

Systemic Immune Network Dysregulation in Oral Squamous Cell Carcinoma: Coordinated Activation of Inflammatory and TNF Superfamily Pathways

Miriam Kokal-Ribaud

University Medical Center Hamburg-Eppendorf

Juliana Sufcak

University Medical Center Hamburg-Eppendorf

Niclas Rénevier

University Medical Center Hamburg-Eppendorf

Xiangyuan Wu

University Medical Center Hamburg-Eppendorf

Katharina Flick

University Medical Center Hamburg-Eppendorf

Tabea-Luise Droste

University Medical Center Hamburg-Eppendorf

Anemone Mannes

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

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Abstract

Background:

Oral squamous cell carcinoma (OSCC) involves complex interactions between tumor-associated inflammation and immune regulatory pathways. This study aimed to characterize circulating inflammatory cytokines and tumor necrosis factor (TNF) superfamily members and their contribution to systemic immune dysregulation in OSCC.

Methods:

Serum samples from OSCC patients and healthy controls were analyzed using multiplex immunoassays. Inflammatory cytokines and TNF superfamily markers were compared between groups. Associations were assessed by Spearman correlation analysis. Diagnostic performance was evaluated using receiver operating characteristic (ROC) curves, and multivariable logistic regression assessed associations with nodal metastasis and UICC stage.

Results:

IL-6 and MCP-1 were significantly elevated in OSCC patients, while OPG, TWEAK, and LIGHT were increased among TNF superfamily members. IL-6 correlated with IL-8, IL-17A, TNF- α , IL-1 β , and IP-10, indicating a coordinated inflammatory network. OPG, TWEAK, and LIGHT also showed structured intercorrelations, while IL-6 and MCP-1 were associated with OPG and TWEAK, suggesting cross-talk between inflammatory and TNF-related pathways. ROC analysis showed modest diagnostic performance for IL-6 (AUC 0.631) and MCP-1 (AUC 0.618), whereas OPG reached an AUC of 0.749. A combined IL-6/OPG/LIGHT score showed the best performance (AUC 0.788). Log10-transformed IL-6 was independently associated with nodal metastasis and advanced UICC stage.

Conclusions:

OSCC is associated with coordinated systemic alterations in inflammatory and TNF-related signaling. Integrated biomarker approaches may improve diagnostic performance and provide greater translational relevance than single markers.

Clinical Relevance:

Circulating immune signatures combining inflammatory and TNF-related markers may complement established clinical parameters in OSCC. Especially, multimarker signatures warrant further validation as non-invasive tools for patient stratification.

Introduction

Oral squamous cell carcinoma (OSCC) remains a major global health burden, with prognosis still largely determined by stage at diagnosis (1, 2). Despite advances in treatment, the biological mechanisms underlying systemic tumor-immune interactions are incompletely understood, and clinically applicable markers capturing these processes remain limited.

Cancer-associated inflammation is increasingly recognized as a dynamic and network-driven process rather than a collection of isolated signaling events. Within the tumor microenvironment, cytokines, chemokines, and immune regulatory molecules form interconnected signaling circuits that coordinate tumor progression, immune evasion, and tissue remodeling (3). In OSCC, these interactions extend beyond the local tumor niche and are reflected in the systemic circulation, where soluble mediators may provide insight into tumor-associated immune activity (4, 5).

Among these mediators, inflammatory cytokines such as interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1/CCL2) have been implicated in tumor progression, immune modulation, and myeloid cell recruitment (5–11). In parallel, members of the tumor necrosis factor (TNF) superfamily regulate key processes including immune activation, apoptosis, and stromal interactions (12, 13). Pathways such as the RANKL–osteoprotegerin (OPG) axis and the TWEAK/Fn14 system highlight the functional diversity of this signaling family in cancer biology (14–16). Furthermore, TNF- α itself has been extensively linked to OSCC pathogenesis, acting as a key mediator of inflammation-driven carcinogenesis and tumor progression (17).

However, most studies have examined inflammatory cytokines and TNF superfamily members separately, thereby overlooking their potential integration within a shared systemic immune network. Given the complexity of tumor-associated immune responses, an integrated, network-oriented analysis may provide a more comprehensive understanding of systemic immune dysregulation in OSCC (18, 19).

In this study, we characterize circulating inflammatory cytokines and TNF superfamily members in OSCC and investigate their coordinated relationships and associations with disease burden. We hypothesize that OSCC is defined by structured and interconnected immune signaling patterns rather than isolated biomarker alterations, reflecting a systems-level organization of tumor-associated immune responses.

Material and methods

Study design and patient cohort

This cross-sectional 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 56 OSCC patients were enrolled. Thirty-one 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, smoking status, alcohol consumption, 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 from healthy control subjects at the time of study inclusion.

Blood samples were allowed to clot at room temperature for 30 minutes and subsequently centrifuged at $2,000 \times g$ 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 cytokines and TNF superfamily 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 TNFSF Family Panel 1**, including OPG, APRIL (TNFSF13), TRAIL (TNFSF10), sCD40L (TNFSF5), TWEAK (TNFSF12), LIGHT (TNFSF14), FasL (TNFSF6), RANKL (TNFSF11), TNF- α (TNFSF2), TNF- β (TNFSF1), BAFF (TNFSF20), CD30L (TNFSF8),
- **LEGENDplex™ Human Essential Immune Response Panel**,
- including IL-4, IL-2, CXCL10 (IP-10), IL-1 β , TNF- α , CCL2 (MCP-1), IL-17A, IL-6, IL-10, IFN- γ , IL-12p70, CXCL8 (IL-8), TGF- β 1 (Free Active Form).

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.

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 associations of IL-6, MCP-1, and OPG with nodal status and UICC stage, adjusted for age and sex. Log10-transformed values were applied. 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 56 patients with oral squamous cell carcinoma (OSCC) and 31 healthy controls were included. The OSCC cohort comprised 30 males (53.6%) and 26 females (46.4%), while the control group included 21 males (67.7%) and 10 females (32.3%). Median age was similar between OSCC patients and healthy controls (71 vs. 65 years).

Tumors were most commonly located in the lower jaw (30.4%), followed by the floor of the mouth (17.9%) and tongue (17.9%). Less frequent sites included the upper jaw (12.5%), buccal mucosa (10.7%), palate (5.4%), and lip (5.4%).

Tumor stages were distributed as T1 (32.1%), T2 (19.6%), T3 (16.1%), and T4a (32.1%). The majority of patients were node-negative (N0: 75.0%), while nodal involvement was observed in N1 (3.6%), N2a/b (10.8%), N2c (1.8%), and N3b (7.1%).

Histological grading was G1 (26.8%), G2 (37.5%), G3 (23.2%), and Gx (12.5%). Lymphovascular, vascular, and perineural invasion were absent in 76.8%, 80.4%, and 67.9% of cases, respectively. All patients underwent complete tumor resection (R0: 100%).

Detailed clinicopathological characteristics are provided in Table S1.

Inflammatory cytokines in OSCC patients and healthy controls

Serum levels of MCP-1 and IL-6 were significantly elevated in OSCC patients compared with healthy controls (MCP-1: 928.02 vs. 828.46 pg/mL, $p = 0.049$; IL-6: 226.13 vs. 167.30 pg/mL, $p = 0.043$).

No significant differences were observed for IL-4, IL-2, IP-10, IL-1 β , TNF- α , IL-17A, IL-10, IFN- γ , IL-12p70, IL-8, or free TGF- β 1 (all $p > 0.05$).

TNF superfamily markers in OSCC patients and healthy controls

Among TNF superfamily markers, OPG, TWEAK, and LIGHT were significantly increased in OSCC patients (OPG: 5855.61 vs. 4629.27 pg/mL, $p < 0.001$; TWEAK: 1625.87 vs. 1472.72 pg/mL, $p = 0.007$; LIGHT: 11770.38 vs. 9143.43 pg/mL, $p = 0.009$).

All other markers, including APRIL, TRAIL, sCD40L, FasL, RANKL, BAFF, and CD30L, showed no significant differences (all $p > 0.05$).

Detailed results are provided in Table 1 and Fig. 1.

Correlations between inflammatory cytokines

Spearman correlation analysis revealed a coordinated inflammatory network centered on IL-6. IL-6 showed strong positive correlations with IL-8 ($r = 0.727$, $p < 0.001$), IL-17A ($r = 0.628$, $p < 0.001$), TNF- α ($r = 0.610$, $p < 0.001$), IL-1 β ($r = 0.465$, $p < 0.001$), IP-10 ($r = 0.440$, $p < 0.001$), and MCP-1 ($r = 0.397$, $p < 0.001$).

Additional associations included MCP-1 with IL-8 ($r = 0.395$, $p < 0.001$) and IFN- γ with IL-12p70 ($r = 0.758$, $p < 0.001$).

Correlations within the TNF superfamily

Within the TNF superfamily, structured correlations were observed among key markers. OPG correlated with TWEAK ($r = 0.283$, $p = 0.008$) and LIGHT ($r = 0.323$, $p = 0.002$), while TWEAK correlated with LIGHT (r

= 0.354, $p < 0.001$). In addition, OPG correlated with BAFF ($r = 0.363$, $p < 0.001$), and TRAIL showed a strong association with FasL ($r = 0.556$, $p < 0.001$).

No significant correlation was observed between APRIL and BAFF ($r = 0.146$, $p = 0.178$).

Cross-talk between inflammatory cytokines and TNF superfamily markers

Cross-group analyses revealed interactions between inflammatory and TNF-related pathways. IL-6 correlated with OPG ($r = 0.214$, $p = 0.045$) and TWEAK ($r = 0.273$, $p = 0.011$), while MCP-1 correlated with OPG ($r = 0.287$, $p = 0.007$).

No significant associations were observed between IL-6 and LIGHT, MCP-1 and LIGHT, IP-10 and LIGHT, or IL-8 and OPG (all $p > 0.05$).

Detailed results are provided in Table S2 and Fig. 2.

Heatmap

A heatmap of z-score-normalized circulating inflammatory cytokines and TNF superfamily markers is shown in Fig. 3.

Clustering patterns suggest coordinated regulation among inflammatory cytokines, particularly within IL-6-associated signaling, as well as structured associations within TNF superfamily markers.

Diagnostic performance of inflammatory cytokines

ROC analysis showed that IL-6 (AUC 0.631, 95% CI 0.507–0.755, $p = 0.039$) and MCP-1 (AUC 0.618, 95% CI 0.500–0.735, $p = 0.049$) demonstrated the highest diagnostic performance among inflammatory cytokines, although overall discriminatory accuracy remained modest. All other cytokines showed AUC values below 0.60 and were not statistically significant.

Diagnostic performance of TNF superfamily markers

Among TNF superfamily markers, OPG exhibited the highest diagnostic accuracy (AUC 0.749, 95% CI 0.641–0.858, $p < 0.001$), followed by TWEAK (AUC 0.677, $p = 0.005$) and LIGHT (AUC 0.672, $p = 0.009$). All other markers were not statistically significant.

Diagnostic performance of biomarker scores

Composite biomarker scores improved diagnostic performance compared with individual inflammatory cytokines. The inflammatory score (IL-6 and MCP-1) achieved an AUC of 0.642 (95% CI 0.521–0.762, $p = 0.021$), while the TNFSF score (OPG, TWEAK, LIGHT) showed higher accuracy (AUC 0.748, 95% CI 0.628–0.868, $p < 0.001$).

Notably, the combined score integrating IL-6, OPG, and LIGHT demonstrated the best overall performance (AUC 0.788, 95% CI 0.684–0.892, $p < 0.001$), indicating improved discriminatory capacity of integrated biomarker approaches.

Detailed results are provided in Fig. 4 and Table S3.

Associations between circulating biomarkers and clinicopathological parameters

IL-6 showed consistent associations with disease burden. IL-6 levels differed between sexes ($p = 0.009$) and were positively correlated with nodal stage ($r = 0.353$, $p = 0.008$) and UICC stage ($r = 0.305$, $p = 0.022$). In addition, IL-6 levels were significantly higher in patients with advanced T stage ($p = 0.032$), nodal metastasis ($p = 0.010$), and advanced UICC stage ($p = 0.017$).

MCP-1 differed between sexes ($p = 0.045$) but showed no significant associations with tumor stage, nodal stage, or UICC stage.

Among TNF superfamily markers, OPG correlated with age ($r = 0.456$, $p < 0.001$), while LIGHT showed a weaker association with age ($r = 0.246$, $p = 0.022$). TWEAK was associated with nodal stage ($r = 0.322$, $p = 0.016$) and differed between nodal groups ($p = 0.010$).

Composite biomarker scores were associated with age and sex but showed no significant relationships with tumor stage, nodal stage, or UICC stage. Detailed results are provided in Table 2.

Multivariable logistic regression analysis

In multivariable models, log10-transformed IL-6 remained independently associated with disease burden. IL-6 was significantly associated with nodal metastasis (OR 13.282, 95% CI 1.372–128.565, $p = 0.026$) and advanced UICC stage (OR 9.818, 95% CI 1.684–57.224, $p = 0.011$).

MCP-1, OPG, age, and sex were not independently associated with either outcome.

Detailed regression results are provided in Tables 3 and 4.

Discussion

The present study provides an integrated characterization of circulating inflammatory cytokines and TNF superfamily members in patients with OSCC and demonstrates that systemic immune alterations are characterized by selective pathway activation and coordinated biomarker networks rather than generalized inflammation. These findings support a dual-axis model of systemic immune dysregulation, in which classical inflammatory cytokines and TNF superfamily members contribute distinct yet interconnected signals reflecting tumor-associated immune activity.

Systemic inflammatory network organization in OSCC

A central finding of this study is the identification of IL-6 as a key regulatory hub within the systemic inflammatory network. Beyond its elevation at the group level, IL-6 showed strong associations with multiple inflammatory mediators, including IL-8, IL-17A, TNF- α , IL-1 β , and IP-10, indicating coordinated activation of a broader inflammatory program.

This observation is consistent with the established role of IL-6 as a central regulator of cancer-associated inflammation, linking innate immune activation with tumor progression through pathways such as JAK/STAT3 signaling and downstream transcriptional programs that promote proliferation, survival, and immune modulation (6, 7, 20–23). In this context, IL-6 may act less as an isolated biomarker and more as a systems-level integrator of inflammatory signaling within a networked immune response (24–26).

MCP-1 (CCL2) complements this inflammatory axis by reflecting myeloid cell recruitment and macrophage-associated tumor responses. CCL2-driven recruitment of monocytes and their differentiation into tumor-associated macrophages represents a key mechanism linking inflammation to tumor progression, angiogenesis, and immune suppression. The observed elevation of MCP-1 in our cohort is therefore consistent with its role as a mediator of tumor-promoting myeloid cell dynamics (8, 27, 28).

However, the limited diagnostic performance of individual inflammatory cytokines observed in this study underscores the redundancy and complexity of inflammatory signaling in cancer. These findings support the concept that single cytokines capture only partial aspects of systemic immune activation and highlight the need for integrated, multi-parameter approaches.

TNF superfamily signaling and tumor-microenvironment interactions

In contrast to classical cytokines, members of the TNF superfamily – particularly OPG, TWEAK, and LIGHT – demonstrated distinct alterations in OSCC, indicating the involvement of additional immune regulatory pathways beyond canonical inflammatory signaling.

OPG exhibited the highest diagnostic performance among the analyzed markers. While traditionally associated with bone metabolism (29), the RANKL/OPG axis has emerged as a critical regulator of tumor–bone interactions and stromal remodeling in OSCC. Beyond its local effects, circulating OPG levels may reflect systemic manifestations of tumor-induced alterations in bone turnover and microenvironmental signaling, particularly in the context of osteolytic activity and tumor invasion (14, 15, 30, 31).

The identification of TWEAK further supports the involvement of TNF superfamily signaling in OSCC. The TWEAK/Fn14 axis has been implicated in inflammation, tissue remodeling, and tumor progression, including processes such as angiogenesis and invasive growth at the tumor front. The observed

systemic elevation suggests that this pathway may contribute to both local tumor dynamics and systemic immune modulation (16, 32).

In contrast, the role of LIGHT (TNFSF14) in OSCC remains incompletely defined. As a regulator of T-cell activation and immune-stromal interactions, LIGHT has been implicated in broader cancer-associated immune processes (33). The elevated circulating levels observed in this study suggest that additional TNF superfamily pathways may contribute to OSCC-associated immune dysregulation, representing a potentially novel finding that warrants further mechanistic investigation.

Integrated immune network and pathway cross-talk

Correlation analyses revealed coordinated regulation within both inflammatory and TNF superfamily compartments, as well as selective cross-talk between these systems. Associations between IL-6, MCP-1, and OPG or TWEAK indicate that inflammatory signaling and TNF superfamily pathways may reflect coordinated systemic regulation rather than independent alterations.

These findings support a model in which OSCC induces a multilayered systemic immune response involving both soluble cytokines and receptor–ligand signaling systems with complementary biological functions. Such network organization is consistent with current concepts of tumor biology, in which cancer-associated inflammation is embedded within complex, interconnected signaling networks rather than linear pathways (3, 34).

Association with disease burden

Among all analyzed biomarkers, IL-6 showed the most consistent associations with clinicopathological parameters and remained independently associated with nodal metastasis and advanced UICC stage in multivariable analyses. These findings are in line with previous reports linking IL-6 to tumor progression, metastatic potential, and adverse clinical outcomes in head and neck cancers (7, 35).

In contrast, other biomarkers, including MCP-1 and OPG, did not retain significance in multivariable models, suggesting that their associations may be context-dependent or secondary to broader immune processes (11, 36). This further supports the concept that individual markers reflect specific components of a larger systemic network rather than independent drivers of disease progression.

Clinical implications

From a translational perspective, the present findings indicate that distinct biomarker classes capture complementary aspects of tumor-associated immune responses. The observed network organization argues against single-marker approaches and instead supports the development of integrated, multi-parameter biomarker signatures combining inflammatory cytokines and TNF superfamily members (37). Notably, the superior performance of the combined biomarker score highlights the potential of such integrative approaches to improve diagnostic accuracy.

Beyond diagnostic applications, these findings may have implications for patient stratification and monitoring of disease progression, as network-based biomarkers could more accurately reflect the complexity of systemic immune dysregulation in OSCC.

In addition, signaling pathways such as RANKL/OPG and TWEAK/Fn14 may represent promising targets for therapeutic intervention, particularly in the context of tumor–stroma interactions, immune modulation, and bone-associated disease progression.

Limitations

Several limitations should be considered. The sample size is moderate and may limit statistical power for subgroup analyses. The cross-sectional design precludes conclusions regarding temporal dynamics and causal relationships. In addition, circulating biomarkers reflect systemic immune responses and do not directly represent the tumor microenvironment. Moreover, potential confounding factors such as systemic comorbidities or treatment-related effects could not be fully controlled and may have influenced circulating biomarker levels.

Conclusion

In conclusion, OSCC is characterized by coordinated reorganization of systemic immune networks linking inflammatory cytokines and TNF superfamily signaling. These findings support a shift from single-marker analysis toward network-based biomarker strategies in translational oncology.

Table 1

Circulating inflammatory cytokines and TNF superfamily markers in OSCC patients and healthy controls

Inflammatory cytokines	Biomarker	OSCC patients (in pg/mL)	Healthy controls (in pg/mL)	p-value
	IL-4	74.29 (35.94-197.02)	158.26 (85.61–287.90)	0.177
	IL-2	35.08 (24.67–60.39)	40.95 (22.35–56.85)	0.594
	IP-10	696.30 (565.36-980.61)	726.73 (464.88-850.33)	0.317
	IL-1β	148.95 (81.35-327.16)	224.87 (128.39-299.79)	0.514
	TNF-α	66.90 (26.75–134.10)	85.35 (25.01-192.55)	0.474
	MCP-1	928.02 (702.92-1151.49)	828.46 (647.04-963.43)	0.049*
	IL-17A	80.70 (46.02-188.13)	83.10 (39.71-200.37)	0.972
	IL-6	226.130 (87.18-379.49)	167.30 (59.70–391.00)	0.043*
	IL-10	69.95 (41.54-118.74)	69.49 (21.15-116.27)	0.343
	IFN-γ	71.96 (19.81-200.09)	118.47 (49.83-225.48)	0.581
	IL-12p70	115.10 (38.79–258.10)	159.39 (35.68-291.64)	0.471
	IL-8	249.96 (62.34-404.22)	137.40 (74.49-411.07)	0.265
	Free TGF-β1	836.67 (413.76-1480.58)	875.62 (464.70-1284.65)	0.986
TNF superfamily markers				
	OPG	5855.61 (4958.24-7884.09)	4629.27 (4017.90-5963.39)	< 0.001*
	APRIL	9271.52 (8329.23-10730.78)	9055.54 (8509.60-10239.06)	0.515
	TRAIL	589.06 (403.97-772.21)	648.75 (460.51-917.85)	0.915

sCD40L	15116.58 (12801.47-17613.28)	14703.33 (10516.92-18079.15)	0.750
TWEAK	1625.87 (1464.10-1951.10)	1472.72 (1268.24-1772.57)	0.007*
LIGHT	11770.38 (9685.38-13181.39)	9143.43 (5977.82-12832.34)	0.009*
FasL	1050.16 (907.44-1151.76)	1086.93 (869.55-1258.36)	0.803
RANKL	1055.76 (483.14-2162.32)	803.80 (600.39-1415.82)	0.369
BAFF	1960.66 (1514.14-2760.19)	1933.36 (1393.68-2283.56)	0.116
CD30L	19416.70 (15791.87-24099.00)	16104.96 (13608.27-23694.10)	0.086

Comparison of circulating inflammatory cytokines and TNF superfamily markers between patients with oral squamous cell carcinoma (OSCC) and healthy controls. Values are presented as median (IQR). Comparisons were performed using the Mann-Whitney U test. P-values < 0.05 were considered statistically significant. Statistically significant differences are marked with an asterisk.

Table 2
Associations between circulating biomarkers and clinicopathological parameters in OSCC

Biomarker	Age ¹	Sex ²	T stage ¹	T stage ²	N stage ¹	N stage ²	UICC stage ¹	UICC stage ²
IL-6	r = 0.159 p = 0.142	U = 614 p = 0.009*	r = 0.242 p = 0.073	U = 261 p = 0.032*	r = 0.353 p = 0.008*	U = 157 p = 0.010*	r = 0.305 p = 0.022*	U = 245 p = 0.017*
MCP-1	r = 0.204 p = 0.058	U = 685 p = 0.045*	r = -0.059 p = 0.664	U = 385 p = 0.915	r = 0.104 p = 0.444	U = 257 p = 0.484	r = -0.004 p = 0.978	U = 376 p = 0.818
OPG	r = 0.456 p < 0.001*	U = 743 p = 0.132	r = -0.022 p = 0.874	U = 389 p = 0.967	r = -0.073 p = 0.592	U = 277 p = 0.748	r = -0.063 p = 0.644	U = 386 p = 0.948
TWEAK	r = 0.081 p = 0.456	U = 784 p = 0.312	r = 0.012 p = 0.929	U = 371 p = 0.737	r = 0.322 p = 0.016*	U = 158 p = 0.010*	r = 0.101 p = 0.459	U = 334 p = 0.362
LIGHT	r = 0.246 p = 0.022*	U = 843 p = 0.618	r = -0.008 p = 0.954	U = 358 p = 0.583	r = 0.010 p = 0.944	U = 281 p = 0.813	r = -0.044 p = 0.745	U = 390 p = 1.00
Inflammatory score	r = 0.188 p = 0.081	U = 620 p = 0.010*	r = 0.044 p = 0.750	U = 345 p = 0.446	r = 0.202 p = 0.136	U = 210 p = 0.112	r = 0.114 p = 0.404	U = 337 p = 0.385
TNFSF score	r = 0.301 p = 0.005*	U = 677 p = 0.077	r = 0.028 p = 0.839	U = 368 p = 0.700	r = 0.221 p = 0.102	U = 202 p = 0.082	r = 0.043 p = 0.754	U = 336 p = 0.375
Combined score	r = 0.404 p < 0.001*	U = 560 p = 0.005*	r = 0.176 p = 0.195	U = 296 p = 0.117	r = 0.218 p = 0.106	U = 203 p = 0.085	r = 0.181 p = 0.182	U = 283 p = 0.079

¹ = Spearman, ² = Mann-Whitney U test

Associations between circulating biomarkers and clinicopathological parameters in patients with oral squamous cell carcinoma (OSCC). Continuous variables (age, T stage, N stage, UICC stage) were analyzed using Spearman's rank correlation (r), while group comparisons (sex and dichotomized stages) were performed using the Mann-Whitney U test. P-values < 0.05 were considered statistically significant. Statistically significant differences are marked with an asterisk.

Table 3
Multivariable logistic regression analysis for association of circulating biomarkers with nodal metastasis in OSCC

Variable	OR	95% CI	p-value
Log10 IL-6	13.282	1.372-128.565	0.026*
Log10 MCP-1	0.818	0.009-74.416	0.931
Log10 OPG	0.243	0.004-14.459	0.497
Age	0.961	0.892-1.036	0.298
Sex	0.950	0.197-4.572	0.949

Multivariable logistic regression analysis of log10-transformed biomarkers associated with nodal metastasis in OSCC, adjusted for age and sex. Results are presented as odds ratios (ORs) with 95% confidence intervals (CIs). A p-value < 0.05 was considered statistically significant. Statistically significant differences are marked with an asterisk.

Table 4
Multivariable logistic regression analysis for association of circulating biomarkers with advanced UICC stage in OSCC

Variable	OR	95% CI	p-value
Log10 IL-6	9.818	1.684-57.224	0.011*
Log10 MCP-1	0.054	0.001-3.072	0.157
Log10 OPG	0.280	0.006-12.608	0.512
Age	0.997	0.938-1.061	0.934
Sex	0.939	0.246-3.581	0.927

Multivariable logistic regression analysis of log10-transformed biomarkers associated with advanced UICC stage in OSCC, adjusted for age and sex. Results are presented as odds ratios (ORs) with 95% confidence intervals (CIs). A p-value < 0.05 was considered statistically significant. Statistically significant differences are marked with an asterisk.

Abbreviations

APRIL

A Proliferation–Inducing Ligand

AUC

Area Under the Curve

BAFF

B–cell Activating Factor

CI

Confidence Interval

Fn14

Fibroblast Growth Factor–Inducible 14

IFN

Interferon

IL

Interleukin

IP

10–Interferon Gamma–Induced Protein 10

IQR

Interquartile Range

LIGHT

Lymphotoxin–like, Exhibits Inducible Expression, and Competes with HSV Glycoprotein D for HVEM, a Receptor Expressed by T Lymphocytes

MCP

1–Monocyte Chemoattractant Protein 1

OPG

Osteoprotegerin

OR

Odds Ratio

OSCC

Oral Squamous Cell Carcinoma

RANKL

Receptor Activator of Nuclear Factor κ B Ligand

ROC

Receiver Operating Characteristic

sCD40L

soluble CD40 Ligand

TGF

Transforming Growth Factor

TNF

Tumor Necrosis Factor

TNFSF

Tumor Necrosis Factor Superfamily

TRAIL

TNF–Related Apoptosis–Inducing Ligand

TWEAK

TNF–like Weak Inducer of Apoptosis

UICC

Union for International Cancer Control

Declarations

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 (Ethics approval number: 2025-101539-BO-ff). Written informed consent was obtained from all subjects involved in the study.

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Author Contribution

Ann-Kristin Struckmeier conceived and supervised the study, performed the statistical analyses, was responsible for project administration and funding acquisition, and drafted the original manuscript. Miriam Kokal-Ribaudo contributed to patient recruitment and sample collection and substantially contributed to manuscript drafting. Juliana Sufcak, Niclas Rénevier, and Xiangyuan Wu contributed to patient recruitment and sample collection. Katharina Flick, Tabea-Luise Droste, Anemone Mannes, Tobias Vollkommer and Ralf Smeets contributed to critical review of the manuscript. All authors contributed to the interpretation of the findings, critically revised the manuscript, and read and approved the final version.

Data availability:

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

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Figures

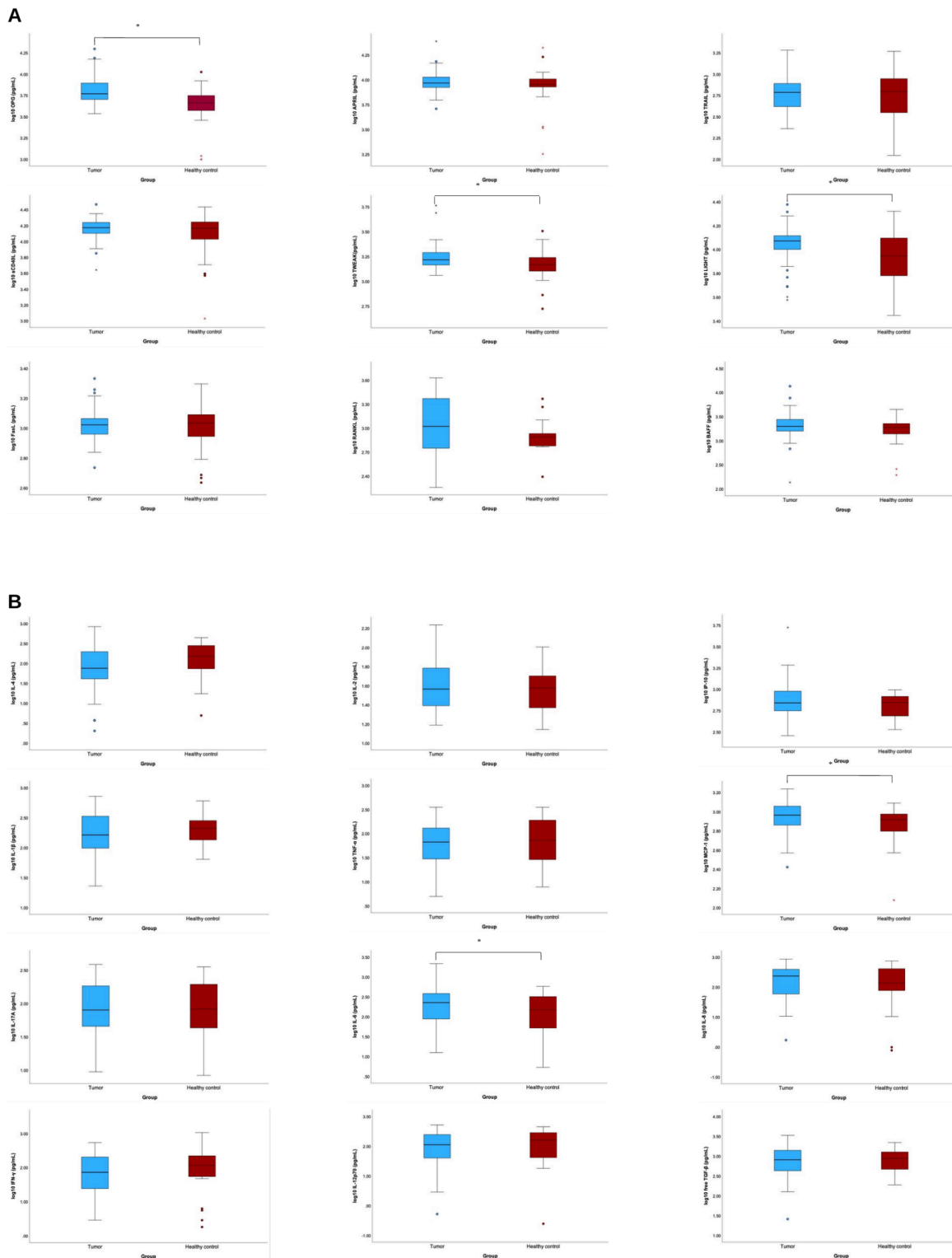


Figure 1

Serum levels of circulating inflammatory cytokines and TNF superfamily markers in patients with oral squamous cell carcinoma (OSCC) and healthy controls.

Boxplots display log10-transformed biomarker concentrations. Boxes represent the interquartile range (IQR) with the median indicated by the horizontal line; whiskers denote the range excluding outliers, and

individual points represent outliers. Statistical comparisons were performed using the Mann-Whitney U test.

(A) Inflammatory cytokines. MCP-1 and IL-6 were significantly elevated in OSCC patients compared with healthy controls, whereas all other cytokines showed no significant differences.

(B) TNF superfamily markers. OPG, TWEAK, and LIGHT were significantly increased in OSCC patients, while the remaining markers showed no significant differences.

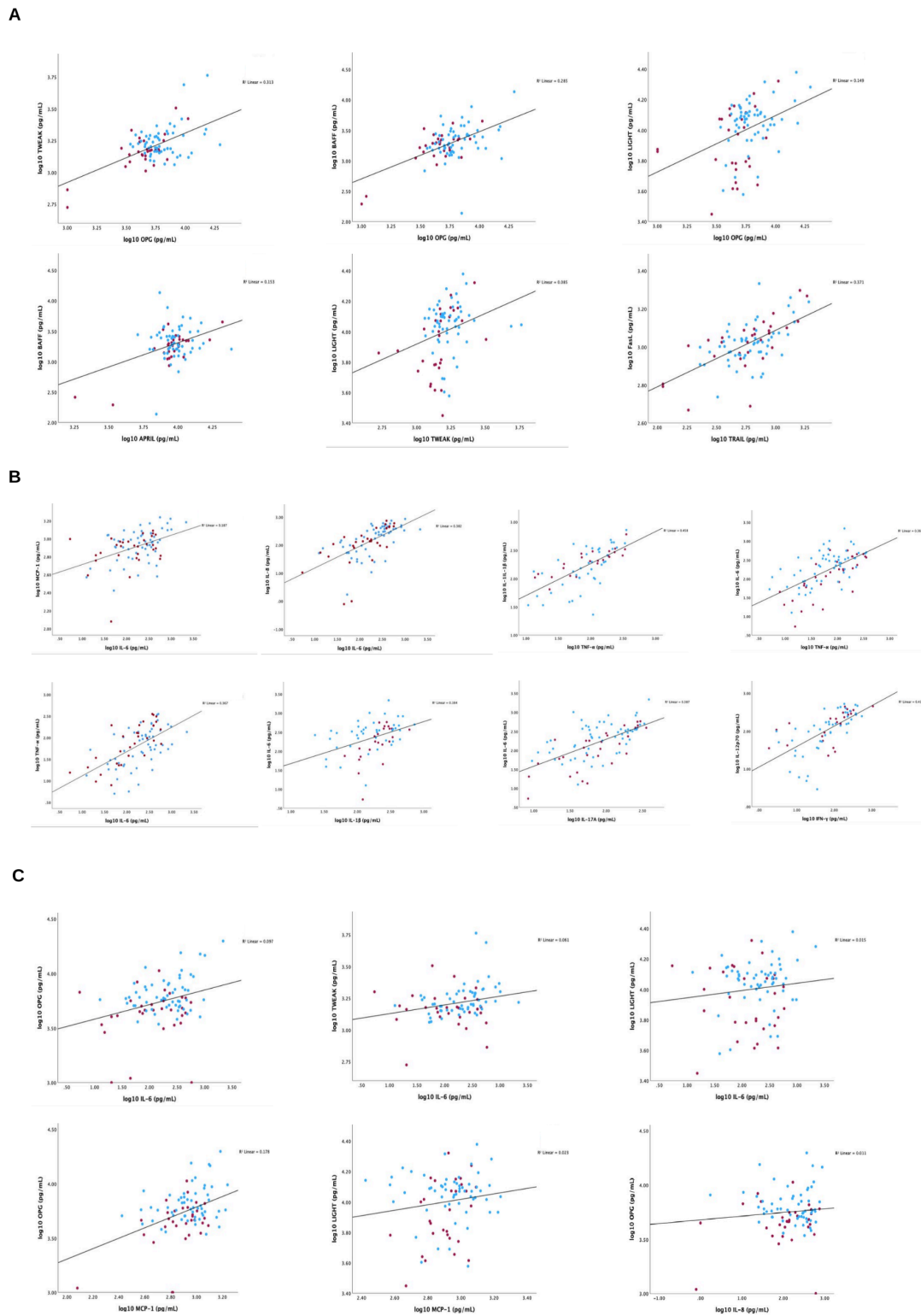


Figure 2

Correlation analysis of circulating inflammatory cytokines and TNF superfamily markers in serum samples from patients with oral squamous cell carcinoma (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 TNF superfamily markers, demonstrating positive associations between OPG, TWEAK, LIGHT, and BAFF, consistent with coordinated regulation within TNF superfamily signaling.

(B)Correlations among inflammatory cytokines, with IL-6 showing positive associations with MCP-1, IL-8, IP-10, TNF- α , IL-1 β , and IL-17A. In addition, a strong positive correlation was observed between IFN- γ and IL-12p70, supporting coordinated regulation within the inflammatory cytokine network.

(C) Cross-group correlations between inflammatory cytokines and TNF superfamily markers, including associations of IL-6 with OPG and TWEAK and of MCP-1 with OPG, suggesting coordinated associations between inflammatory and TNF superfamily mediators.

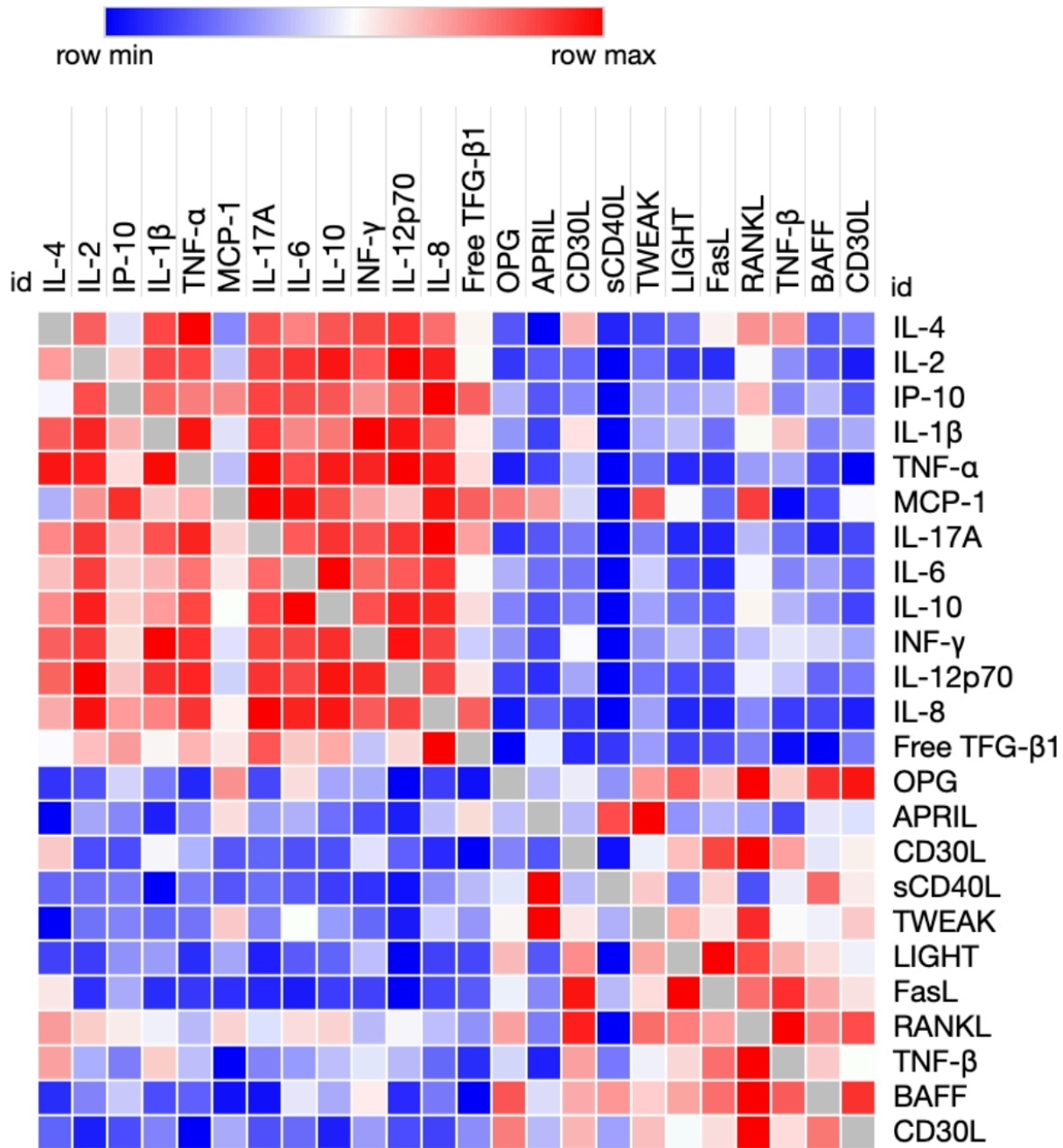


Figure 3

Heatmap of circulating immune mediator profiles in OSCC patients and healthy controls

Heatmap of z-score-normalized circulating inflammatory cytokines and TNF superfamily markers in OSCC patients and healthy controls, illustrating distinct patterns of systemic immune dysregulation. Red indicates higher and blue lower relative expression levels.

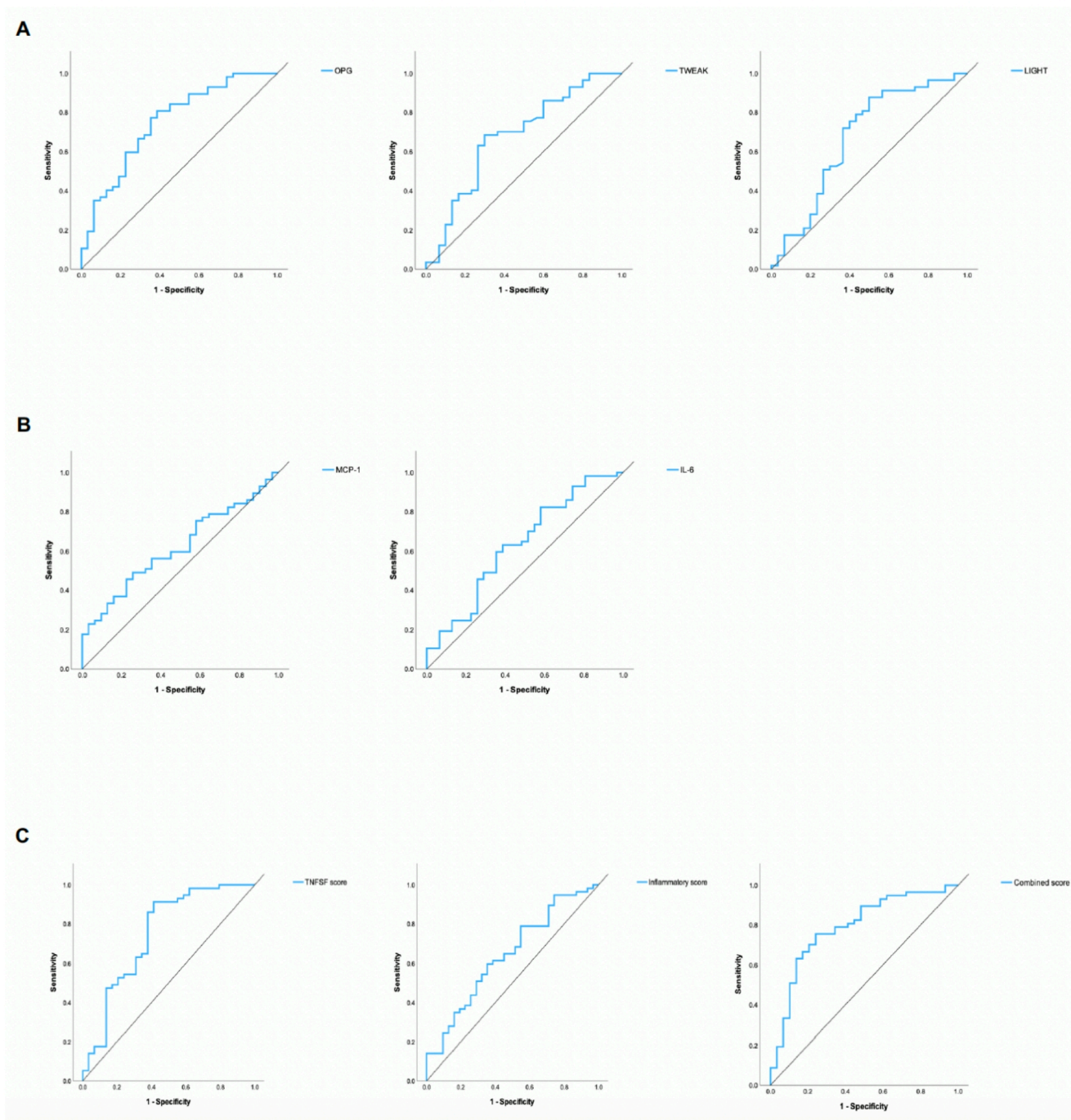


Figure 4

Receiver operating characteristic (ROC) curve analysis evaluating the diagnostic performance of circulating biomarkers for distinguishing patients with oral squamous cell carcinoma (OSCC) from healthy controls.

Sensitivity is plotted against 1–specificity, and the diagonal line represents a non-informative classifier (AUC = 0.5).

(A) TNF superfamily markers with AUC > 0.60 (OPG, TWEAK, LIGHT). OPG showed the highest diagnostic accuracy among TNF-related biomarkers.

(B) Inflammatory cytokines with AUC > 0.60 (MCP-1, IL-6).

(C) Composite biomarker scores, including the TNFSF score, inflammatory score, and a combined score. The combined score demonstrated the highest discriminatory performance.

Supplementary Files

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