INTRODUCTION

Haematopoietic stem cell transplantation (HSCT) is a potentially curative treatment for malignant and non-malignant diseases. An HSCT can be performed either with autologous or allogeneic stem cells. In an autologous HSCT, patient’s own stem cells are collected, stored and reinfused after suppressing the immune system with high-dose chemotherapy. For example, high-dose melphalan (HDM) is the standard care treatment regimen for multiple myeloma (MM) (Voorhees & Usmani, 2016).

A common adverse effect of HDM is oral mucositis (OM), characterized by pain, erythema, swollen mucosa, xerostomia and ulcerations (Peterson & Cariello, 2004). Severe OM requires narcotics and parenteral feeding and increases hospital stay. Furthermore, the ulcerative lesions may allow the penetration of bacteria into the bloodstream resulting in a systemic infection (Peterson & Cariello, 2004). Oral mucositis to some degree affects almost all patients treated with high-dose chemotherapy. A multicentre study to assess the incidence, severity and duration of OM found that 90% of the MM patients treated with...
HDM and autologous HSCT was affected. Severe OM (WHO score 3 and 4) occurred in 46% of the MM patients and had a duration of 5 days (Blijlevens et al., 2008). Peak OM scores were found approximately 9 days after HSCT (Blijlevens et al., 2008). To prevent OM, cryotherapy is used for patients undergoing HDM and HSCT (Marchesi et al., 2017). Cryotherapy, the use of ice chips during the infusion of chemotherapy, results in local vasoconstriction, which reduces the amount of oncolytic drugs that reach the oral mucosa. Although it significantly reduces the incidence, some patients still develop severe OM (Marchesi et al., 2017). Moreover, there are no good treatment options for OM.

One factor that may influence the severity of OM is a decrease in the normal defence mechanisms in saliva (Epstein, Tsang, Warkentin, & Ship, 2002). Several salivary constituents are thought to have a protective function such as immunoglobulins, histatins, cystatins, defensins and lactoferrin (Amerongen & Veerman, 2002). A study evaluating changes in the salivary proteome after allogeneic HSCT found that levels of lactoferrin and secretory leucocyte protease inhibitor were increased at 6 months compared to pre-HSCT levels (Imanguli et al., 2007). The levels of secretory IgA (sIgA) were decreased 1 month after HSCT, but returned to pre-HSCT levels 6 months after HSCT (Imanguli et al., 2007). Decreased sIgA levels after HSCT were also found in another study examining salivary changes in MM patients undergoing autologous HSCT (Avivi et al., 2009). In the latter study, sIgA levels were significantly reduced 7 days after HSCT and OM was associated with a decrease in sIgA, uric acid levels and salivary antioxidant activity.

As OM is most severe around 9 days after transplantation, the aim of this study was to examine early salivary changes in relation to OM in MM patients treated with HDM and autologous HSCT. To determine salivary changes caused by leakage from the oral mucosae, unstimulated whole-mouth saliva (UWS) and stimulated (SWS) whole-mouth saliva were used in this explorative observational study performed in two parts. Due to protocol changes in regular care at the hospital based on the MASCC/ISOO clinical practice guideline in 2014 for the prevention of OM, patients in the last part received cryotherapy during infusion of HDM (Lalla et al., 2014).

2.1 Study population
Adult MM patients undergoing HDM (200 mg/m², infused during 1 hr) and autologous HSCT at the Department of Hematology of the Radboud University Medical Centre were asked to participate. Patients who did not understand the Dutch language were excluded. In total, 12 (of 18) eligible patients were included from March 2013 to October 2013 for the first part (A) and 8 (of 11) eligible patients were included from May 2015 to July 2015 for the second part (B).

Patients brushed their teeth 4 times a day and rinsed with physiological salt solution when required per standard protocol. To maintain fluid balance during admission, patients received intravenous NaCl 0.9% solution. Analgesics, parenteral feeding and other medications were administered if necessary per standard protocol. In the second part, cryotherapy was used by consuming ice chips or crushed ice starting 5 min before HDM infusion (day –2) until 30 min after infusion. Melted ice was swallowed or spit out before the use of new ice chips. The medical charts were used to obtain the neutrophil counts and albumin levels in blood and to obtain the administered dose of melphalan in mg/kg.

2.2 Oral examination and saliva collection
During the hospitalization period, saliva was collected and the severity of OM was scored using the OMNI score (Oral Mucositis Nursing Instrument) on day –3 (admission day), day 0 (day of autologous HSCT), day 4, day 7, day 11 and day 14 (Potting, Blijlevens, Donnelly, Feuth, & van Achterberg, 2006). The total OMNI score was calculated by the sum of the individual scores (ranging between 0 and 3) for erythema, edema, ulcerations, pain, mouth dryness and saliva viscosity.

The protocols for the collection of UWS and SWS were based on the guidelines for saliva collection of the University of Southern California School of Dentistry (Navazesh & Kumar, 2008). For the collection of UWS and SWS, patients were asked to refrain from eating or drinking 1 hr before collection and to swallow immediately before collection. For UWS, patients were asked to spit the saliva in a previously weighed plastic cup for 5 min without making any effort to increase the saliva flow. For SWS, patients were asked to chew 1 min on a piece of paraffin film to stimulate saliva flow. After swallowing, SWS collection was started by spitting saliva, while continuing chewing, in a plastic cup for 2-5 min. Directly after collecting, samples were weighed and stored on ice. UWS and SWS flow rates were estimated by assuming 1 ml of saliva is 1 gram. Directly after collection, pH was determined using pH strips (pH-indicator strips 4.0 – 7.0, Merck® Germany), and saliva samples were centrifuged for 5 min at 9,800 g (mini spin plus, Eppendorf, Germany). The supernatant was stored separately from pellets at −80°C. For analysis, samples were defrosted and divided into aliquots and stored at −80°C.
2.3 | Saliva analysis

Total protein was determined using the BCA protein assay kit according to the manufacturer's protocol (Pierce™ BCA protein assay kit, Thermo Scientific, USA). Salivary proteins and glycoproteins were analysed by SDS-PAGE gels stained with Coomassie Brilliant Blue R250 (CBB) and Periodic Acid-Schiff (PAS) to determine mucin 5b (MUC5B). Western blotting was used to determine MUC5B and albumin levels, and enzyme-linked immunosorbent assay (ELISA) was used to determine total IgA, myeloperoxidase (MPO) and lactoferrin levels.

2.4 | SDS-PAGE and CBB/PAS staining

For SDS-PAGE gels, equal amounts of total protein were loaded onto gels, UWS and SWS separately. Samples were prepared in LDS containing sample buffer (1:4) and DTT (1:10), incubated at 95°C and centrifuged. Protein standards with predefined molecular weights (5 μl, SeeBlue® Plus2 prestained protein, Thermo Scientific, USA) and 10 μl of sample were loaded on 4%-12% Bis-Tris gels (NuPage®, Thermo Scientific, USA) and run in MES SDS running buffer as per manufacturer’s recommendations. Gels were stained in CBB staining solution (Brilliant Blue R Staining Solution in 10% acetic acid, Sigma-Aldrich, UK) and destained in 10% acetic acid. The CBB-stained gels (after photographing) were used for PAS staining after 1 hr incubation in Schiff reagent (VWR® Prolabo®, UK). Images were taken and band intensities were determined using a Bio-Rad® Chemidoc™ Imaging system and Image Lab™ software.

2.5 | Western blots

Using a sandwich technique, proteins were transferred from gel to a nitrocellulose membrane (Invitrogen, XCell II™ Blot Module, USA) in transfer buffer (Novex® NuPage Transfer Buffer 20x, USA, in methanol and H₂O) for 1 hr at constant voltage. After transfer, the membrane was blocked in 5% skimmed milk in TBS-T. Blots were incubated with primary mouse monoclonal anti-human MUC5B (1:500 in TBS-T, Abcam, Ab105460, UK) or mouse anti-albumin (1:1,000 in TBS-T Sigma, A6684, UK). Blots were washed 3x 5 min with TBS-T and incubated with second antibody, polyclonal goat anti-mouse HRP (1:2,000 in TBS-T, Dako PO447, UK). Results were visualized using a chemiluminescence Western blotting reagent (Bio-Rad® ClarityTM Western C Kit Western ECL Subrate, USA). Images were taken, and intensities were determined using Chemidoc and Image Lab™ software.

2.6 | Sandwich ELISA

Ninety-six well plates were coated overnight at 4°C (MPO at RT) with 100 μl rabbit anti-human IgA (1:1,000 in Na₂CO₃ buffer pH 9.6, Dako A0262, UK), rabbit anti-human lactoferrin (1:2,000 in Na₂CO₃ buffer pH 9.6, Abcam Ab15811, UK) or MPO coating antibody (1:180 in PBS, Duoset DY3174, R&D systems, UK). The first antibody solution was discarded, and plates were washed 3x with PBS-T. Blocking with 300 μl of PBS with 1% BSA (1 hr at RT) was performed for MPO. After washing, 100 μl samples/standard was added in duplo to the plates and incubated at 37°C for 2 hr. IgA from colostrum I2636 (1 – 0.0156 μg/ml in PBS-T, Sigma-Aldrich, UK), lactoferrin from human milk LO520 (125 – 1.95 ng/ml in PBS-T, Sigma-Aldrich, UK) and MPO standard (16 – 0.25 ng/ml in PBS + 1% BSA, Duoset DY3174, R&D systems, UK) were used as standard. For total IgA and lactoferrin, samples were diluted 1:50 – 1:800 in PBS-T. For MPO, samples were diluted 1:20 – 1:400 in PBS + 1% BSA. After incubation, plates were washed 3x with PBS-T and incubated at 37°C for 1 hr (total IgA and lactoferrin) or at RT for 2 hr (MPO) with 100 μl of polyclonal anti-human IgA/HRP (1:4,000 in PBS-T, Dako 0216, UK), polyclonal rabbit anti-human lactoferrin/HRP (1:25000 in PBS-T, Abcam ab24264, UK) or detecting antibody (1:180 in PBS + 1% BSA, Duoset DY3174, UK). For MPO, plates were washed with PBS-T and incubated with 100 μl HRP solution (1:200 in PBS + 1% BSA, Duoset DY3174, UK) for 30 min at RT. After incubation with the second antibody or HRP solution, plates were washed with PBS-T and were incubated with 100 μl TMB (TMB liquid substrate system, Sigma-Aldrich, UK) for 30 min at RT in dark. Reaction was stopped with 50 μl 2 N sulphuric acid, and plates were read using a microplate reader at 492 nm (iMark™ Microplate Absorbance Reader Bio-Rad®, USA). Protein concentrations were calculated with Excel software using the standard curve.

2.7 | Data analysis

Statistical analysis was performed in SPSS (version 22) and R (version 3.4.0), and p < .05 was considered as statistical significant. Descriptive analysis was used for patient characteristics. To test the difference between the OMNI scores in part A and part B, a multilevel regression model was used with the OMNI score as dependant variable and “study part” as independent variable. To model the changes over time, both T and T² were included as independent variables as well, while a random intercept was added to the model to allow for the clustering of measurements within patients.

All quantitative results of MUC5B, albumin, total IgA, lactoferrin and MPO are based on part B only, as part A was used to optimize the protocols for quantitative analysis of saliva samples. Results from the salivary compositional analysis of the first part (A) were only used to compare trends between both studies. To correct for interindividual variation between patients, scaled scores were calculated per variable per patient by subtraction of day −3 score and dividing by the SD. These scaled scores were used for paired t tests to compare days 0, 4, 7, 11 and 14 with day −3. Differences are graphically labelled at α-levels of 5%, 1% and 0.1% separately to allow for evaluation of significance in view of multiple testing. For easier interpretation, most figures contain the absolute values. Correlations between peak OMNI scores and salivary parameters were calculated...
by applying meta-analytical techniques (inverse variance weighting) on the individual correlations within patients over time.

3 | RESULTS

3.1 | Patient characteristics

In total, 20 patients were included (part A: n = 12, part B: n = 8). No important differences in study population were observed between both groups (Table 1). Due to hyposalivation or nausea, it was not possible to collect saliva from all patients on all study days. This resulted in missing samples with a peak at day 7 in both parts (Figure 1).

An important difference between both groups is the use of cryotherapy during the infusion of HDM in the second part (B). This resulted in significantly lower total OMNI scores in part B (95% CI [-3.87, -1.29], p < .001; Figure 2). Peak OMNI scores were seen at day 7 after HSCT, and the individual OMNI variables, mouth dryness and edema, contributed most to the total OMNI score each day (Figures 2 and 3). The highest individual OMNI scores for pain and lesions were found in part A (Figure 3).

### TABLE 1  Patient characteristics

<table>
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<tr>
<th></th>
<th>First study (n = 12)</th>
<th>Second study (n = 8)</th>
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<td>62.5%</td>
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<tr>
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<td>63</td>
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<tr>
<td>Range</td>
<td>43 – 68</td>
<td>53 – 67</td>
</tr>
<tr>
<td>Dentition (% dentate)</td>
<td>75%</td>
<td>62.5%</td>
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<td>Melphalan doses in mg/kg (mean ± SD)</td>
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<td>4.65 ± 0.86</td>
</tr>
<tr>
<td>Median</td>
<td>4.96</td>
<td>4.88</td>
</tr>
</tbody>
</table>

**FIGURE 1** Percentage of missing samples over time for unstimulated whole-mouth saliva (UWS), stimulated whole-mouth saliva (SWS) and total (UWS + SWS) for part A (grey lines) and part B (black lines)

**FIGURE 2** Total Oral Mucositis Nursing Instrument (OMNI) score. Mean ± 95% CI of total OMNI score for part A (grey line) and part B (black line). Numbers in the graph represent the number of patients per time point for part A (grey) and part B (black). Total OMNI scores in part B were significantly lower compared to part A (95% CI [-3.87, -1.29], p < .001)

**FIGURE 3** Contribution of four Oral Mucositis Nursing Instrument (OMNI) variables to total OMNI score per day. For each variable, mean score ± 95% CI is shown for part A (a) and part B (b)
3.2 | Salivary flow rate, pH and total protein

Both parts showed similar changes in flow rate reductions for UWS and SWS over the study period, with generally lower flow rates being consistently shown for UWS and SWS at day 0, 4, 11 and 14, compared with day −3. The reductions were most clear in part B: SWS flow rate was significantly decreased compared to day −3, 95% CI of scaled scores [−2.01, −0.84], p < .001; [−2.14, −0.77], p = .002; [−2.53, −1.96], p < .001; [−2.57, −1.37], p < .001, respectively. Salivary pH was determined directly after collection, showing a similar immediate reduction in UWS and SWS in part A at day 0 as seen in the flow rates: 95% CI of scaled scores [−2.11, −0.09], p = .038; [−2.51, −0.52], p = .009; [−2.57, −0.65], p = .008; [−2.18, −0.24], p = .023, for UWS and SWS part A and part B, respectively (Figure 5).

Although there is some indication of increasing total protein levels in both parts of the study, the effect is only consistent and statistically significant in SWS at day 0: 95% CI of scaled scores [0.92, 2.18], p < .001 for part A and [0.47, 2.37], p = .011 for part B (Figure 6).

3.3 | Salivary composition

3.3.1 | MUC5B + albumin

Figure 7a-c shows the changes in UWS and SWS MUC5B and albumin levels over time with a representative CBB- and PAS-stained SDS-PAGE and Western blot for MUC5B and albumin from one patient. Although in part A an increasing concentration of MUC5B was observed in UWS, this was not confirmed in part B (Figure 7b). Albumin levels showed a clear trend for both UWS and SWS, mimicking the curve of OMNI scores. The albumin levels were significantly increased at day 7 in UWS and day 11 in SWS: 95% CI of scaled scores [0.57, 2.77], p = .018 and [0.61, 2.09], p = .005, respectively (Figure 7c).

3.3.2 | Total IgA, lactoferrin and MPO

Figure 8a-c shows the changes in UWS and SWS levels of total IgA, lactoferrin and MPO over time. A trend for decreasing and recovering concentration is seen in UWS and SWS total IgA levels; however, only at day 14 in UWS, the decrease was found statistically significant: 95% CI of scaled scores [−2.44, −0.23], p = .032 (Figure 8a). For lactoferrin, from day 4 an increasing trend was observed, which is almost parallel in UWS and SWS. The peak increase at day 11 is highly significant in both UWS and SWS: 95% CI of scaled scores [1.74, 2.56], p < .001 and [1.97, 2.58], p < .001, respectively. The preliminary part A showed similar trends for total IgA and lactoferrin. For MPO, a fluctuating trend is seen with only limited statistical significance of differences observed.

Myeloperoxidase was used as an indirect measurement for the polymorphonuclear leucocyte (PMN) levels in saliva. The PMN levels in blood are shown in Figure 8d. During the whole study period, a similar trend was observed between both parts. At day 0, an increase in PMN levels in blood was seen while the MPO levels in UWS were decreased. Directly after this increase in blood PMNs, a decreasing trend is seen reaching undetectable levels at day 6 after HSCT as expected (Figure 8c and d). At day 4, MPO levels in UWS showed an increase compared to day 0, while the PMN levels in blood were decreased compared to day 0 (Figure 8c and d). At day 7, no PMNs were detected in blood and the lowest MPO levels in UWS were found. Interestingly, the UWS MPO levels started to increase at day 11, while the PMNs were still undetectable in blood (Figure 8c and d).
Correlations between OMNI scores and salivary parameters are shown in Table 2. A positive correlation was found between total OMNI scores and SWS MUC5B, SWS albumin and SWS lactoferrin levels ($R^2 = .318$, $p = .043$; $R^2 = .56$, $p = .029$ and $R^2 = .49$, $p = .043$, respectively). In addition, total OMNI scores and UWS albumin levels were close to a statistical significant positive correlation ($R^2 = .47$, $p = .05$), and total OMNI scores and SWS flow rate were close to a statistical significant negative correlation ($R^2 = -.39$, $p = .089$).

As oral dryness is the largest contributor to OMNI scores in this study (Figure 2b), correlations were also determined between the dryness component of the OMNI score and flow rate and MUC5B levels in UWS and SWS (Table 2). A negative correlation was found between UWS flow rate and the dryness component of the OMNI score ($R^2 = -.57$, $p = .003$), and a positive correlation was found between SWS MUC5B levels and the dryness component of the OMNI score ($R^2 = .51$, $p = .015$), indicating that the increased feeling of a dry mouth starting from day 0 was correlated with the trend of increasing SWS MUC5B levels between days 0 and 7.

**3.4 | Correlations**

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**4 | DISCUSSION**

This study was performed to determine early changes in salivary parameters in relation to OM in MM patients undergoing autologous HSCT. After HSCT, salivary albumin and lactoferrin levels were increased, whereas salivary flow rate showed a decreasing trend. A positive correlation was found between OM and MUC5B, albumin and lactoferrin levels in SWS. Furthermore, due to protocol changes it was possible to observe the effect of cryotherapy on OM.
development, which resulted in significantly lower max OMNI scores during the whole study period in the second part with cryotherapy.

Consistent with the published literature, peak OM was reached around day 7 and had almost returned to baseline at the end of the study period (Blijlevens et al., 2008). Oral mucositis caused by HDM was reduced by the use of cryotherapy in this study. As melphalan is found in saliva, the local vasoconstriction caused by the cryotherapy may reduce the melphalan concentration in saliva (Epstein et al., 2002; Slavik, Wu, & Riley, 1993). Similar to a retrospective study, cryotherapy resulted in reduced OM, but did not reduce the duration of OM (Batlle et al., 2014).

Although a direct effect of HDM on salivary glands is unclear, conditioning therapy before HSCT causes salivary hypofunction (Epstein et al., 2002). Furthermore, salivary gland dysfunction by lymphocyte infiltration is found using gland biopsies and a scintigraphic method several weeks after HSCT with conditioning regimens consisting of cyclophosphamide, busulfan and TBI (Coracin et al., 2006; Lindahl, Lönnquist, & Hedfors, 1988). Most other studies measuring salivary flow rate after HSCT found a decrease in salivary flow rate (Amaral et al., 2012; Avivi et al., 2009; Boer, Correa, Tenuta, Souza, & Vigorito, 2015; Elad et al., 2006; Nagler, Barness-Hadar, Lieba, & Nagler, 2006). However, one study found no changes in UWS flow rate in autologous HSCT patients, while a study in allogeneic patients showed an increase in UWS flow rate 8-10 days after HSCT (Avivi et al., 2009; Boer et al., 2015). This increase might be related to dysphagia resulting in an increased resting volume of saliva as suggested by the authors (Boer et al., 2015).

One indication for glandular dysfunction caused by HDM is the similar decrease in flow rate and pH at day 4 in this study. However, the protein secretory functions of the glands are not affected as suggested by the increase in total protein and lactoferrin. Lactoferrin in saliva has a glandular origin although it is also a product of PMNs (Reitamo, Konttinen, & Segerberg-Konttinen, 1980). The increase in lactoferrin observed in this study may be derived from glandular origin as the PMN levels in blood decrease after HSCT resulting in neutropenia. To further investigate protein output of the salivary glands, salivary amylase levels could be studied or glandular saliva could be used to determine changes in specific glandular-derived proteins.

Myeloperoxidase is secreted by PMNs and could therefore be used as an indirect measurement of the PMN levels in saliva. The decrease in MPO found in this study at day 7 probably reflects neutropenia. Interestingly, the MPO levels in saliva showed an increasing trend some days before the PMNs were again detectable in blood. This finding is supported by two other studies measuring PMN levels directly after HSCT and may indicate that PMNs from the stem cells directly reach the site of inflammation before they circulate at detectable levels in blood (Lieschke et al., 1992; Pink et al., 2009).
Besides a decreasing trend in salivary flow rate, a decrease in salivary antimicrobial defence is also described in the literature mainly by a decrease in total or secretory IgA levels after HSCT (Avivi et al., 2009; Chaushu et al., 1994; Dens et al., 1996; Norhagen-Engstrom, Hammarstrom, Lonnqvist, Soder, & Smith, 1988). One mechanism to overcome this decreased antimicrobial defence might be an increased secretion of lactoferrin as most salivary proteins act in synergy for the antimicrobial function (Avivi et al., 2009; Imanguli et al., 2007). This hypothesis is supported in our study by a decreasing trend in total IgA levels and increased levels of lactoferrin. Furthermore, the increase in lactoferrin and albumin levels was also positively correlated with OM. Salivary albumin levels were increased during peak OM, while blood levels of albumin show a decreasing trend. This supports the previously described hypothesis of decreased integrity of the oral mucosa, which may result in leakage of blood substances into saliva during OM (Avivi et al., 2009; Izutsu et al., 1981).

Another OM-related complaint after HSCT is xerostomia, the feeling of a dry mouth. As suggested by the dryness component of the OMNI score, xerostomia was present during the whole study period. Xerostomia has been linked to changes in the lubricating protein MUC5B (Chaudhury, Shirlaw, Pramanik, Carpenter, & Proctor, 2015; Dijkema et al., 2012). The dryness component of the OMNI score was negatively correlated with UWS flow rate and, although no significant changes in MUC5B concentrations were observed, positively correlated with SWS MUC5B levels. This counterintuitive finding is supported by another study where patients with xerostomia had similar or higher MUC5B and MUC7 concentrations, combined with differences in the glycosylation of the mucins (Chaudhury et al., 2015). We conclude that the correlation may be based on reduced total MUC5B availability or changed lubricating capabilities of the mucins.

The differences in the levels of proteins between UWS and SWS found in this study can be explained by the normal composition of...
In resting conditions, saliva is mainly formed by secretions from the submandibular and sublingual glands. In stimulated saliva, the parotid gland contributes more, which results in more watery saliva compared to UWS (Proctor, 2016).

One limitation of several studies evaluating salivary changes in HSCT recipients is the mixed patient population. Only one earlier study evaluated early salivary changes in a homogenous group of MM patients receiving HDM and autologous HSCT (Avivi et al., 2009). Our observational study also included a homogenous patient population. However, both studies included small numbers of patients and lost power due to missing samples caused by nausea and hyposalivation especially during the OM period. Furthermore, due to logistic difficulties it was not possible to collect saliva at the same day during the whole study period. It was tried to collect saliva at the same time per patients, but also due to logistic difficulties, this was not always possible.

Salivary changes may influence the severity of OM. For proper symptom management, local salivary changes may be indicative. This study found decreased total IgA levels in saliva resulting in decreased antimicrobial defence, which may be compensated by other antimicrobial proteins in saliva such as lactoferrin. However, further research is necessary to confirm and expand these results.

In conclusion, our observational study showed compositional changes in saliva reflecting inflammation of the salivary glands and the oral mucosae in the first days after autologous HSCT, of which the changes in albumin and lactoferrin were correlated with OM development. Although cryotherapy in the second study resulted in decreased OM severity, both studies showed similar trends in altered salivary protein composition.

TABLE 2 Correlation between Oral Mucositis Nursing Instrument (OMNI) scores and salivary parameters, based on inverse variance weighting of correlations within patients over time from part B

<table>
<thead>
<tr>
<th>Variable</th>
<th>Heterogeneity $\hat{r}^2$ (%)</th>
<th>Correlation coefficient</th>
<th>95% CI</th>
<th>Significance (two-tailed)</th>
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<tr>
<td>Flow rate</td>
<td>0; 0.96</td>
<td>−0.33</td>
<td>−0.79, 0.14</td>
<td>.138</td>
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<td>MUC5B</td>
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<td>−0.33, 0.62</td>
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<td>.050</td>
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<tr>
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<td>Lactoferrin</td>
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<td>0.24</td>
<td>−0.23, 0.72</td>
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<td>MPO</td>
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<td><strong>Total OMNI - SWS</strong></td>
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<tr>
<td>Flow rate</td>
<td>0; 0.92</td>
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<td>MUC5B</td>
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<td>Albumin</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flow rate</td>
<td>0; 0.59</td>
<td>−0.57</td>
<td>−0.87, −0.28</td>
<td>.003 **</td>
</tr>
<tr>
<td>MUC5B</td>
<td>0; 0.65</td>
<td>0.3</td>
<td>−0.05, 0.65</td>
<td>.081</td>
</tr>
<tr>
<td><strong>OMNI dryness - SWS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flow rate</td>
<td>49; 0.07</td>
<td>−0.28</td>
<td>−0.73, 0.16</td>
<td>.168</td>
</tr>
<tr>
<td>MUC5B</td>
<td>36; 0.15</td>
<td>0.51</td>
<td>0.14, 0.87</td>
<td>.015 **</td>
</tr>
</tbody>
</table>

MPO, myeloperoxidase; SWS, stimulated whole-mouth saliva; UWS, unstimulated whole-mouth saliva.

$p < .05$.

**$p < .01$.**

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CONFLICT OF INTEREST

None to declare.

AUTHOR CONTRIBUTION

The study was designed by C.M.J. Potting, N.M.A. Blijlevens, M.C.D.N.J.M. Huysmans and L.F.J. van Groningen. S. ten Hoopen had a substantial contribution in the data collection for the clinical and the laboratory part. The laboratory part was performed by S. ten Hoopen at the lab of G.B. Proctor. For the optimization of the laboratory protocols G.B. Proctor had a substantial contribution.
The data analysis was performed by S.J.M. van Leeuwen, G.B. Proctor, S. ten Hoopen and E.M. Bronkhorst. S.J.M. van Leeuwen drafted the paper, which was reviewed by G.B. Proctor, C.M.J. Potting, N.M.A. Blijlevens, M.C.D.N.J.M. Huysmans and L.F.J. van Groningen.

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REFERENCES


