research article

Plasma sICAM-1 correlates with tumor volume before primary radiochemotherapy of head and neck squamous cell carcinoma patients

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Background. Biomarkers are of major interest to optimize diagnosis, prognosis and to guide treatment in head and neck cancer patients. Especially blood-based biomarkers appear promising as they can be easily collected and repeatedly analyzed during the course of radiochemotherapy.

Patients and methods. At first, for a broad overview, multiple immune markers were evaluated in six plasma samples of three head and neck squamous cell carcinoma (HNSCC) patients at the beginning and the end of radiochemotherapy. In this pre-selection, the soluble Intercellular Adhesion Molecule 1 (sICAM-1) appeared most promising. Thus, this marker was measured in multiple samples (n = 86) during treatment and follow-up in a cohort of eleven patients and correlated with tumor features and clinical data.

Results. We found a strong correlation between the initial levels of sICAM-1 in the plasma and the gross tumor volumes of the primary tumor and the involved lymph nodes. However, during the course of treatment no systematic dynamics could be identified. Toxicity or infections did not seem to influence sICAM-1 concentrations.

Conclusions. sICAM-1 appears to reflect the pre-treatment total tumor burden (primary tumor and involved lymph nodes) in head and neck tumor patients. However, it does not seem to be a dynamic marker reflecting response during radiochemotherapy. Thus, if our findings are confirmed in future, sICAM-1 could be used as a staging marker: if high sICAM-1 levels but low tumor burden are found it might be reasonable to intensify staging investigations to rule out further, yet undetected, tumor sites.

Key words: head and neck cancer; biomarker; radiotherapy; tumor volume; gross tumor volume, sICAM-1

Introduction

Biomarkers are a promising feature to personalize radiotherapy treatment of head and neck squamous cell carcinoma (HNSCC). To some extent the focus has already shifted from sole anatomical tumor stage to biological features[1] with HPV being integrated in the 8th edition of the AJCC staging manual.[2] The superior discrimination of patient outcomes by combined anatomical and biological factors have been validated in the US national cancer database.[3] HPV / p16 positive HNSCC might be

treated differently than HPV / p16 negative cancers in the near future.[4] Due to the superior prognosis treatment de-escalation has been proposed in this subgroup of patients.[5] Blood-based biomarkers have not been developed this far yet, however, there is major interest, as blood samples can be easily obtained not only at time of diagnosis, but also throughout treatment and follow-up. Different classes of blood biomarkers have been described in HNSCC such as circulating tumor cells and nucleic acids (e.g. circulating cell-free DNA (cfDNA), circulating cell-free tumor DNA (ctDNA), exosomal RNA).[6] In addition, microRNAs, long non-coding RNAs and DNA methylation patterns have been described as potential biomarkers in blood and saliva of HNSCC patients.[7] Proteomics approaches have been used to identify protein biomarkers in HNSCC tumors and body fluids of patients.[8] For radiation oncology, different clinical settings and treatment modifications based on circulating biomarkers have been hypothesized.[9,10] Possible approaches are patient stratification for more or less intense treatment based on prognostic markers or adaptive approaches tailoring treatment to biomarker responses e.g. during fractionated radio(chemo)therapy.[9,10]

Cytokines and chemokines play a crucial role in the intercellular communication of cancer and immune cells and can be measured in serum / plasma of patients with different tumor entities. Different cytokine profiles have been reported to be altered in cancer patients compared to healthy volunteers (e.g. in breast cancer[11], nasopharyngeal carcinoma[12] and HNSCC[13]). Clinical response to systemic therapy has been linked to cytokine profiles for metastatic renal cell carcinoma[14], non-small cell lung cancer treated with tyrosine kinase inhibitor[15] and nasopharyngeal carcinoma.[12,16] For HNSCC, several reports focusing on different cytokines have been published. Osteopontin has been linked to initial tumor burden and response to radiochemotherapy.[17] CXCL12 (SDF-1) but not its receptor CXCR4 was elevated in the serum of HNSCC patients compared to healthy volunteers.[18] Radiochemotherapy for HNSCC significantly decreased TGFβ levels[19], whereas high plasma C-reactive protein (CRP) and TNFα levels were found in patients and was associated with worse prognosis.[20]

In this prospective pilot study, at first, diverse plasma cytokine levels were evaluated in HNSCC patients undergoing definitive radiochemotherapy. Subsequently, focusing on the soluble Intercellular Adhesion Molecule 1 (sICAM-1), dynamics during and after radiochemotherapy as well as associations with clinical patient and tumor characteristics and patient outcome were evaluated.

Patients and methods

In this prospective pilot biomarker study, patients with newly diagnosed, locally advanced HNSCC were included. All patients declared their informed written consent and the study was approved by the local ethics committee (reference number 064/2016BO2). The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments.

As described previously[21], eleven patients were included in this study, who underwent primary radiochemotherapy. Radiotherapy to 54 / 60 / 70 Gy to elective nodal regions / high risk regions / macroscopic primary tumor and lymph node metastases was combined with cisplatin in eight cases or 5-fluorouracil and mitomycin C in three patients, respectively. For every patient initial tumor volumes, as contoured for radiotherapy including the primary tumor and involved lymph nodes were recorded, as well as disease free survival (DFS, local or distant recurrence or death of any cause). Clinically manifest infections and toxicity graded according to the Radiation Therapy Oncology Group (RTOG) were recorded and correlated with sICAM-1 levels in the plasma.

Blood sampling was planned weekly on Mondays before the application of the radiotherapy fraction during radiochemotherapy as well as at every available follow-up time point. For one patient, the initial sample was taken on day 2 of radiochemotherapy. In total, 86 samples were analyzed, 62 during treatment, 24 during follow-up, respectively. A median of six samples (range: 4–7) were analyzed per patient during treatment. Blood was collected in EDTA tubes (Sarstedt, Nümbrecht, Germany), plasma isolation was performed by centrifugation. Plasma samples were stored in -80°C in aliquots until further use.

For three patients, plasma samples before radiochemotherapy and at end of treatment were analyzed by the human cytokine array Proteome ProfilerTM Array, Human Cytokine Array Panel A (R&D Systems Europe, Abingdon, UK) analyzing CCL1, CCL2, CCL3, CCL5, CD40L, C5/C5a, CXCL1, CXCL10, CXCL11, CXCL12 (SDF1), G-CSF, GM-CSF, sICAM-1, IFNγ, IL-1F1, IL-1F2, IL-1F3, IL2, IL4, IL5, IL6, IL8, IL10, IL12, IL13, IL16, IL17A,

IL17E, IL18, IL21, IL27, IL32α, MIF, SerpinE1, TNFα and sTREM-0. Plasma samples were incubated on the membranes and washing and staining steps were performed according to manufacturer's instructions. After development of films, semi-quantitative analysis was performed by densitometric assessment of the films with ImageJ normalized to control regions adjacent to analyzed areas. Arbitrary densitometry units were analyzed for all six samples, mean of technical duplicates were used for further analyses.

For further analysis of sICAM-1, all available samples of all time points were analyzed with Enzyme-linked Immunosorbent Assay (ELISA) according to manufacturer's instruction (Human ICAM1 ELISA Kit (CD54) (ab174445), Abcam, Cambridge, UK). Standard curves were measured with the following concentrations of ICAM-1: 0 pg/ml, 19.53 pg/ml, 39.06 pg/ml, 78.13 pg/ml, 156 pg/ml, 312 pg/ml, 625 pg/ml, 1250 pg/ml, 2500 pg/ml, 5000 pg/ml ($R^2 = 0.99$). Every sample was measured in technical duplicates, means were used for further analyses. For every patient, absolute sICAM-1 concentrations at each time point as well as relative sICAM-1 concentrations normalized to the baseline value of the respective patient were recorded. Patients stratified by median initial sICAM-1 values were analyzed for DFS. Pooled values of all patients were used for the analysis of time points with and without manifest infection (53 available time points) as well as RTOG graded toxicity (58 available time points). Plasma sICAM-1 concentrations at the beginning of radiochemotherapy were correlated with gross tumor volumes (GTVs) for the primary tumor, lymph node metastases and hull of both.

Statistical analysis included Kaplan Meier method of DFS and comparison by log-rank-test. Means were compared by t-test or Mann-Whitney test depending on whether values passed normality test. In case of multiple testing Bonferroni correction was performed. Pearson correlation coefficients were used to characterize correlations of continuous variables (moderate correlation defined as 0.4–0.7; strong correlation defined as > 0.7). Level of significance was defined with $p < 0.05$. Analyses were done with IBM SPSS Version 26 and GraphPad Version 8.

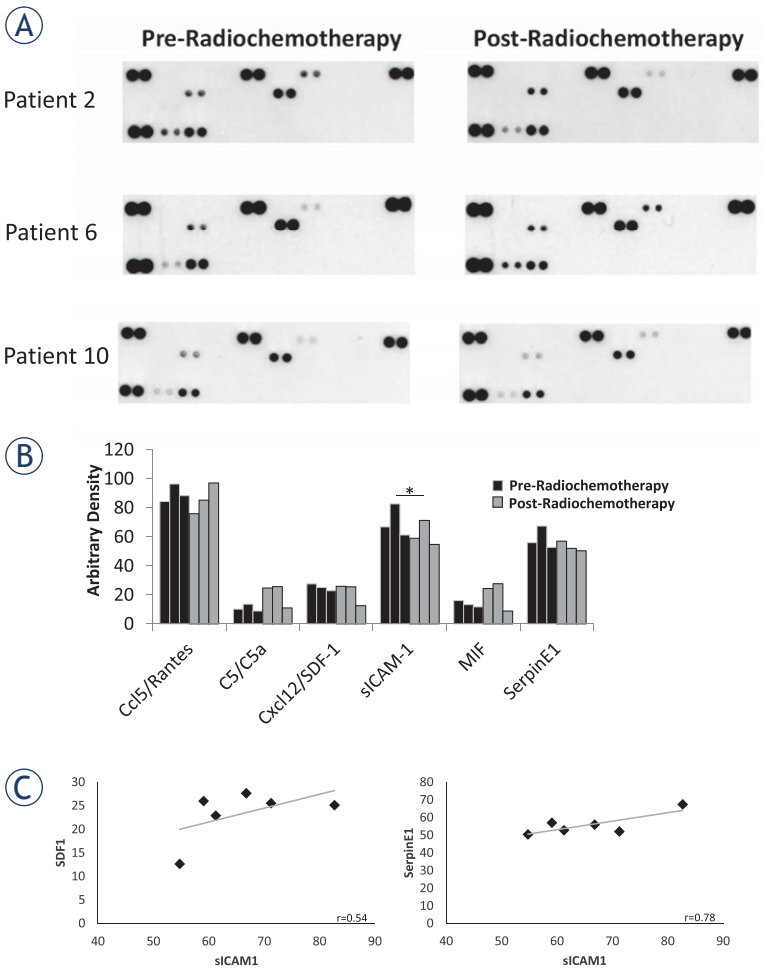


FIGURE 1. Plasma of three patients before and at end of radiochemotherapy for head and neck squamous cell carcinoma were analyzed with a human cytokine array. Of the tested cytokines, only six were present in detectable concentrations (Ccl5, Complement Component, SDF1, sICAM-1, MIF and SerpinE1). sICAM-1 was the only cytokine with a significant difference between the tested time points decreasing after treatment. sICAM-1 abundance showed moderate and strong correlations with SDF1 and SerpinE1, respectively.

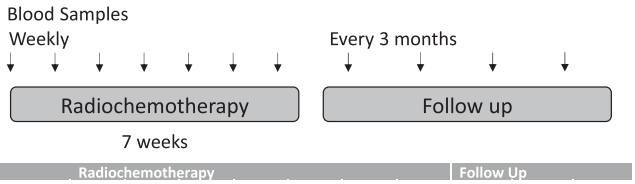
Results

The patient cohort has been described previously in Clasen *et al*.[21] Patients exhibited typical features for primary radiochemotherapy of HNSCC. Three female and eight male patients were included and the tumors were located in the oropharynx (n = 5), hypopharynx (n = 5) and larynx (n = 1). With three of eleven patients (27%) developing recurrences or metastases (range of follow-up: 2.5 to 4.0 years (mean 3.7)), oncologic outcomes seem in the range of published data. At the time point of the analysis, all patients were alive.

Cytokine abundances

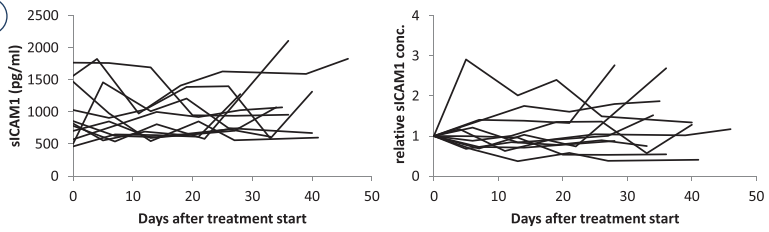
Six of the 36 cytokines measured by the human cytokine array (CCL5/ Rantes, C5/C5a, CXCL12 / SDF-1, sICAM-1, MIF, SerpinE1) showed measur-

A



	Radiochemotherapy							Follow Up		
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Follow Up 1	Follow Up 2	Follow Up 3
Patient 1	x		x	x	x	x		x	x	x
Patient 2	x	x	x	x	x	X			x	x
Patient 3	x	x	x	x	x		x	x	x	
Patient 4	x	x	x	x	x	X	x	x	x	x
Patient 5	x	x	x	x	x	x	x		x	x
Patient 6	x	x	x	x	x		x			
Patient 7	x	x	x		x	x		x	x	x
Patient 8	x	x	x		x			x	x	x
Patient 9	x	x	x	x	x			x	x	x
Patient 10	x	x	x	x		x			x	x
Patient 11	x	x	x	x	x	x			x	

B



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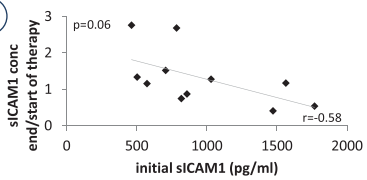

FIGURE 2. Blood samples were taken weekly during radiochemotherapy and at every follow-up visit of the patients. In total, 86 samples were evaluated at the different time points as shown in the table for every single patient included in the study **(A)**. sICAM-1 concentrations measured by ELISA in plasma samples of 11 patients differed significantly between patients. Over the course of treatment and compared intraindividually before and after treatment sICAM-1 concentrations did not show significant changes **(B)**. Initial sICAM-1 concentrations showed a moderate negative correlation with relative sICAM-1 levels at the end of treatment **(C)**.

able abundances in the plasma of three HNSCC patients before and at the end of radiochemotherapy (Figure 1A, B). The only cytokine with a significant difference over the course of radiotherapy was sICAM-1 (Figure 1B). sICAM-1 abundances decreased significantly at the end of radiochemotherapy. sICAM-1 levels in all six samples showed a moderate and strong correlation with SDF1 and SerpinE1, respectively (Figure 1C). SDF-1 was not associated with GTV volumes, whereas SerpinE1 showed a strong positive correlation (r = 0.92, data not shown).

sICAM-1 levels during radiochemotherapy

sICAM-1 concentrations at 86 time points (Figure 2A) during and after radiochemotherapy of eleven patients measured by ELISA differed significantly at baseline with a median of 818.4 pg/ml (range: 462.0–1767.5 pg/ml). Over the course of treatment, no systematic changes were observed (Figure 2B). Relative sICAM-1 levels also did not change significantly during radiochemotherapy (Figure 2B). Patients with high initial sICAM1 concentrations tended to have decreasing levels over the course of therapy as demonstrated by a moderate negative correlation between initial sICAM-1 concentrations and relative sICAM-1 levels at end of radiochemotherapy (Figure 2C).

Disease free survival

Mean disease-free survival for the whole cohort was 3.3 ± 0.4 years. All patients experiencing a recurrence had N2 disease before radiochemotherapy (p = 0.23, data not shown). Median initial sICAM-1 concentration was used to stratify the patient cohort in two groups. The two patients experiencing early recurrences in the first year were in the group of high initial sICAM-1 concentrations. The patient experiencing a late recurrence had low initial sICAM-1 values. Overall, no statistical significance was observed (Figure 3).

sICAM-1 levels and infection and toxicity

A pooled analysis of relative sICAM-1 concentrations normalized to baseline values at all time points of all patients was performed. sICAM-1 levels were slightly higher at time points of clinically manifest infections, although without statistical significance (Figure 4A). No difference was observed for sICAM-1 levels at time points with toxicity graded according to RTOG (Figure 4B).

Tumor volumes (GTV) and initial sICAM-1 concentrations

For the analysis of tumor volumes contoured for radiotherapy planning, one patient with an exceptionally large lymph node metastasis was excluded (GTV LN of 150.2 cm^3, compared to 3.3–23.9 cm^3 for the other patients). Primary tumor volume (GTV PT) as well as volume of lymph node metastases (GTV LN) showed a moderate correlation with sICAM-1 concentrations measured at the start of radiochemotherapy (Figure 5A, B). GTV PT

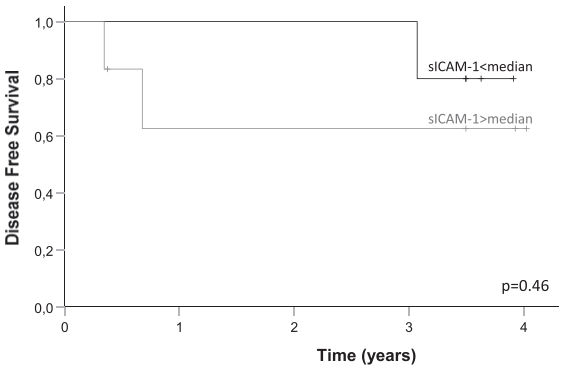

FIGURE 3. Kaplan Meier analysis of disease-free survival stratified by median initial sICAM-1 concentration showed that the two patients with early recurrence in the first year after treatment were in the group with high sICAM-1 levels. Disease-free survival in the small patient cohort did not differ significantly for high and low sICAM-1 concentrations.

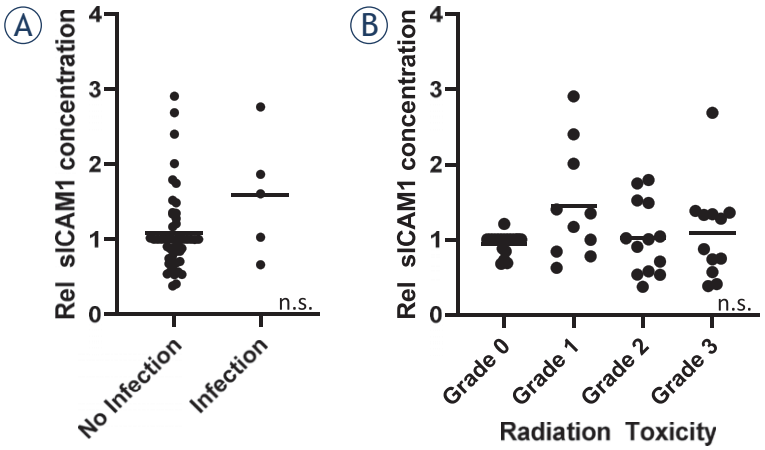

FIGURE 4. sICAM-1 levels at time points with manifest infections showed a tendency to higher concentrations compared to all other time points, however, without statistical significance **(A)**. No difference in sICAM-1 concentrations was observed comparing time points with different documented Radiation Therapy Oncology Group (RTOG) toxicity grades **(B)**.

and GTV LN were not correlated with each other (r = -0.17, data not shown). The hull of GTV PT and GTV LN reflecting the total tumor burden of the patient before the start of radiochemotherapy showed a strong correlation with initial sICAM-1 concentrations (Figure 5C). With a cut-off of 50 cm^3, sCIAM1 levels were significantly higher for larger tumors with 1600.8 ± 87.5 pg/ml vs 718.2 ± 79.3 pg/ml ($p < 0.01$).

Discussion

In this study, sICAM-1 was identified as a plasma cytokine that significantly decreased during definitive radiochemotherapy of three head and neck cancer patients in a cytokine array of 36 cytokines. In a larger patient cohort of eight additional patients (eleven in total) and additional time points during therapy and during follow-up these findings could not be confirmed. No conclusions can be drawn concerning sICAM-1 and oncological outcomes. The fact that both patients developing early recurrences presented with high initial sICAM-1 levels might be of notice. No correlation of sICAM-1 levels with infection and toxicity were observed (in contrast to recent findings for HMGB1 in the same patient cohort).[21] The most prominent finding is a strong correlation of initial sICAM-1 concentrations with the tumor burden of the patients at the start of radiochemotherapy as contoured for radiotherapy planning. This finding is in line with a report on hepatocellular carcinoma, which also found a correlation of sICAM-1 levels with tumor volume and tumor stage.[22] In colorectal cancer, sICAM1 levels correlated with tumor diameter.[23]

ICAM-1 (CD54) on endothelial cells is a crucial mediator of leukocyte adhesion to blood vessel walls.[24] In its soluble form, sICAM-1 is involved in autoimmune and inflammatory diseases, as well as infections and cancer.[25] Elevated sICAM-1 levels have been described in various cancer entities and have been linked to tumor stage and prognosis in HCC[22], gastric cancer[26,27] and cervical cancer.[28] The largest body of evidence was found for breast cancer with an association with tumor stage but no effect on immune function[29,30,31], colorectal cancer with an association with tumor stage and prognosis[32,33,34] and meta-analyses for lung cancer.[35,36] In HNSCC, elevated levels of sICAM-1 were found in comparison to healthy controls, without significant changes after radiochemotherapy[37], which is in line with our findings. The positive correlation of sICAM-1 with SerpinE1 might be explained by the link of both parameters to total tumor burden. SerpinE1 has been established as a prognostic marker in breast cancer and seems to be associated with cancer spread and metastasis.[38] The association with SDF1 is not that easily explainable as SDF1 was not associated with tumor size.

sICAM-1 might be a soluble plasma marker for initial tumor burden. In contrast to cfDNA[39] and HMGB1[21], sICAM-1 levels were not significantly influenced by infection or toxicity. As these con-

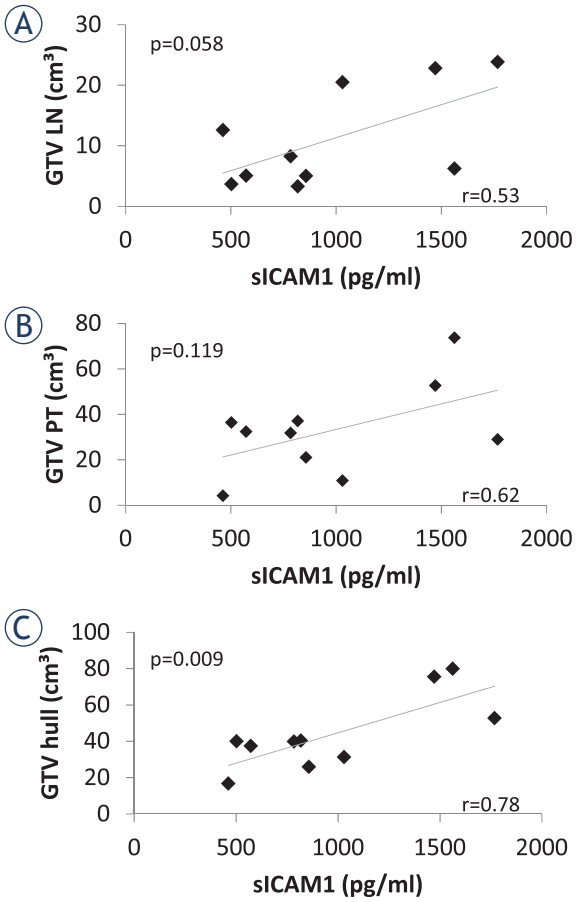

FIGURE 5. After exclusion of one patient with a large, mostly necrotic lymph node metastasis, initial pretherapeutic sICAM-1 levels showed moderate positive correlations with primary tumor volumes **(A)** and volumes of lymph node metastases **(B)** contoured for radiotherapy planning and a strong correlation with the sum of these volumes (gross tumor volume [GTV] hull, **(C)**) indicating the total tumor burden of the patient at the time point of initiation of radiotherapy.

founders do not seem to play a major role in measuring sICAM-1, sICAM-1 levels might be evaluated at any time point during radiochemotherapy. However, we did not find conclusive changes of s-ICAM-1 concentrations during therapy. Thus, further investigation is needed to confirm and explain the missing decline during treatment as some tumor shrinkage is usually already observed during the course of radiochemotherapy. Maybe our cohort was too small for significant findings or confounders other than inflammation and infection might play a role in measuring sICAM-1 during cancer treatment. However, if our results can be confirmed in larger patient cohorts, sICAM-1 might become a tumor marker for patients with HNSCC at initial diagnosis with exceedingly high values and low clinical tumor burden prompting further staging imaging due to suspicion of further undetected tumor manifestations. This might also be in line with the two patients developing early recurrences with high initial sICAM-1 levels as large tumor mass or micrometastases might limit curative treatment options.

Therefore, sICAM-1 seems to be a biomarker for total tumor burden (primary tumor and lymph node metastases) in head and neck cancer patients prior to definitive radiochemotherapy. No systematic changes were observed during radiochemotherapy and with toxicity or infections. High sICAM-1 concentrations initially or during follow-up might hint at higher tumor burden than clinically suspected and might prompt further investigations after validation in larger cohorts.

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