1 was scored as no ulceration present and mucositis WHO grade 2 - 4 was scored as ulcerative mucositis present. In view of the repeated measurements of ulcerative mucositis and ulcerations on keratinized mucosa within patients, the outcome was modeled as a repeated measures logistic regression. Parameters were estimated using the generalized estimating equations procedure in SPSS with first-order autoregressive correlation structure and a robust estimation proce-
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dure. Univariable analysis of potential predictors was performed with p-values <0.20 as a criterion for possible inclusion in a multivariable model.

Results

Patient characteristics and oral assessment outcomes

The characteristics of the 49 patients participating in this study are summarized in Table 1. The mean duration of hospitalization was 27 days. During the assessment phase, 33 patients (67%) developed ulcerative mucositis and maximum WHO grades 2, 3 and 4 occurred in 23, 7 and 3 patients, respectively. During the assessment phase, 23 out of 48 (due to missing data on 1 patient) patients (48%) developed an ulceration on the keratinized intraoral mucosa or the vermilion lip. There was a weak, but significant, association between the peak mucositis score and the length of stay in the hospital (Spearman’s ρ=0.31, p=0.036).

Oral ulcerations and viral loads

HSV-1 could be analyzed in 191 samples and was detected in 23 patients (47%) starting at a median of 2 days before HSCT (range -8 days to +6 days). Most HSV-1-positive samples occurred between days 4 and 6 after HSCT. The median HSV-1 DNA load in the 55 positive samples was 105.3 copies/mL (range $10^{2.1}$ - $10^{7.4}$ copies/mL). HSV-1 DNA was detectable in 24 of 61 samples (39%) from patients with ulcerations on non-keratinized mucosa (‘ulcerative mucositis’) and in 30 of 129 samples (23%) from patients without ulcerative mucositis (Figure 1). In 31 of 45 samples (69%) from patients with ulcerations on the keratinized areas of the mouth HSV-1 DNA was detected, whereas it was detectable in 24 of 140 samples (17%) from patients without ulcerations on keratinized mucosa (Figure 1). Of the 55 HSV-1-positive samples, 7 (13%) were from patients with ulcerative mucositis, 14 (25%) from patients with ulcerations on keratinized mucosa, 17 (31%) from patients with ulcerations at both locations and 17 (31%) from patients without any ulceration at the time of sampling. In the case of 15 of the 17 samples (88%) from patients without ulcerations, ulcerations occurred directly preceding or following sampling within days. There was no difference in the presence or the load of HSV-1 between patients in the MAC and reduced intensity conditioning regimens (data not shown).

From 18 patients at 24 sampling moments both oral rinsing samples and swabs from oral lesions were available; 20 samples (83%) showed concordant results (12 were HSV-1 positive and 8 were HSV-1 negative in both samples types), whereas 4 showed discordant results (all were negative oral rinsing samples with a positive swab). In two of the discrepant cases, swabs were weakly positive (C<sub>T</sub>-value >34) and in the other two cases swabs were from ulcerations on the lip. EBV and CMV could be analyzed in 186 samples. EBV was detected in
12 patients (24%) with a median load of $10^{3.8}$ copies/mL (range $10^{2.1}$-$10^{5.7}$ copies/mL) in the 35 positive samples. CMV was detected in six patients (12%) with a median load in the nine positive samples of $10^{2.5}$ copies/mL (range $10^{2.1}$-$10^{4.1}$ copies/mL). EBV was detected in 6 of 59 samples (10%) from patients with ulcerative mucositis, in 29 of 126 samples (23%) from patients without mucositis, in 12 of 44 samples (27%) from patients with ulcerations on keratinized mucosa and in 19 of 136 samples (14%) from patients without ulcerations on keratinized mucosa (Figure 1). For CMV, these percentages were 10.2, 2.4, 6.8 and 4.4%, respectively (Figure 1).

<table>
<thead>
<tr>
<th>Table 1 Patient characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of patients</strong></td>
</tr>
<tr>
<td><strong>Male</strong></td>
</tr>
<tr>
<td><strong>Female</strong></td>
</tr>
<tr>
<td><strong>Age (mean; s.d.)</strong></td>
</tr>
<tr>
<td><strong>Diagnosis</strong></td>
</tr>
<tr>
<td>Acute myeloid leukemia</td>
</tr>
<tr>
<td>Multiple myeloma</td>
</tr>
<tr>
<td>Acute lymphoblastic leukemia</td>
</tr>
<tr>
<td>Non-Hodgkin’s lymphoma</td>
</tr>
<tr>
<td>Myelodysplastic syndrome</td>
</tr>
<tr>
<td>Chronic myeloid leukemia</td>
</tr>
<tr>
<td>Hodgkin’s lymphoma</td>
</tr>
<tr>
<td>Chronic lymphocytic leukemia</td>
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<tr>
<td>Others</td>
</tr>
<tr>
<td><strong>Donor type</strong></td>
</tr>
<tr>
<td>Matched sibling</td>
</tr>
<tr>
<td>Matched unrelated</td>
</tr>
<tr>
<td>Other*</td>
</tr>
<tr>
<td><strong>Conditioning regimen</strong></td>
</tr>
<tr>
<td>Myeloablative</td>
</tr>
<tr>
<td>Reduced intensity</td>
</tr>
<tr>
<td><strong>Length of stay in the hospital (mean; s.d.)</strong></td>
</tr>
<tr>
<td><strong>HSV serostatus (IgG pre-HSCT)</strong></td>
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<tr>
<td>Positive</td>
</tr>
<tr>
<td>Negative</td>
</tr>
<tr>
<td>Unknown</td>
</tr>
<tr>
<td><strong>EBV serostatus (IgG pre-HSCT)</strong></td>
</tr>
<tr>
<td>Positive</td>
</tr>
<tr>
<td>Negative</td>
</tr>
<tr>
<td>Unknown</td>
</tr>
<tr>
<td><strong>CMV serostatus (IgG pre-HSCT)</strong></td>
</tr>
<tr>
<td>Positive</td>
</tr>
<tr>
<td>Negative</td>
</tr>
<tr>
<td>Unknown</td>
</tr>
</tbody>
</table>

*Cord blood donor for two patients, mismatched related donor for one patient, autologous transplant in one patient.
Figure 1 Detection of herpesviruses and presence of oral ulcerations. Percentage of HSV-1 (top), EBV (middle) and CMV (bottom) DNA-positive samples from patients with or without oral ulcerations at the time of sampling.
Predictors of oral ulcerations

In uni- and multivariable analyses, the presence of HSV-1 DNA in a sample (Table 2) was a significant positive predictor of having ulcerative mucositis (multivariable odds ratio (OR) = 2.62, p = 0.049) and of having ulcerations on the keratinized mucosa (multivariable OR = 4.37, p = 0.003). The HSV-1 DNA load was a significant predictor of both ulcerative mucositis and of ulcerations on keratinized mucosa in the univariable analysis (OR = 1.17, p = 0.023 and OR = 1.41, p<0.001, respectively). In multivariable analysis, HSV-1 DNA load was a positive predictor of ulcerations on keratinized mucosa (OR = 1.35, p = 0.0005), but it was no predictor of ulcerative mucositis (OR = 1.18, p = 0.08). No threshold of the HSV-1 DNA load could be established above which all samples were derived from patients with oral ulcerations (Table 3).

The presence of EBV DNA, but not the EBV DNA load, was a positive predictor of ulcerations on keratinized mucosa (multivariable OR = 3.82, p = 0.02, Table 2). Neither the presence nor the load of EBV DNA was significant predictor of ulcerative mucositis in the multivariable analysis.

CMV was not a significant predictor of ulcerative mucositis or of ulcerations on keratinized mucosa. Interestingly, conditioning regimen was not a significant predictor of ulcerative mucositis or ulcerations on the keratinized mucosa. Sex, age, body mass index, donor type and time after HSCT were not significant predictors of oral ulcerations either.

Table 3 The number and percentage of samples coming from patients with oral ulcerations at the time of sampling given for various HSV-1 load thresholds

<table>
<thead>
<tr>
<th>viral load in oral washing sample</th>
<th>total</th>
<th>ulcerative mucositis</th>
<th>ulcerations on keratinized mucosa</th>
<th>ulceration anywhere in the mouth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>&lt; 1000 copies/ml</td>
<td>136</td>
<td>34</td>
<td>25.2</td>
<td>17</td>
</tr>
<tr>
<td>≥ 1000 copies/ml</td>
<td>49</td>
<td>22</td>
<td>44.9</td>
<td>28</td>
</tr>
<tr>
<td>≥ 10,000 copies/ml</td>
<td>39</td>
<td>17</td>
<td>43.6</td>
<td>23</td>
</tr>
<tr>
<td>≥ 100,000 copies/ml</td>
<td>32</td>
<td>13</td>
<td>40.6</td>
<td>19</td>
</tr>
<tr>
<td>≥ 1,000,000 copies/ml</td>
<td>13</td>
<td>7</td>
<td>53.8</td>
<td>8</td>
</tr>
<tr>
<td>≥ 10,000,000 copies/ml</td>
<td>6</td>
<td>4</td>
<td>66.7</td>
<td>4</td>
</tr>
<tr>
<td>total</td>
<td>185</td>
<td>56</td>
<td>45</td>
<td>81</td>
</tr>
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</table>
### Table 2: Uni- and multivariable GEE analyses of predictors of ulcerative mucositis and of ulcerations on non-keratinized mucosa

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Univariable Analysis</th>
<th>Multivariable Analysis</th>
<th>Univariable Analysis</th>
<th>Multivariable Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
<td>p-value</td>
<td>OR</td>
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<tr>
<td><strong>sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>1.16</td>
<td>0.62-2.19</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>female</td>
<td>reference category</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>age (years, mean)</strong></td>
<td>0.98</td>
<td>0.95-1.00</td>
<td>0.048*</td>
<td>0.97</td>
</tr>
<tr>
<td><strong>body mass index (kg/m², mean)</strong></td>
<td>0.94</td>
<td>0.86-1.04</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td><strong>underlying disease</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>myeloablative</td>
<td>1.77</td>
<td>0.97-3.22</td>
<td>0.06</td>
<td>1.26</td>
</tr>
<tr>
<td>non-myeloablative</td>
<td>reference category</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>conditioning regimen</strong></td>
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<tr>
<td>myeloablative</td>
<td>1.77</td>
<td>0.97-3.22</td>
<td>0.06</td>
<td>1.26</td>
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<td><strong>donor type</strong></td>
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<td>matched sibling</td>
<td>0.64</td>
<td>0.16-2.56</td>
<td>0.53</td>
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<tr>
<td>matched unrelated</td>
<td>1.46</td>
<td>0.40-5.35</td>
<td>0.57</td>
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<tr>
<td>other</td>
<td>reference category</td>
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<tr>
<td><strong>time of sampling</strong></td>
<td>1.08</td>
<td>1.02-1.14</td>
<td>0.008*</td>
<td>1.06</td>
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<tr>
<td>(day after HSCT, median)</td>
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<tr>
<td><strong>HSV-1 present in sample</strong></td>
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<td></td>
</tr>
<tr>
<td>yes</td>
<td>2.54</td>
<td>1.24-5.18</td>
<td>0.01*</td>
<td>2.62</td>
</tr>
<tr>
<td>no</td>
<td>reference category</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>CMV present in sample</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>3.05</td>
<td>0.96-9.87</td>
<td>0.06</td>
<td>1.80</td>
</tr>
<tr>
<td>no</td>
<td>reference category</td>
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<tr>
<td><strong>EBV present in sample</strong></td>
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<tr>
<td>yes</td>
<td>0.31</td>
<td>0.11-0.92</td>
<td>0.04*</td>
<td>0.38</td>
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<tr>
<td>no</td>
<td>reference category</td>
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<tr>
<td><strong>total</strong></td>
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<tr>
<td>clinical data available</td>
<td>232</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>samples available</td>
<td>185</td>
<td></td>
<td></td>
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</tbody>
</table>

1 In multivariable analysis either virus detectability or virus load (results shown in text) were included as potential predictors. Beta-globin load (10log transformed) was included in the multivariable analysis to correct for cell count in the sample (ulcerative mucositis: OR=0.93, p=0.78; ulcerations on keratinized mucosa: OR=1.56, p=0.12).

2 not determined because of insufficient power for analysis of various small strata * p-value < 0.05
**Persistent HSV-1 and viral resistance**

Twenty-nine patients had at least one HSV-1-positive oral rinsing sample or oral swab, of whom 28 received antiviral treatment for 5 days and 12 (43%) still had detectable levels of HSV-1 after 5 days of antiviral treatment. Clinical data were available in 11 of the 12 patients and all patients suffered from oral ulcerations. No mutations were found in pre-treatment samples, but resistance-associated mutations in the TK gene were found in on-treatment isolates from five patients with treatment failure (Table 4); a frameshift mutation (insG@436) known to confer acyclovir (ACV) resistance (26) was found in four patients and a substitution (G59W) in one patient (GenBank accession number JN191624). The G59W substitution has not been described before in a clinical isolate. Phenotypical susceptibility testing showed ACV resistance in the isolates with mutation G59W (IC\textsubscript{50}-value 20-fold of pretherapy isolates without mutation). No resistance-associated mutations were found in the viral DNA-polymerase gene of these samples.

**Discussion**

The aim of our study was to prospectively investigate the relationship between herpesvirus excretion and the presence of oral ulcerations. In our study the presence of HSV-1 was a significant positive predictor for ulcerations on non-keratinized and keratinized mucosa. Primary HSV-1 infection may cause ulcerative stomatitis resembling the typical presentation of HSV-1 reactivation in immunocompromised patients; therefore, a causative role for HSV-1 in oral ulcerations in these patients is very plausible. Our findings support the use of antiviral prophylaxis in HSV-seropositive HSCT recipients (27). A previous study found an association between HSV-1 and stomatitis in autologous HSCT recipients as well (28), whereas another study failed to establish a relationship between the presence of HSV-1 and mucositis in HSCT recipients (5). The use of antiviral prophylaxis probably limited the role of HSV in this latter study (5). No other studies performed standardized and prospective quantitative sampling in all patients, with and without oral ulcers or used sensitive real-time PCR.

Although the HSV-1 load was a predictor of ulcerations on the non-keratinized mucosa, a clear quantitative relationship was difficult to establish. No cut-off value of the HSV-1 load could be established above which all samples were derived from patients with oral ulcerations, irrespective of correction for the b-globin load. Various factors may contribute to this phenomenon, such as the presence of non-cell-associated virus in the sample, the unknown interval between virus detection and the development of ulcerations and the unknown significance of viral dynamics. Possibly the increase in load is more predictive...
than the actual load. However, truly asymptomatic shedding without preceding or subsequent oral ulceration was found in only two samples. Therefore, in the absence of antiviral prophylaxis, prompt initiation of antiviral treatment upon detection of HSV-1 DNA in an oral sample may be warranted, regardless of the virus quantity.

Persistent HSV-1 shedding despite treatment occurred in 43% of the treated patients, and resistance-associated mutations were found in 18% of the treated patients. Of course, sensitive detection of HSV-1 DNA by real-time PCR after ulcerations have healed may account for part of the persistence. Nevertheless, resistance to antivirals appears to have a role in persistent infections in a relevant proportion of patients. The prevalence of resistant HSV-1 in our study as assessed by sequence analysis is comparable with previous studies in HSCT recipients, using different methodologies to detect resistance. In a setting of antiviral prophylaxis, HSV-1 resistance was demonstrated by viral culture in 27% of patients with HSV (13). In other studies, a prevalence of resistance of 14% was found using a colorimetric yield reduction assay (14) and of 18% using another colorimetric assay (12).

The retrospective analysis of resistance in our study hampers addressing the clinical significance of the infections with resistant HSV-1. We can only speculate that faster healing of oral ulcerations would have occurred if treatment had been switched early and in all patients with resistant isolates, but a role for viral susceptibility testing in cases of persistent symptomatic HSV-1 infection seems clear.

Mutation G59W has not been described in a clinical isolate before. The mutation is located in the ATP-binding site of the TK protein and it has been selected \textit{in vitro} under selection pressure with brivudin (29) and ACV (30), and an ACV-resistant HSV-2 isolate with mutation G59P has been found in an AIDS patient (31). This makes the G59W mutation the likely cause of the ACV-resistant phenotype of the isolate in our study.

The presence of EBV in a sample was an independent predictor of oral ulcerations on the keratinized mucosa, but not of ulcerative mucositis. The rate of EBV shedding of 25% was comparable with a previous study using DNA hybridization, which detected EBV in mouth washes from 19% of HSCT patients (32). EBV has clearly been associated with oral hairy leukoplakia in various categories of immunocompromised patients (33). Its role in oral ulcerations is not well known. Recently, EBV-positive oral ulcers with Hodgkin’s-like features that responded well to reduction of immunosuppression have been described in immunosuppressed patients, including one HSCT recipient (34). Also, EBV-associated oral lesions resembling oral hairy leukoplakia with extensive mucosal
ulceration have been described in an HSCT recipient (16). In our patient group, oral EBV shedding was not a predictor of systemic EBV complications, as there was no difference in the occurrence of EBV plasma DNAemia or the occurrence of post-transplant lymphoproliferative disorder between patients with or without EBV in the oral samples (data not shown). The possible role for EBV in oral lesions in HSCT recipients is currently very unclear, but certainly merits further study.

No relationship between the presence of CMV in the oral cavity and oral ulcerations was found. Furthermore, the occurrence of oral CMV shedding and the viral loads were low, despite the fact that half of the patients were seropositive for the virus prior to HSCT.

In our patient group, 67% of the patients developed ulcerative mucositis during hospitalization, which is a lower percentage than reported previously (1-5). Although Takahashi et al. (2) reported a profound decrease in the presence of ulcerative mucositis when patients underwent reduced intensity conditioning instead of a MAC regimen, the conditioning regimen was not a significant predictor of oral ulcerations in our study. The important role of HSV-1 in the development of oral ulcerations in the absence of antiviral prophylaxis in our study possibly masks the effects of the conditioning regimen. In addition, the fact that reduced intensity conditioning and GVHD prophylaxis regimens vary between our hospital and that of Takahashi et al. (2) may account for some differences as well.

In order to obtain a standardized and quantitative oral sample, oral rinsing samples were used. Results from oral rinsing samples were concordant with results from swabs from intraoral ulcerations, with discrepancies only in the case of lesions on the lips or in the case of weakly positive swabs. As taking a rinsing sample is less painful than swabbing an ulceration, oral washings might be a suitable alternative for viral diagnostics of intraoral ulcerations. A possible limitation of our study is the co-occurrence of various types of oral ulcerations with a different etiology simultaneously, which is expected to occur in this severely immunocompromised patient category. In patients in whom both types of ulcerations occur, risk factors for each type of oral ulceration, including viral shedding, may be interrelated.

In conclusion, reactivation of HSV-1 is an important factor in ulcerations involving the non-keratinized and keratinized oral mucosa after HSCT in the absence of antiviral prophylaxis. Persistent HSV-1 replication despite antiviral treatment is common and is due to resistance in a relevant proportion of patients. Oral shedding of EBV may have a role in ulcerations on the keratinized mucosa, but the mechanism of action remains to be elucidated.
**Table 4** Details on samples from patients with ACV resistant isolates

<table>
<thead>
<tr>
<th>patient</th>
<th>day after SCT</th>
<th>clinical &amp; treatment details at time of sampling</th>
<th>mutations in thymidine kinase gene</th>
<th>mutations in DNA polymerase gene</th>
<th>ACV IC50(^1), mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>1</td>
<td>Pre-treatment</td>
<td>S23N, E36K, Q89R, <strong>insG@436</strong>, G240E, R281Q, G371E, V372M</td>
<td><strong>S920P</strong></td>
<td>n.d.</td>
</tr>
<tr>
<td>60</td>
<td>1</td>
<td>Pre-treatment</td>
<td>C6G, R41H, Q89R, A192V, G251C, V267L, P268T, D286E, Y305T, G371E, V372M, N376H</td>
<td>V905M, S920P 0.07</td>
<td>1.4 (^2)</td>
</tr>
</tbody>
</table>

Resistance-associated mutations in bold. \(^1\) ACV IC50 as determined by DNA reduction assay (25).
\(^2\) average from 2 independent experiments on two viral isolates with G59W mutation. ins = insertion, n.d. = not determined
References


