Research Square

Preprints are preliminary reports that have not undergone peer review. They should not be considered conclusive, used to inform clinical practice, or referenced by the media as validated information.

Systemic Immune Reprogramming After Tumor Resection: Perioperative Changes in Chemokines and Immune Checkpoint Markers in Oral Squamous Cell Carcinoma

Miriam Kokal-Ribaudo
University Medical Center Hamburg-Eppendorf
Niclas Rénevier
University Medical Center Hamburg-Eppendorf
Juliana Sufcak
University Medical Center Hamburg-Eppendorf
Xiangyuan Wu
University Medical Center Hamburg-Eppendorf
Ole Moritz Block
University Medical Center Hamburg-Eppendorf
Katharina Besch
University Medical Center Hamburg-Eppendorf
Christina Bäcker
University Medical Center Hamburg-Eppendorf
Maximilian Horst Rudolf Olivier
University Medical Center Hamburg-Eppendorf
Tobias Vollkommer
University Medical Center Hamburg-Eppendorf
Ralf Smeets
University Medical Center Hamburg-Eppendorf
Ann-Kristin Struckmeier
a.struckmeier@uke.de

University Medical Center Hamburg-Eppendorf

Research Article

Keywords: oral squamous cell carcinoma, tumor resection, systemic immune reaction, immune checkpoint markers

Posted Date: October 16th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10970110/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Systemic Immune Reprogramming After Tumor Resection: Perioperative Changes in Chemokines and Immune Checkpoint Markers in Oral Squamous Cell Carcinoma

Miriam Kokal-Ribaudo[1], Niclas Rénevier[1], Juliana Sufcak[1], Xiangyuan Wu[1], Ole Moritz Block[1], Katharina Besch[1], Christina Bäcker[1], Maximilian Horst Rudolf Olivier[1], Tobias Vollkommer[1], Ralf Smeets[1,2], Ann-Kristin Struckmeier[1,*]

[1] Department of Oral and Maxillofacial Surgery, University Medical Center Hamburg-Eppendorf, Martinistraße 52, 20246 Hamburg, Germany.
[2] Department of Oral and Maxillofacial Surgery, Division of Regenerative Orofacial Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany.

* Corresponding author: Ann-Kristin Struckmeier, e-mail: a.struckmeier@uke.de,
ORCID: 0000-0002-4015-1466

Keywords: oral squamous cell carcinoma; tumor resection; systemic immune reaction; immune checkpoint markers

Abstract

Background

Oral squamous cell carcinoma (OSCC) develops within a complex immunological environment shaped by chronic inflammation and tumor-induced immune regulation. This study aimed to characterize systemic immune alterations in OSCC and their perioperative changes following tumor resection.

Methods

Serum samples from OSCC patients were collected preoperatively and one and two weeks after surgery and compared with healthy controls. Inflammatory chemokines and soluble immune checkpoint molecules were quantified using multiplex immunoassays. Statistical analyses included paired comparisons, correlation analyses, and receiver operating characteristic (ROC) curve analysis.

Results

IL-8, IP-10, Eotaxin, TARC, MIG, and MIP-1β were elevated preoperatively and decreased significantly within one week after tumor resection, remaining reduced thereafter (all $p < 0.05$). In contrast, MCP-1 increased postoperatively. Immune checkpoint markers, including PD-1, PD-L1, CTLA-4, TIM-3, and LAG-3, also decreased significantly, with the most pronounced changes during the first postoperative week. Correlation analyses showed coordinated immune networks, including associations between IP-10 and MIG ($r = 0.654$) and PD-1 and LAG-3 ($r = 0.685$). Most markers were not significantly associated with tumor stage. ROC analysis showed good diagnostic performance for selected chemokines, checkpoint markers, and composite immune scores.

Conclusions

OSCC is associated with coordinated systemic immune alterations that change rapidly after tumor resection, particularly within the first postoperative week. These findings support tumor-driven systemic immune activation and highlight the relevance of perioperative immune dynamics. Composite immune signatures warrant further investigation as potential non-invasive tools for disease detection and monitoring.

Clinical Relevance

Perioperative dynamics and integrated biomarker signatures provide important insights into systemic immune regulation in OSCC patients.

Abbreviations

4-1BB – 4-1BB Receptor (CD137)

AUC – Area Under the Curve

B7.2 – B7-2 (CD86)

CCL – C-C Motif Chemokine Ligand

CI – Confidence Interval

CTLA-4 – Cytotoxic T-Lymphocyte-Associated Protein 4

CXCL – C-X-C Motif Chemokine Ligand

ENA-78 – Epithelial Neutrophil-Activating Peptide 78

GROα – Growth-Regulated Oncogene Alpha

IL – Interleukin

IP-10 – Interferon Gamma-Induced Protein 10

IQR – Interquartile Range

I-TAC – Interferon-Inducible T-cell Alpha Chemoattractant

LAG-3 – Lymphocyte-Activation Gene 3

MCP-1 – Monocyte Chemoattractant Protein 1

MIG – Monokine Induced by Gamma Interferon

MIP – Macrophage Inflammatory Protein

OR – Odds Ratio

OSCC – Oral Squamous Cell Carcinoma

PD-1 – Programmed Cell Death Protein 1

PD-L1 – Programmed Death-Ligand 1

ROC – Receiver Operating Characteristic

sCD25 – Soluble CD25

TARC – Thymus and Activation-Regulated Chemokine

TGF-β1 – Transforming Growth Factor Beta 1

Th1 – T Helper 1

TIM-3 – T-cell Immunoglobulin and Mucin-Domain Containing 3

UICC – Union for International Cancer Control

Introduction

Oral squamous cell carcinoma (OSCC) represents the most common malignancy of the oral cavity and remains associated with substantial morbidity and an unfavorable prognosis, particularly in advanced stages. Despite advances in surgical and multimodal treatment strategies, survival rates have improved only modestly, underscoring the need for a better understanding of tumor–host interactions and novel biomarkers for disease detection and monitoring (1, 2).

Cancer development is increasingly recognized as a consequence of dynamic interactions between malignant cells and the immune system. Chronic inflammation constitutes a hallmark of cancer and contributes to tumor initiation, progression, angiogenesis, and metastatic dissemination. In this context, chemokines play a central role by orchestrating immune cell recruitment, trafficking, and spatial organization within tissues. Depending on the cellular context and receptor engagement, chemokines can promote either antitumor immunity or immunosuppressive tumor microenvironments, highlighting their dual and context-dependent function in cancer biology (3, 4).

In parallel, immune checkpoint pathways represent a major mechanism of tumor immune evasion. In head and neck squamous cell carcinoma (HNSCC), inhibitory axes such as programmed cell death protein 1 (PD-1) and its ligand PD-L1, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), and lymphocyte activation gene-3 (LAG-3) contribute to T-cell dysfunction and impaired antitumor immunity. These pathways have become central therapeutic targets, and immune checkpoint inhibition has significantly improved outcomes in subsets of patients with advanced disease (5, 6).

While the tumor microenvironment has been extensively characterized, tumor immunity is not confined to the local tissue. Increasing evidence indicates that cancer induces systemic immune reprogramming, affecting circulating immune cells, soluble mediators, and inter-organ immune communication. This concept of systemic cancer immunity highlights that peripheral blood biomarkers may reflect tumor-associated immune activity and provide clinically relevant information beyond tissue-based analyses (7).

In OSCC, several studies have demonstrated altered circulating inflammatory mediators compared with healthy individuals, including increased levels of cytokines such as IL-8 and changes in chemokines such as CCL2 (MCP-1) and CCL3 (MIP-1α) (8-10). Similarly, studies in HNSCC have reported systemic alterations in chemokines involved in T-cell recruitment,

including CXCL10 (IP-10) and CCL4 (MIP-1β), supporting the presence of tumor-associated immune changes in circulation. (11). However, most of these investigations are limited to cross-sectional comparisons and do not address dynamic changes over time.

The perioperative period represents a unique biological window to study systemic immune dynamics. Surgical tumor resection removes a major source of tumor-derived signals while simultaneously inducing tissue injury and inflammatory responses. Previous studies in head and neck cancer have shown that tumor treatment can rapidly modulate systemic cytokine profiles, indicating that circulating immune mediators are sensitive to both tumor burden and treatment-related effects (12). More recently, the perioperative phase has been recognized as a critical period of immune modulation with potential implications for tumor progression and therapeutic intervention (13, 14).

Despite these advances, the perioperative systemic immune response in OSCC remains insufficiently characterized, particularly regarding the interplay between inflammatory chemokines and soluble immune checkpoint markers. While both pathways are known to interact within the tumor microenvironment, their coordinated behavior in the systemic circulation and their short-term dynamics following tumor removal are poorly understood.

Therefore, the aim of the present study was to comprehensively characterize perioperative changes in circulating inflammatory chemokines and soluble immune checkpoint molecules in patients with OSCC and to evaluate their diagnostic potential. By integrating longitudinal sampling, correlation analyses, and composite immune scores, this study seeks to provide a systems-level understanding of systemic immune alterations associated with OSCC.

Material and methods

Study design and patient cohort

This longitudinal study included patients with histologically confirmed OSCC who were treated at the Department of Oral and Maxillofacial Surgery, University Medical Center Hamburg-Eppendorf, between 2025 and 2026. A total of 62 OSCC patients were enrolled. Thirty healthy control subjects without a history of malignant disease or acute or chronic inflammatory conditions were included for comparison.

Exclusion criteria for both patients and controls comprised a history of other malignancies, acute infection, chronic inflammatory or autoimmune diseases, immunosuppressive medication, and insufficient serum sample volume.

The study was conducted in accordance with the Declaration of Helsinki and approved by the local ethics committee. Written informed consent was obtained from all participants prior to inclusion.

Clinical and pathological data collection

Baseline clinical and pathological parameters were retrieved from medical records for all OSCC patients. Collected variables included: age, sex, tumor localization, pathological T category, pathological N category, UICC stage, histological grading, perineural invasion, lymphatic invasion, and vascular invasion.

Serum sample collection and processing

Peripheral venous blood samples were collected from OSCC patients prior to surgical or oncologic treatment and at one and two weeks after tumor resection (i.e., 5–7 and 10–14 days postoperatively, respectively). Blood samples from healthy control subjects were collected at the time of study inclusion.

Blood samples were allowed to clot at room temperature for 30 minutes and subsequently centrifuged at 2,000 × g for 10 minutes. Serum was aliquoted and stored at −80 °C until further analysis. Freeze-thaw cycles were avoided.

Multiplex serum biomarker analysis

Serum concentrations of inflammatory chemokines and soluble immune checkpoint markers were quantified using bead-based multiplex immunoassays (LEGENDplex™, BioLegend, San Diego, CA, USA) according to the manufacturer's instructions.

The following panels were applied:

- **LEGENDplex™ Human Proinflammatory Chemokine Panel 1**, including MCP-1 (CCL2), IP-10 (CXCL10), Eotaxin (CCL11), TARC (CCL17), MIP-1α (CCL3), MIP-1β

(CCL4), MIG (CXCL9), MIP-3α (CCL20), ENA-78 (CXCL5), GROα (CXCL1), I-TAC (CXCL11) and IL-8 (CXCL8).

- **LEGENDplex™ Human Immune Checkpoint Panel 1**, including sCD25 (IL-2Rα), 4-1BB, B7.2 (CD86), Free Active TGF-β1, CTLA-4, PD-L1, PD-1, TIM-3, LAG-3, and Galectin-9.

Samples were processed using the recommended dilution factors. Data acquisition was performed using a BD LSRFortessa™ flow cytometer in the Cytometry and Cell Sorting Core Facility at Universitätsklinikum Hamburg-Eppendorf, and data were analyzed using the LEGENDplex™ data analysis software. Concentrations were calculated based on standard curves generated for each analyte.

Statistical analysis

Statistical analyses were performed using SPSS for Mac (Version 31, IBM Corp., Armonk, NY, USA). Continuous variables are presented as median and interquartile range (IQR), and categorical variables as absolute numbers and percentages.

Due to skewed distributions, biomarker concentrations were log10-transformed prior to analysis. For multimarker analyses, z-score normalization was applied.

Group differences between OSCC patients and healthy controls were assessed using the Mann-Whitney U test. Paired comparisons between perioperative time points were performed using the Wilcoxon signed-rank test.

Associations between biomarkers and clinicopathological variables were evaluated using Spearman's rank correlation for continuous or ordinal variables and Mann-Whitney U test for binary comparisons.

Correlations between biomarkers were assessed using Spearman's rank correlation coefficient.

Diagnostic performance was evaluated using receiver operating characteristic (ROC) curve analysis, with calculation of the area under the curve (AUC) and 95% confidence intervals (CIs).

Multivariable logistic regression models were used to assess differences between squamous cell carcinoma patients and healthy controls. Results are reported as odds ratios (ORs) with 95% confidence intervals (CIs).

All tests were two-sided, and p-values < 0.05 were considered statistically significant.

Heatmaps were generated using Morpheus (Broad Institute, Cambridge, MA, USA) based on z-score-normalized data.

Results

Patient characteristics

A total of 62 patients with oral squamous cell carcinoma (OSCC) and 30 healthy controls were included (Table S1). The OSCC cohort comprised 33 males (53.2%) and 29 females (46.8%), while the control group included 21 males (70.0%) and 9 females (30.0%).

Tumors were most commonly located in the lower jaw (30.6%), followed by the tongue (19.4%) and floor of the mouth (16.1%). Less frequent sites included the upper jaw (14.5%), buccal mucosa (9.7%), palate (4.8%), and lip (4.8%).

Tumor stages were distributed as T1 (29.0%), T2 (21.0%), T3 (16.1%), and T4a (33.9%). Most patients were node-negative (N0: 72.6%), while nodal involvement was observed in N1 (4.8%), N2a/b (each 6.5%), N2c (1.6%), and N3b (6.5%).

Histological grading was G1 (27.4%), G2 (37.1%), G3 (22.6%), and Gx (12.9%). Lymphovascular, vascular, and perineural invasion were absent in 75.8%, 79.0%, and 67.7% of cases, respectively. All patients underwent complete tumor resection (R0: 100%).

Perioperative dynamics of inflammatory chemokines

Serum levels of inflammatory chemokines were analyzed at baseline, one week, and two weeks after tumor resection and compared with healthy controls (Table S2).

One week after surgery, most chemokines showed reduced levels compared with baseline, including IL-8, IP-10, Eotaxin, TARC, MIP-1α, MIG, MIP-3α, GROα, I-TAC, and MIP-1β (all $p \leq 0.017$), whereas MCP-1 increased ($p < 0.001$). ENA-78 remained unchanged ($p = 0.110$) (Figure 1A). At two weeks postoperatively, the majority of chemokines remained significantly different from baseline, including IL-8, IP-10, Eotaxin, TARC, MCP-1, MIP-1α, MIG, MIP-3α, GROα, I-TAC, and MIP-1β (all $p < 0.001$), while ENA-78 again showed no significant change ($p = 0.953$). No significant differences were observed between week 1 and week 2 for most chemokines, except for Eotaxin ($p = 0.010$). Compared with healthy controls, IP-10 ($p = 0.021$) and MCP-1 ($p = 0.012$) differed significantly, whereas all other chemokines showed no significant differences.

Perioperative dynamics of soluble immune checkpoint markers

Perioperative changes in soluble immune checkpoint markers are summarized in Table S3.

One week after surgery, several markers were reduced compared with baseline, including 4-1BB, B7.2, TGF-β1, CTLA-4, PD-L1, PD-1, TIM-3, LAG-3, and Galectin-9 (all $p \leq 0.048$), whereas sCD25 remained unchanged ($p = 0.239$) (Figure 1B). At two weeks, multiple markers remained significantly different from baseline, including 4-1BB, B7.2, CTLA-4, PD-L1, PD-1, TIM-3, and LAG-3 (all $p \leq 0.047$). TGF-β1, Galectin-9, and sCD25 were not significantly different at this time point. No significant differences were observed between week 1 and

week 2 for any marker (all $p > 0.05$). Compared with healthy controls, only sCD25 differed significantly ($p = 0.047$), whereas all other markers showed no significant differences.

Correlations among inflammatory chemokines

Correlation analysis revealed structured associations among circulating chemokines (Table S4). IL-8 showed a moderate negative correlation with MCP-1 ($r = -0.430$), and positive correlations with GROα ($r = 0.530$) and ENA-78 ($r = 0.267$) (Figure 2A). MCP-1 was negatively correlated with MIP-1α ($r = -0.451$) and MIP-1β ($r = -0.655$). Strong positive correlations were observed between IP-10 and MIG ($r = 0.654$), as well as between TARC and Eotaxin ($r = 0.594$) (all $p < 0.001$).

Correlations among soluble immune checkpoint markers

Significant associations were also observed among soluble immune checkpoint markers (Table S4).

A strong positive correlation was found between PD-1 and LAG-3 ($r = 0.685$), while TIM-3 correlated with Galectin-9 ($r = 0.516$) (Figure 2B). PD-L1 showed a strong correlation with CTLA-4 ($r = 0.616$), and weaker correlations were observed between PD-1 and TIM-3 ($r = 0.274$) and between sCD25 and PD-1 ($r = 0.273$) (all $p \leq 0.003$).

Correlations between inflammatory chemokines and immune checkpoint markers

Cross-pathway analysis revealed significant associations between inflammatory chemokines and immune checkpoint markers (Table S4).

IL-8 correlated positively with PD-1 ($r = 0.367$) and TIM-3 ($r = 0.228$), while IP-10 showed a strong positive correlation with PD-1 ($r = 0.531$) (Figure 2C). In contrast, MCP-1 demonstrated negative correlations with PD-L1 ($r = -0.389$) and TGF-β1 ($r = -0.458$) (all $p \leq 0.004$).

Correlations between composite biomarker scores

In addition to the individual biomarker correlations, composite biomarker scores were calculated based on the markers listed in Table S7. Spearman correlation analysis revealed significant correlations between the Myeloid and Exhaustion score ($r = 0.396$), the Myeloid and Immunosuppression score ($r = 0.393$), and the Exhaustion and Immunosuppression score ($r = 0.954$) (Table S5).

Global correlation patterns

A correlation heatmap demonstrated distinct clusters of co-regulated biomarkers (Figure 3). Immune checkpoint markers, including PD-1, TIM-3, LAG-3, CTLA-4, and Galectin-9, formed a strongly correlated cluster. Chemokines involved in T-cell recruitment, including IP-10, MIG,

and I-TAC, also clustered together. IL-8, GROα, and ENA-78 formed a separate group, whereas MCP-1 showed inverse correlations with multiple markers.

Associations with clinicopathological parameters

Associations between circulating biomarkers and clinicopathological characteristics are summarized in Table S6. IP-10 differed significantly between sexes ($p = 0.002$), but showed no statistically significant association with age, T stage, N stage, or UICC stage in this cohort. MIP-1β, LAG-3, and PD-1 were not associated with any clinical parameters. The Th1 recruitment score correlated positively with age ($r = 0.303$, $p = 0.026$) and differed between sexes ($p = 0.008$). The immune exhaustion score differed between sexes ($p = 0.011$) but was not associated with other parameters.

Diagnostic performance of circulating chemokines

ROC analysis of circulating chemokines is presented in Table S7.

MIP-1β (AUC 0.788), Eotaxin (AUC 0.783), and IP-10 (AUC 0.756) showed the highest diagnostic performance (Figure 4A). Additional chemokines, including I-TAC, MIP-3α, MIG, TARC, GROα, IL-8, and MIP-1α, demonstrated moderate discriminative ability (all $p < 0.001$). ENA-78 did not show significant discrimination (AUC 0.559, $p = 0.193$), whereas MCP-1 showed an inverse pattern (AUC 0.288).

Diagnostic performance of soluble immune checkpoint biomarkers

ROC analysis of immune checkpoint markers is summarized in Table S7.

LAG-3 (AUC 0.732), B7.2 (AUC 0.712), and CTLA-4 (AUC 0.709) showed the highest diagnostic performance (Figure 4B). Additional markers, including PD-1, TGF-β1, 4-1BB, PD-L1, TIM-3, and Galectin-9, demonstrated moderate discriminative ability (all $p \leq 0.003$), whereas sCD25 was not significant (AUC 0.579, $p = 0.086$).

Diagnostic performance of biomarker scores

Composite biomarker scores are summarized in Table S7.

The Th1 recruitment score (AUC 0.754) and immune exhaustion score (AUC 0.748) showed the highest diagnostic performance (Figure 4C). Additional scores, including the immune checkpoint score (AUC 0.733), tumor immune escape score (AUC 0.708), and T-cell exhaustion-dominant score (AUC 0.746), also demonstrated good discrimination. The myeloid score showed moderate performance (AUC 0.631, $p = 0.004$).

Multivariable logistic regression analysis

Multivariable logistic regression analysis is presented in Table S8. The Th1 recruitment score was significantly associated with OSCC (OR 1.891, 95% CI 1.213–2.948, $p = 0.005$), whereas the immune exhaustion score was not ($p = 0.473$). Age was significantly associated with OSCC ($p < 0.001$), while sex was not ($p = 0.478$).

Discussion

The present study provides a comprehensive analysis of systemic immune alterations in patients with OSCC and demonstrates that tumor presence is associated with dynamic and coordinated changes in circulating inflammatory chemokines and soluble immune checkpoint markers. By integrating perioperative analyses, correlation patterns, and diagnostic modeling, this study offers a systems-level perspective on tumor–host immune interactions in the peripheral blood.

A central finding of this study is the pronounced perioperative modulation of inflammatory chemokines, with the most marked changes occurring within the first postoperative week. Several chemokines, including IL-8, IP-10, Eotaxin, TARC, MIP-1α, MIG, MIP-3α, GROα, I-TAC, and MIP-1β, showed significantly higher concentrations at baseline compared with postoperative levels and decreased markedly already one week after tumor resection. This rapid decline suggests that these mediators are closely linked to tumor presence and that systemic inflammatory signaling is promptly attenuated following tumor removal.

At two weeks postoperatively, most chemokines remained significantly altered compared with baseline but did not show further significant changes compared with week one, indicating that the major systemic immune shift occurs early after surgery, followed by relative stabilization. However, perioperative immune changes are unlikely to be exclusively tumor-driven, as surgery itself induces acute inflammatory responses associated with wound healing and tissue repair, which may partially contribute to the observed dynamics (15-17).

In contrast to the overall decrease of most chemokines, MCP-1 exhibited a distinct pattern, with significantly increased levels one week after surgery that remained elevated at two weeks. Moreover, MCP-1 was among the few markers that differed significantly between OSCC patients and healthy controls at baseline. This divergent behavior likely reflects the dual role of MCP-1 in tumor-associated immune modulation and postoperative inflammatory responses, particularly monocyte recruitment and tissue repair. In line with this, Wen et al. reported that postoperative MCP-1 dynamics are associated with clinical outcome, with increasing levels indicating poorer prognosis and decreasing levels associated with improved survival (18). Together, these findings suggest that MCP-1 reflects both tumor-related and surgery-induced immune processes and may carry prognostic relevance depending on its perioperative trajectory.

When comparing our findings with previous studies, a heterogeneous picture emerges for circulating chemokines. For MIP-1β, Kaskas et al. similarly reported reduced levels in HNSCC

patients, whereas Trellakis et al. described increased concentrations, possibly due to differences in cohort composition and stage distribution (11, 19). For IP-10, several studies have demonstrated elevated levels in tumor tissue and peripheral blood, particularly in nasopharyngeal carcinoma, as well as in oral fluids of orolaryngeal cancer patients (20-22).

In contrast, our data showed only limited differences between OSCC patients and healthy controls despite pronounced perioperative changes, suggesting that IP-10 reflects dynamic immune regulation rather than consistent baseline elevation across all patient populations.
A similar pattern was observed for IL-8. While previous studies consistently reported elevated IL-8 levels in OSCC patients (8, 10, 23, 24), our data did not demonstrate a significant baseline difference, despite marked postoperative decreases. Bao et al. showed that IL-8 contributes to diagnostic discrimination, particularly in combination with other cytokines (10), while Shree et al. suggested that IL-8 correlates with tumor progression (23). Together, these findings indicate that IL-8 may be more relevant as a marker of disease activity or progression rather than as a standalone diagnostic marker.

More broadly, several studies have reported elevated systemic cytokines in OSCC patients, including IL-1β, IL-6, IL-8, IL-10, and TNF-α (8, 24). However, some studies observed limited changes after treatment, suggesting that systemic inflammation may also be influenced by host-related factors beyond tumor presence (8). In contrast, our perioperative data demonstrate a rapid decline of multiple mediators within the first postoperative week, supporting the concept of tumor-associated immune activation that is at least partially reversible.

Data from tissue-based analyses consistently demonstrate upregulation of several markers investigated in this study, including PD-1, PD-L1, CTLA-4, 4-1BB, and multiple chemokines, in tumor tissue compared with healthy controls (25-31). However, this pattern is not uniformly reflected in peripheral blood. For example, Glajzer et al. found no difference in circulating 4-1BB levels between cancer patients and healthy controls (28), which is consistent with our findings of limited baseline differences for most soluble immune checkpoint markers. This discrepancy highlights the importance of distinguishing between local tumor microenvironment effects and systemic immune responses.

Despite limited baseline differences, soluble immune checkpoint markers demonstrated pronounced perioperative dynamics, with several markers decreasing significantly already within the first postoperative week. At two weeks, most markers remained reduced but did not change further, indicating early stabilization. Only sCD25 differed significantly between

patients and controls at baseline, suggesting that soluble checkpoint markers primarily reflect dynamic immune regulation rather than stable disease-specific differences.

The concept of coordinated systemic immune regulation is supported by both our correlation analyses and previous studies. Strong associations between chemokines and checkpoint markers, including IP-10–MIG and PD-1–LAG-3, as well as cross-pathway interactions, indicate integrated immune signaling networks. Similar coordinated patterns have been described in broader cytokine analyses, where composite biomarker signatures may capture complementary information across functionally related immune pathways (32, 33). Consistent with this concept, several composite biomarker scores in our study demonstrated good discriminatory performance, including the Th1 recruitment and immune exhaustion scores. However, their diagnostic performance did not exceed that of the best-performing individual chemokines, suggesting that their potential value may lie in capturing coordinated immune alterations rather than in improving diagnostic accuracy per se (10, 32).

Importantly, most circulating biomarkers were not significantly associated with tumor stage or other clinicopathological parameters, suggesting that systemic immune alterations reflect broader host immune responses rather than tumor burden alone. Similar findings have been reported in previous studies of circulating biomarkers in head and neck cancer (11, 12).

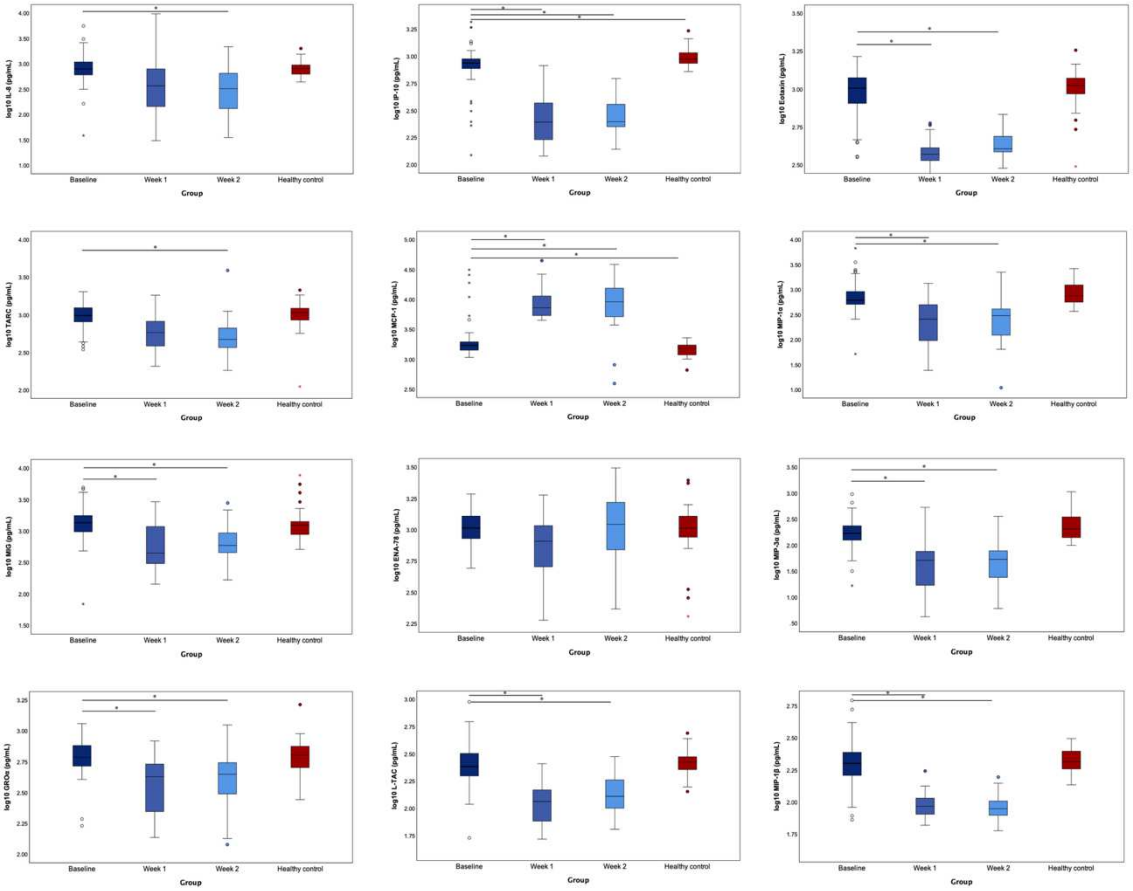
The perioperative time course highlights the first postoperative week as a critical window of systemic immune reprogramming. The rapid decline of multiple chemokines and immune checkpoint markers during this period supports the concept of tumor-driven immune activation that is promptly attenuated after tumor removal, while the divergent behavior of MCP-1 suggests overlapping effects of surgery-induced inflammation.

Several limitations should be considered. First, the sample size limits statistical power, particularly for subgroup analyses. Second, circulating biomarkers reflect systemic immune responses and do not directly represent the tumor microenvironment. Third, the follow-up period was limited to the early postoperative phase, and longer-term immune dynamics remain to be elucidated.

In conclusion, this study demonstrates that OSCC is associated with dynamic and coordinated systemic immune alterations involving both inflammatory chemokines and soluble immune checkpoint markers. The most pronounced systemic immune changes occur within the first postoperative week, identifying this period as a critical window of tumor-associated immune reprogramming. While only a limited number of markers differed between patients and healthy

controls at baseline, perioperative dynamics and integrated biomarker signatures provide important insights into systemic immune regulation and warrant further investigation as potential biomarkers for non-invasive disease monitoring.

A


log10 IL-8 (pg/mL)
log10 IP-10 (pg/mL)
log10 Eotaxin (pg/mL)
log10 TARC (pg/mL)
log10 MCP-1 (pg/mL)
log10 MIP-1α (pg/mL)
log10 MIG (pg/mL)
log10 ENA-78 (pg/mL)
log10 MIP-3α (pg/mL)
log10 GROα (pg/mL)
log10 I-TAC (pg/mL)
log10 MIP-1β (pg/mL)
Baseline
Week 1
Week 2
Healthy control
Group

B

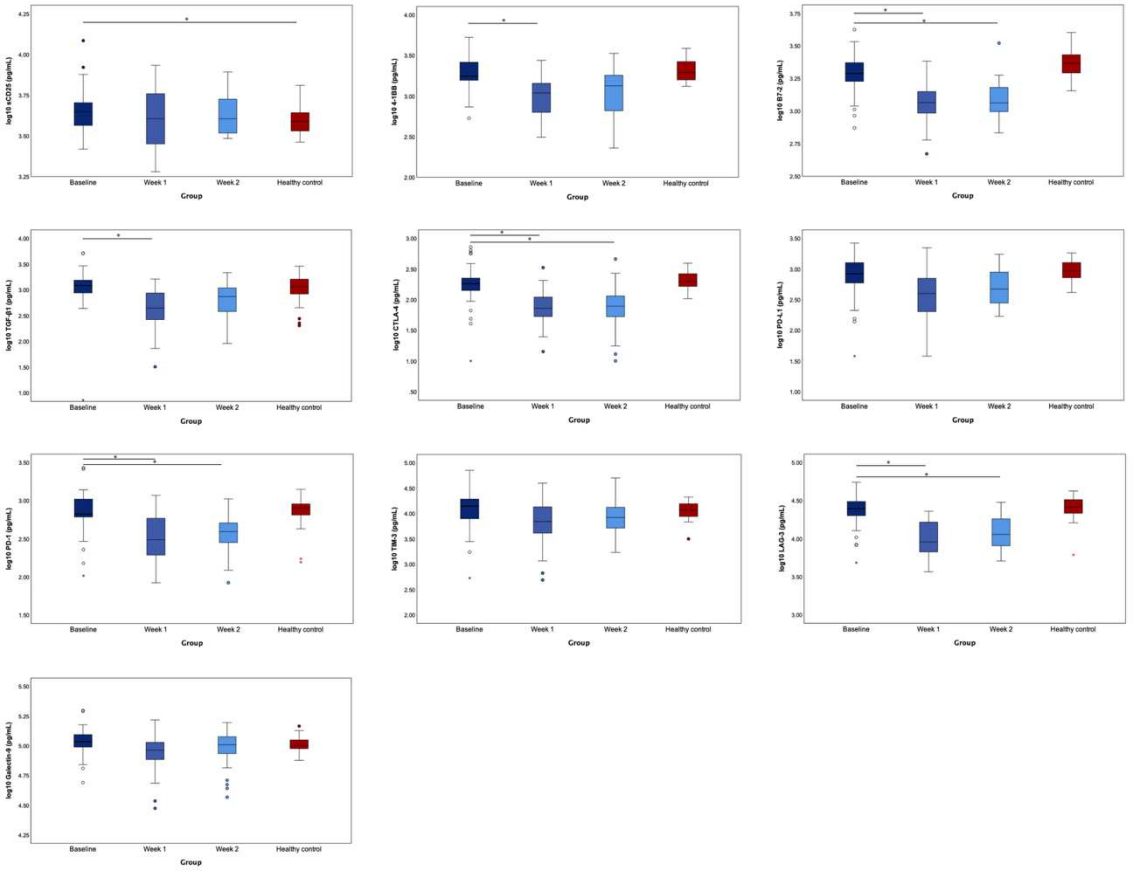

log10 sCD25 (pg/mL)
log10 4-1BB (pg/mL)
log10 B7-2 (pg/mL)
log10 TGF-β1 (pg/mL)
log10 CTLA-4 (pg/mL)
log10 PD-L1 (pg/mL)
log10 PD-1 (pg/mL)
log10 TIM-3 (pg/mL)
log10 LAG-3 (pg/mL)
log10 Galectin-9 (pg/mL)
Baseline
Week 1
Week 2
Healthy control
Group

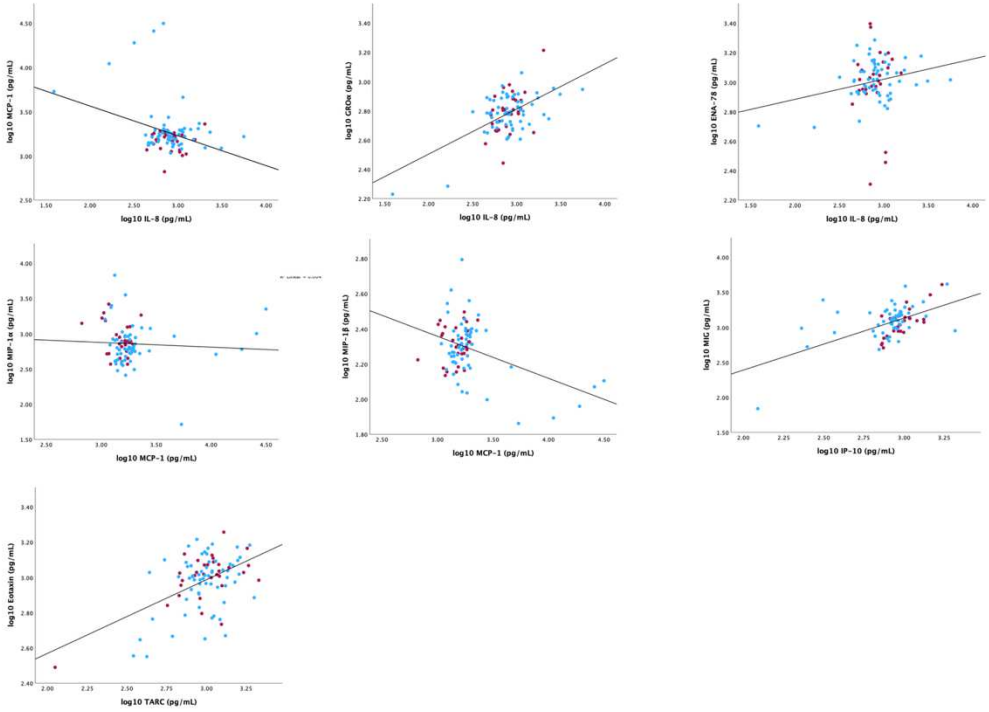
Figure 1. Temporal dynamics of circulating chemokines and soluble immune checkpoint markers in OSCC patients and healthy controls.

Boxplots illustrate log10-transformed serum concentrations of inflammatory chemokines and soluble immune checkpoint markers in OSCC patients at baseline (preoperative), one week after surgery, and two weeks after surgery, compared with healthy controls. Boxes represent the interquartile range (IQR), with the median indicated by the horizontal line, while whiskers show the range excluding outliers. Individual points represent extreme values. Paired perioperative comparisons were performed using the Wilcoxon signed-rank test, whereas comparisons between OSCC patients and healthy controls were performed using the Mann-Whitney U test.

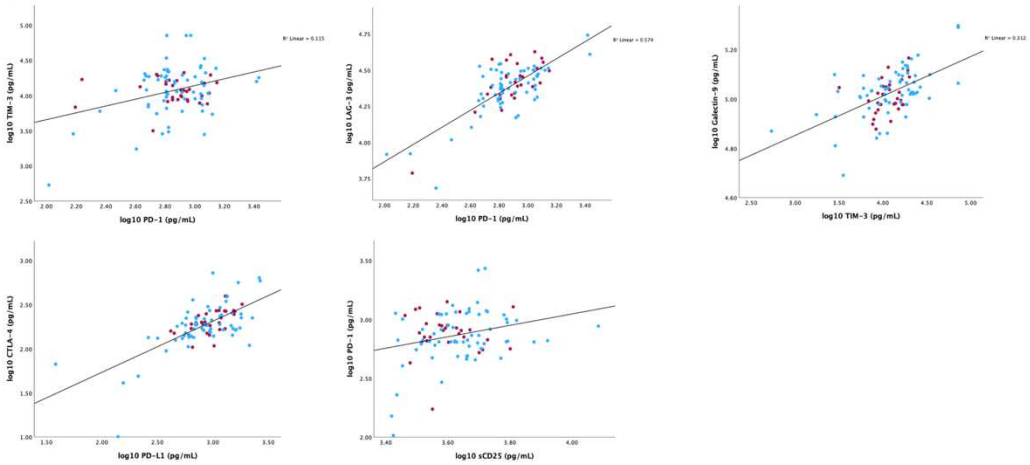
(A) Several chemokines, including IP-10, Eotaxin, TARC, MIP-1α, MIG, MIP-3α, GROα, I-TAC, and MIP-1β, showed significant decreases after tumor resection, particularly one week postoperatively. In contrast, MCP-1 increased significantly after surgery, whereas ENA-78 did not show significant temporal changes.

(B) Several immune checkpoint markers, including 4-1BB, B7.2, TGF-β1, CTLA-4, PD-L1, PD-1, TIM-3, and LAG-3, showed a significant decrease after tumor resection, particularly one week postoperatively. In contrast, sCD25 levels remained relatively stable across time points. Compared with healthy controls, baseline sCD25 concentrations were significantly elevated in OSCC patients ($p = 0.047$).

A


log10 MCP-1 (pg/mL)
log10 IL-8 (pg/mL)
log10 GROα (pg/mL)
log10 IL-8 (pg/mL)
log10 ENA-78 (pg/mL)
log10 IL-8 (pg/mL)
log10 MIP-1α (pg/mL)
log10 MCP-1 (pg/mL)
log10 MIP-1β (pg/mL)
log10 MCP-1 (pg/mL)
log10 MIG (pg/mL)
log10 IP-10 (pg/mL)
log10 Eotaxin (pg/mL)
log10 TARC (pg/mL)

B


log10 TIM-3 (pg/mL)
log10 PD-1 (pg/mL)
log10 LAG-3 (pg/mL)
log10 PD-1 (pg/mL)
log10 Galectin-9 (pg/mL)
log10 TIM-3 (pg/mL)
log10 CTLA-4 (pg/mL)
log10 PD-L1 (pg/mL)
log10 PD-1 (pg/mL)
log10 sCD25 (pg/mL)

C

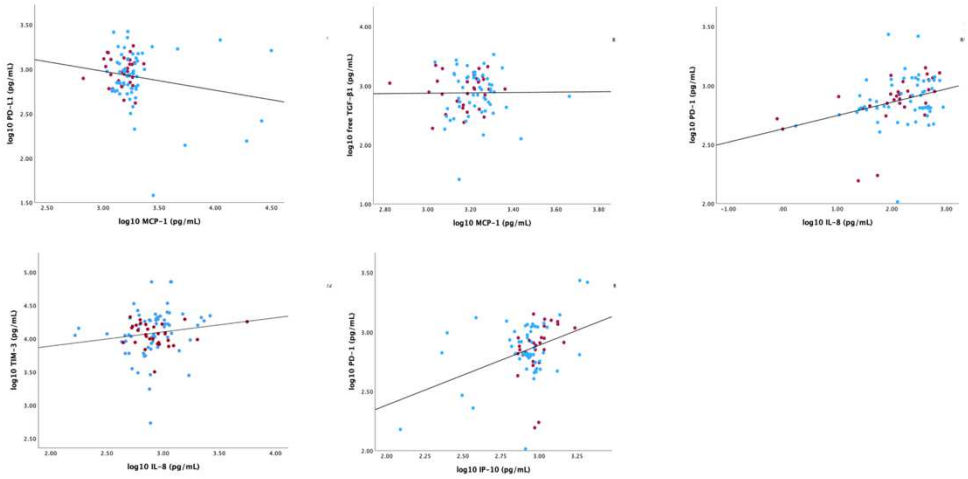

log10 PD-L1 (pg/mL)
log10 MCP-1 (pg/mL)
log10 free TGF-β1 (pg/mL)
log10 MCP-1 (pg/mL)
log10 PD-1 (pg/mL)
log10 IL-8 (pg/mL)
log10 TIM-3 (pg/mL)
log10 IL-8 (pg/mL)
log10 PD-1 (pg/mL)
log10 IP-10 (pg/mL)

Figure 2. Correlation analysis of inflammatory chemokines and soluble immune checkpoint markers in serum samples from patients with OSCC and healthy controls.
Scatter plots show log10-transformed biomarker concentrations with linear regression lines indicating the direction of associations. Each point represents an individual sample, with colors indicating OSCC patients (blue) and healthy controls (red).

(A) Correlations among inflammatory chemokines, including positive associations of IL-8 with GROα and ENA-78, IP-10 with MIG, and TARC with Eotaxin. MCP-1 showed negative correlations with MIP-1α and MIP-1β.

(B) Correlations among soluble immune checkpoint markers, including positive associations of TIM-3 with Galectin-9, PD-L1 with CTLA-4, and PD-1 with LAG-3.

(C) Cross-pathway correlations between inflammatory chemokines and soluble immune checkpoint markers, including positive associations of IL-8 with PD-1 and TIM-3 and of IP-10 with PD-1. MCP-1 showed negative associations with PD-L1 and TGF-β1.

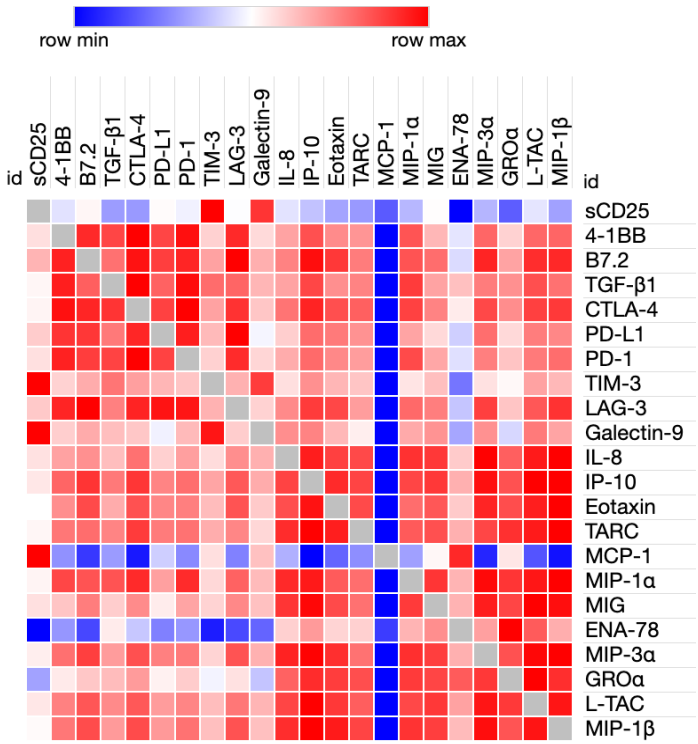
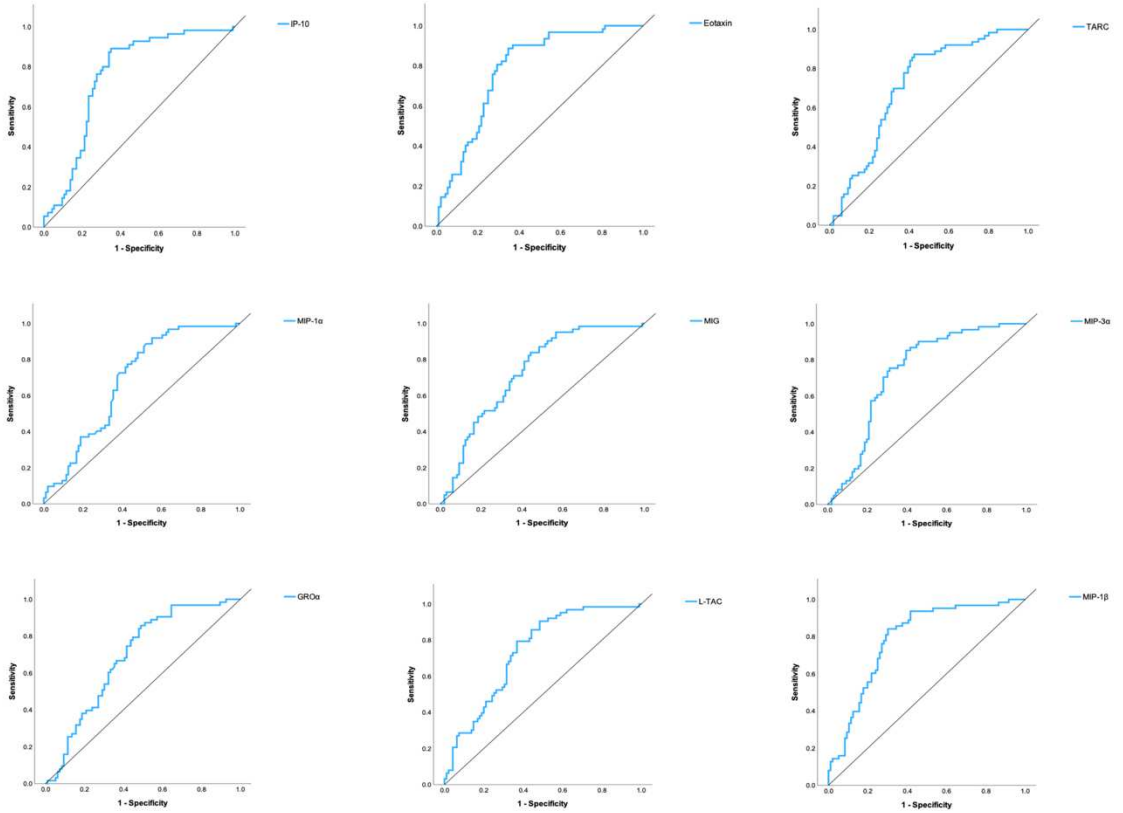


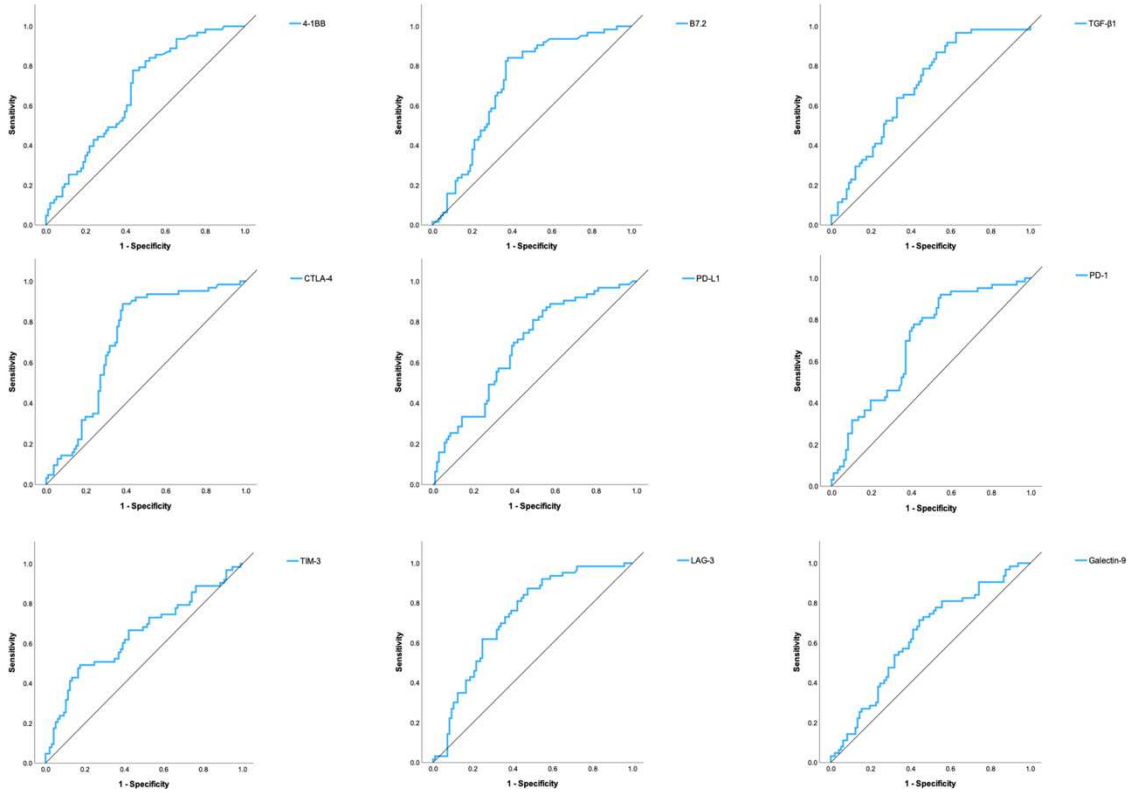
Figure 3. Heatmap of correlations among inflammatory chemokines and soluble immune checkpoint markers.

Heatmap illustrates pairwise correlations between inflammatory chemokines and soluble immune checkpoint markers measured in serum samples. Biomarker concentrations were log10-transformed prior to analysis and standardized using row-wise Z-scores for visualization. Colors indicate the relative correlation strength, ranging from blue (lower values) to red (higher values). Distinct clusters of biomarkers can be observed, reflecting coordinated regulation of immune activation, chemokine signaling, and immune checkpoint pathways.

A


IP-10
Eotaxin
TARC
MIP-1α
MIG
MIP-3α
GROα
L-TAC
MIP-1β
Sensitivity
1 - Specificity

B


4-1BB
B7.2
TGF-β1
CTLA-4
PD-L1
PD-1
TIM-3
LAG-3
Galectin-9
Sensitivity
1 - Specificity

C

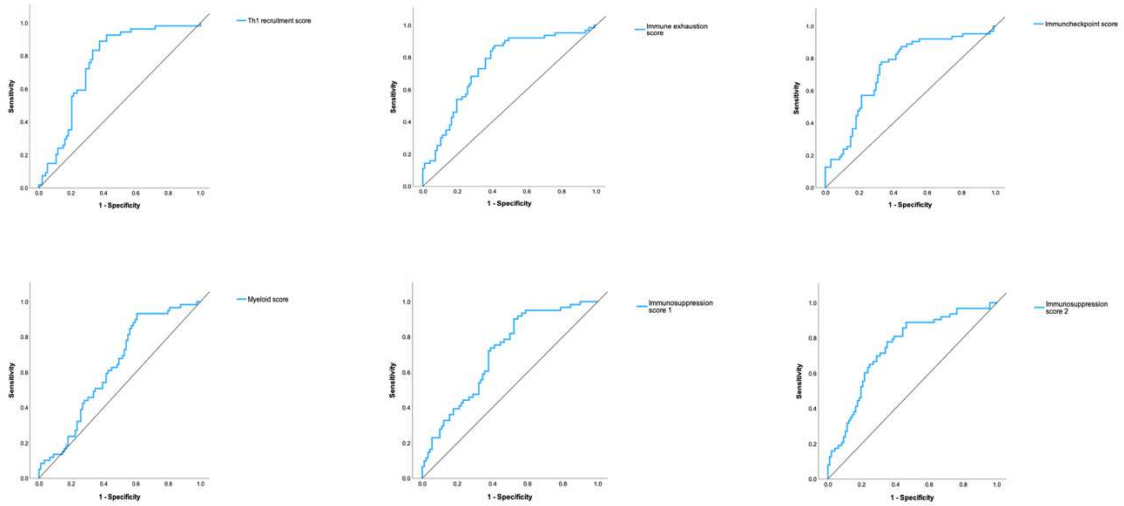


Figure 4. **Receiver operating characteristic (ROC) curve analysis evaluating the diagnostic performance of inflammatory chemokines and soluble checkpoint markers for distinguishing patients with OSCC from healthy controls.**

Only biomarkers demonstrating an area under the curve (AUC) >0.60 are shown for clarity. The diagonal line represents the reference line indicating no discriminative power (AUC = 0.5).

(A) The inflammatory chemokines MIP-1β, Eotaxin, and IP-10 showed the highest diagnostic accuracy.

(B) The soluble immune checkpoint markers LAG-3, B7.2, and CTLA-4 showed the highest diagnostic performance.

(C) Composite biomarker scores, including the Th1 recruitment score, immune exhaustion score, immune checkpoint score, tumor immune escape score, and T-cell exhaustion-dominant score. The Th1 recruitment and immune exhaustion scores demonstrated the highest discriminatory performance among the composite scores.

References:

1. Struckmeier AK, Buchbender M, Lutz R, Kesting M. Improved recurrence rates and progression-free survival in primarily surgically treated oral squamous cell carcinoma - results from a German tertiary medical center. Clin Oral Investig. 2024;28(5):262. https://doi.org/10.1007/s00784-024-05644-z
2. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74(3):229-63. https://doi.org/10.3322/caac.21834
3. Hanahan D. Hallmarks of Cancer: New Dimensions. Cancer Discov. 2022;12(1):31-46. https://doi.org/10.1158/2159-8290.CD-21-1059
4. Yi M, Li T, Niu M, Zhang H, Wu Y, Wu K, et al. Targeting cytokine and chemokine signaling pathways for cancer therapy. Signal Transduct Target Ther. 2024;9(1):176. https://doi.org/10.1038/s41392-024-01868-3
5. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12(4):252-64. https://doi.org/10.1038/nrc3239
6. Tufail M, Jiang CH, Li N. Immune evasion in cancer: mechanisms and cutting-edge therapeutic approaches. Signal Transduct Target Ther. 2025;10(1):227. https://doi.org/10.1038/s41392-025-02280-1
7. Hiam-Galvez KJ, Allen BM, Spitzer MH. Systemic immunity in cancer. Nat Rev Cancer. 2021;21(6):345-59. https://doi.org/10.1038/s41568-021-00347-z
8. Caruntu A, Scheau C, Codrici E, Popescu ID, Calenic B, Caruntu C, et al. The Assessment of Serum Cytokines in Oral Squamous Cell Carcinoma Patients: An Observational Prospective Controlled Study. J Clin Med. 2022;11(18). https://doi.org/10.3390/jcm11185398
9. Ding L, Li B, Zhao Y, Fu YF, Hu EL, Hu QG, et al. Serum CCL2 and CCL3 as potential biomarkers for the diagnosis of oral squamous cell carcinoma. Tumour Biol. 2014;35(10):10539-46. https://doi.org/10.1007/s13277-014-2306-1
10. Bao Z, Yi B. Analysis of preoperative serum cytokine levels in patients with oral squamous cell carcinoma. Sci Rep. 2025;15(1):13537. https://doi.org/10.1038/s41598-025-89816-1
11. Kaskas NM, Moore-Medlin T, McClure GB, Ekshyyan O, Vanchiere JA, Nathan CA. Serum biomarkers in head and neck squamous cell cancer. JAMA Otolaryngol Head Neck Surg. 2014;140(1):5-11. https://doi.org/10.1001/jamaoto.2013.5688
12. Green VL, Michno A, Greenman J, Stafford ND. Effect of treatment on systemic cytokines in head and neck squamous cell carcinoma patients. Results Immunol. 2011;2:1-6. https://doi.org/10.1016/j.rinim.2011.12.001
13. Galmiche A, Saidak Z, Bettoni J, Ouendo M, Testelin S. Therapeutic Perspectives for the Perioperative Period in Oral Squamous Cell Carcinoma (OSCC). Front Oral Health. 2021;2:764386. https://doi.org/10.3389/froh.2021.764386
14. Brammer B, Gross JH, Boyce BJ, Saba NF, Schmitt NC. Implementing perioperative immunotherapy for head and neck cancer after KEYNOTE-689: questions and challenges. Transl Cancer Res. 2025;14(12):8221-7. https://doi.org/10.21037/tcr-2025-2042
15. Lin E, Calvano SE, Lowry SF. Inflammatory cytokines and cell response in surgery. Surgery. 2000;127(2):117-26. https://doi.org/10.1067/msy.2000.101584
16. Marik PE, Flemmer M. The immune response to surgery and trauma: Implications for treatment. J Trauma Acute Care Surg. 2012;73(4):801-8. https://doi.org/10.1097/TA.0b013e318265cf87

17. Barrientos S, Stojadinovic O, Golinko MS, Brem H, Tomic-Canic M. Growth factors and cytokines in wound healing. Wound Repair Regen. 2008;16(5):585-601. https://doi.org/10.1111/j.1524-475X.2008.00410.x
18. Wen S, Tan H. The prognostic significance of CCL2 in oral squamous cell carcinoma: insights from tissue expression and perioperative serum profiling. Frontiers in Oncology. 2026;16. https://doi.org/10.3389/fonc.2026.1764317
19. Trellakis S, Bruderek K, Dumitru CA, Gholaman H, Gu X, Bankfalvi A, et al. Polymorphonuclear granulocytes in human head and neck cancer: enhanced inflammatory activity, modulation by cancer cells and expansion in advanced disease. Int J Cancer. 2011;129(9):2183-93. https://doi.org/10.1002/ijc.25892
20. Chang KP, Chang YT, Wu CC, Liu YL, Chen MC, Tsang NM, et al. Multiplexed immunobead-based profiling of cytokine markers for detection of nasopharyngeal carcinoma and prognosis of patient survival. Head Neck. 2011;33(6):886-97. https://doi.org/10.1002/hed.21557
21. Li J, Zhang XS, Xie D, Deng HX, Gao YF, Chen QY, et al. Expression of immune-related molecules in primary EBV-positive Chinese nasopharyngeal carcinoma: associated with latent membrane protein 1 (LMP1) expression. Cancer Biol Ther. 2007;6(12):1997-2004. https://doi.org/10.4161/cbt.6.12.5160
22. Michiels K, Schutyser E, Conings R, Lenaerts JP, Put W, Nuyts S, et al. Carcinoma cell-derived chemokines and their presence in oral fluid. Eur J Oral Sci. 2009;117(4):362-8. https://doi.org/10.1111/j.1600-0722.2009.00644.x
23. Hema Shree K, Gayathri R, Vishnu Priya V, Selvaraj J, Pratibha R. Pro-inflammatory mediator and cancer-associated chemokine CXCL8 as a prognostic indicator of tumor aggressiveness, angiogenesis, and disease progression with focus on therapeutic implications and recurrence monitoring. Oral Oncology Reports. 2025;13. https://doi.org/10.1016/j.oor.2025.100723
24. Huang W, Lin Y, Xu E, Ji Y, Wang J, Liu F, et al. Predictive value of preoperative pan-immune-inflammation value index in the prognosis of oral cancer patients undergoing radical resection. BMC Oral Health. 2025;25(1):132. https://doi.org/10.1186/s12903-025-05477-6
25. Li Y, Wu T, Gong S, Zhou H, Yu L, Liang M, et al. Analysis of the Prognosis and Therapeutic Value of the CXC Chemokine Family in Head and Neck Squamous Cell Carcinoma. Front Oncol. 2020;10:570736. https://doi.org/10.3389/fonc.2020.570736
26. Chang KP, Kao HK, Yen TC, Chang YL, Liang Y, Liu SC, et al. Overexpression of macrophage inflammatory protein-3alpha in oral cavity squamous cell carcinoma is associated with nodal metastasis. Oral Oncol. 2011;47(2):108-13. https://doi.org/10.1016/j.oraloncology.2010.11.012
27. Lu CC, Tsai HC, Yang DY, Wang SW, Tsai MH, Hua CH, et al. The Chemokine CCL4 Stimulates Angiopoietin-2 Expression and Angiogenesis via the MEK/ERK/STAT3 Pathway in Oral Squamous Cell Carcinoma. Biomedicines. 2022;10(7). https://doi.org/10.3390/biomedicines10071612
28. Glajzer J, Ries J, Trumet L, Matek C, Kesting M, Weber M. Expression of the immune checkpoint CD137/CD137L in oral squamous cell carcinoma (OSCC) and association with histopathological parameters. Virchows Arch. 2026. https://doi.org/10.1007/s00428-026-04430-4
29. Ganesh A, Ashikha Shirin Usman PP, K.P A, Thomas P, Ganapathy DM, Sekar D. Expression analysis of transforming growth factor beta (TGF-β) in oral squamous cell carcinoma. Oral Oncology Reports. 2024;9. https://doi.org/10.1016/j.oor.2024.100195

30. Feldmeier F, Weber M, Pacelli F, Vogl C, Glajzer J, Trumet L, et al. Investigation of the CTLA-4-CD28 Axis in Oral Squamous Cell Carcinoma. J Clin Med. 2025;14(14). https://doi.org/10.3390/jcm14145171
31. Ries J, Trumet L, Hahn A, Kunater L, Lutz R, Geppert C, et al. The Immune Checkpoint BTLA in Oral Cancer: Expression Analysis and Its Correlation to Other Immune Modulators. Int J Mol Sci. 2024;25(12). https://doi.org/10.3390/ijms25126601
32. Saif MSI, Sehgal K, Zhang Z, Butler RA, Wiencke JK, Molinaro AM, et al. A cytokine-based serum proteomic score is a prognostic biomarker for immunotherapy in head and neck cancer. Cancer Immunol Immunother. 2025;75(1):30. https://doi.org/10.1007/s00262-025-04271-1
33. Buranapraditkun S, Mahattanasakul P, Diloktaweewattana A, Bhummaphan N, Siriwattanakankul C, Bin-Alee F, et al. Immune-associated plasma proteins in oral and oropharyngeal cancer patients. Heliyon. 2022;8(9):e10753. https://doi.org/10.1016/j.heliyon.2022.e10753

Statements and Declarations

Disclosure of potential conflicts of interest: The authors have no financial or non-financial conflicts of interest to disclose.

Compliance with Ethical Standards: This study was performed in line with the principles of the 1964 Helsinki Declaration and its later amendments. The Ethics Committee of the Ärztekammer Hamburg approved the study's design and methods (Ethic vote: 2025-101539-BO-ff). Written informed consent was obtained from all subjects involved in the study.

Funding: This research was supported by the Deutsche Forschungsgemeinschaft **(**STR 1941/1-1; 549224982), Brigitte und Dr. Konstanze Wegener Stiftung, Hector-Stiftungen, and Vaillant-Stiftung (grants to AKS).

Author contributions: MKR: interpretation of data, drafting the manuscript. NR, XW, JS: data acquisition. AKS: conception and design of the study, funding acquisition, data acquisition, analysis and interpretation of data, drafting the manuscript, and revising it critically for important intellectual content and scientific integrity. OMB, KB, CB, MHRO, TV and RS revised the manuscript critically for important intellectual content and scientific integrity. All authors read and agreed to the final version of the manuscript.

Data availability: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplemental.docx