Eur Arch Otorhinolaryngol (2016) 273:3371–3384
DOI 10.1007/s00405-016-3950-4

HEAD AND NECK

Standardized pretreatment inflammatory laboratory markers and calculated ratios in patients with oral squamous cell carcinoma

Martin Grimm[1] · Johan Rieth[1] · Sebastian Hoefert[1] · Michael Krimmel[1] · Sven Rieth[1] · Peter Teriete[2] · Susanne Kluba[1] · Thorsten Biegner[3] · Adelheid Munz[1] · Siegmar Reinert[1]

Received: 17 December 2015 / Accepted: 22 February 2016 / Published online: 29 February 2016

Abstract Analyzing the inflammatory microenvironment has become an important issue in the management of oral squamous cell carcinoma (OSCC). Pretreatment C-reactive protein (CRP) levels, leucocytes, monocytes, lymphocytes, neutrophils, basophils, eosinophils, platelets, neutrophil-to-lymphocyte ratio (NLR), derived NLR (dNLR), lymphocyte-to-monocyte ratio (LMR), and platelet-to-lymphocyte ratio (PLR) derived from the peripheral blood were analyzed. Receiver operating characteristic (ROC) curves determined a cut-off value for each parameter in 146 patients with OSCC compared with 93 controls and the results were associated with clinicopathological characteristics. CRP expression of tumors was measured by immunohistochemistry. ROC analysis determined cut-off values for CRP levels, leucocytes, monocytes, lymphocytes, neutrophils, NLR, dNLR, LMR, PLR and showed significant differences between the OSCC and control group. Compared with single laboratory tests calculated ratios were superior in measuring sensitivity and specificity of OSCC disease. NLR was significant directly associated and correlated with PLR. LMR was significant inversely associated and correlated with NLR and PLR. Immunohistochemical analysis did not show CRP expression of OSCCs. This study highlights the first analysis for cut-off values of pretreatment single laboratory tests and calculated ratios, which are strongly needed for a follow-up of cancer patients. Additionally, the calculated baselines can be used as a goal for successful immunotherapies in the future. The links between NLR, LMR, and PLR might be helpful for the clinical course (monitoring) of cancer patients and have been first described for OSCC in this study. Taken together, analyzing these data provides an additional practical guideline of further postoperative OSCC management.

Keywords Oral squamous cell carcinoma · NLR · DNLR · LMR · PLR

Abbreviations

OSCC	Oral squamous cell carcinoma
CP	Clinicopathological parameters
CRP	C-reactive protein
WBCs	White blood cells
ROC	Receiver operating characteristic
NLR	Neutrophil-to-lymphocyte ratio
dNLR	Derived NLR
LMR	Lymphocyte-to-monocyte ratio
PLR	Platelet-to-lymphocyte ratio

✉ Martin Grimm
dr.dr.martingrimm@googlemail.com

[1] Department of Oral and Maxillofacial Surgery, University Hospital Tuebingen, Osianderstrasse 2-8, 72076 Tuebingen, Germany

[2] Cancer Research Center, Sanford-Burnham Medical Research Institute, 10901 North Torrey Pines Road, La Jolla, CA 92037, USA

[3] Department of Pathology, University Hospital Tuebingen, Liebermeisterstrasse 8, 72076 Tuebingen, Germany

Introduction

Traditional prognostic factors have been well established for prognosis in oral squamous cell carcinoma (OSCC) [1]. Beside advanced International Union Against Cancer (UICC) stages of the disease [2] one of the most important predictors of patient survival rates is lymph node

metastasises [3–5]. Other prognostic factors have also been proposed in the literature. Hence, analyzing well-established clinicopathological parameters in association with other prognostic parameters concerning the inflammatory microenvironment has become an important issue in the management of OSCC [6–11].

The link between inflammation and the development of cancer is known since 1863 as Virchow hypothesized that the origin of cancer occurs at sites of chronic inflammation, in part based on his hypothesis that some classes of irritants, together with the tissue injury and ensuing inflammation they cause, enhance cell proliferation [7]. Following, the importance of molecular and cellular pathways linking cancer and inflammation has been clearly demonstrated [7–13]. Based on the assumption that the processes underlying such a response plays important roles in the progression of OSCC in the presence of a systemic inflammatory response has been thought to indicate poor prognosis in OSCC [10, 11].

In the pathogenesis of OSCC pretreatment measurement of elevated inflammatory serum C-reactive protein (CRP) levels [11, 14] and increased leucocytes/white blood cell count (WBC) [11, 15] are associated with adverse prognosis in OSCC. In addition, evidence for the use of other hematologic markers of inflammation as predictors of clinical outcome in OSCC is available. In this context pretreatment monocytes [16, 17], lymphocytes [17–19], neutrophils [17, 20, 21], basophils [22], eosinophils [22–25], platelets [26–28], neutrophil-to-lymphocyte ratio (NLR) [14, 29–40], derived NLR (dNLR) [36], lymphocyte-to-monocyte ratio (LMR) [19, 36, 41, 42], and platelet-to-lymphocyte ratio (PLR) [30, 32, 33, 35, 36, 43, 44] derived from the peripheral blood emerged as simple, easily accessible, and cost-effective tool screening cancer diseases. Moreover, the characterization of these novel markers is easily reproducible and they have the potential to identify patients at high risk for disease recurrence and tumor conditional death.

Concerning this survey the following questionnaires should be addressed: first, no data regarding basophils, eosinophils, dNLR, LMR, and PLR are disposable for OSCC to date. Second, cut-off values for all pretreatment single laboratory tests and calculated ratios have not been determined for OSCCs compared with healthy individuals as yet, which are strongly needed for a follow-up (monitoring [35, 45]) of cancer patients. Third, we analyzed CRP expression in tumor tissues as a potential source of serum CRP elevation that may identify a more aggressive biological phenotype of the disease [11]. Taken together, analyzing these data might be helpful for providing a practical guideline of further postoperative OSCC management.

Given this background the purpose of this study was to examine an extension of clinicopathological parameters for prognosis, follow-up (monitoring [35, 45]), and treatment of patients with OSCC compared with controls based on routine pretreatment single laboratory tests and calculated ratios that have been not determined as yet.

Materials and methods

Patients, blood samples, and tumor specimen

Written informed consent to participate was obtained from all patients (Ethics Committee Tuebingen, Germany, approval number: 562-2013BO2). We retrospectively reviewed the records of 146 patients with histopathological confirmed OSCC in our department between 2011 and 2015 and 93 healthy controls. Patients with preoperative antineoplastic therapies (chemoradiation) were excluded from the study. Further selective criteria for the patients were: complete clinical and laboratory data, no evidence of sepsis, no hematological disorders or treatment that may influence laboratory parameters, no autoimmune disease or treatment with steroids [36]. Tumor and patient characteristics are summarized in Table 1. The material was archival formalin-fixed, paraffin-embedded (FFPE) tissue from routine histopathologic work-up and had been performed under standardized conditions.

Blood samples (2.7 ml) were collected in ethylenediaminetetraacetic acid (EDTA) treated vials prior to surgery or palliative treatment. The blood samples were blinded to the clinical data and analyzed by standard laboratory techniques to determine pretreatment CRP value, leucocyte/WBC count, monocyte count, lymphocyte count, neutrophil count, basophil count, eosinophil count, and platelet count. Moreover, pretreatment NLR (quotient of neutrophil count to lymphocyte count), dNLR (quotient of WBC count–neutrophil count to lymphocyte count), LMR (quotient of lymphocyte count to monocyte count), and PLR (quotient of platelet count to lymphocyte count) were calculated from peripheral blood cell count.

For immunohistochemistry of CRP staining, the records of healthy individuals (normal oral mucosal tissues, $n = 14$), patients with oral precursor lesions (simple hyperplasia, $n = 21$; squamous intraepithelial neoplasia SIN I, $n = 5$; SIN II, $n = 9$; SIN III, severe dysplasia, $n = 10$; SIN III, carcinoma in situ, $n = 11$), and patients with invasive OSCC ($n = 46/146$) were retrospectively assessed if available and the patient agreed to tissue investigation [46]. The diagnosis of normal oral mucosal tissues, precursor lesions, and invasive squamous cell carcinoma was confirmed by the Department of Pathology, University Hospital Tuebingen. Tumor blocks of FFPE tissue were selected by experienced pathologists, based on routine H&E stained sections. Oral precursor lesions were classified according to WHO criteria [47]. Tumor staging

Table 1 Clinicopathological characteristics and prognostic factors in patients ($n = 146$) with OSCC

Characteristics	Number of patients
Age (years)	
<64	74 (50.7 %)
≥64	72 (49.3 %)
Gender	
Female	48 (32.9 %)
Male	98 (67.1 %)
Site distribution of OSCC	
Tongue	56 (38.4 %)
Floor of the mouth	29 (19.9 %)
Palate	10 (6.8 %)
Buccal mucosa	5 (3.4 %)
Alveolar ridge	31 (21.2 %)
Sections overlapping	15 (10.3 %)
Histological grading	
G1	22 (15.1 %)
G2	95 (65.1 %)
G3	28 (19.2 %)
G4	1 (0.7 %)
Tumor size	
pT1	45 (41.7 %)
pT2	31 (28.7 %)
pT3	7 (6.5 %)
pT4	25 (23.1 %)
Cervical lymph node metastasis	
pN0	76 (70.4 %)
pN1–3	32 (29.6 %)
Tumor size	
cT1	5 (13.2 %)
cT2	9 (23.7 %)
cT3	5 (13.2 %)
cT4	19 (50.0 %)
Cervical lymph node metastasis	
cN0	16 (42.1 %)
cN1–3	22 (57.9 %)
Distant metastasis	
cM0	144 (98.6 %)
cM1	2 (1.4 %)
UICC stage	
UICC I	42 (28.8 %)
UICC II	24 (16.4 %)
UICC III	17 (11.6 %)
UICC IV	63 (43.2 %)

G grading, *UICC* International Union against Cancer

was performed according to the 7th edition of the TNM staging system by the UICC/AJCC of 2010 [48]. Grading of OSCC was defined according to WHO criteria [49].

Staining procedure and quantification of immunohistochemistry

We stained for CRP (LSBio, Eching, Germany, mouse mAb, LS-B7859, dilution 1:500, 5 µg/ml) in tissue sections. Staining was performed on serial sections of 2 µm thickness, which were deparaffinized in xylene and ethanol and rehydrated in water. Heat induced epitope retrieval (HIER) was performed with citrate buffer pH 6.0 (Dako, Hamburg, Germany). Endogenous peroxidase activity was quenched with 0.3 % hydrogen peroxide. Endogenous biotin activity was blocked using the streptavidin/biotin blocking kit (Vector Laboratories, Burlingame, CA, USA). After incubation with the primary or mouse control antibody (BD Pharmingen, Heidelberg, Germany [50]) the Dako LSAB2 peroxidase System (Dako, Hamburg) was used. Slides were subsequently incubated for 3–5 min in DAB (3,3′-diaminobenzidine, Biogenex) counterstained with haemalaun and mounted with Glycergel (Dako).

Five representative high power fields (1 HPF = 0.237 mm^2, original magnification: 200×-fold) were analyzed for CRP expression in normal tissue, oral precursor lesions, tumor tissue and averaged, respectively. The extent of the staining, defined as the percentage of positive staining areas of tumor cells in relation to the whole tissue area, was semi-quantitatively scored on a scale of 0–3 as the following: 0, <10 %; 1, 10–30 %; 2, 30–60 %; 3, >60 %. The intensities of the signals were scored as 1+, 2+, and 3+. Then, a combined score (0–9) for each specimen was calculated by multiplying the values of these two categories [51]. Cases were classified as negative, 0 points, or positive, 1–9 points. Liver tissues were used as a representative positive controls. Two observers blinded to the diagnosis performed scoring on identical sections marked by circling with a water-resistant pencil and finally with diamond-tipped pencil on the opposite side of the microscopic slide. Pictures were analyzed using a Canon camera (Krefeld, Germany). The photographed images were imported into the Microsoft Office Picture Manager.

Statistical analysis

Statistical analysis was performed with MedCalc Statistical Software version 15.11.0 (MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2015).

Receiver operating characteristic curves (ROC) [11] were plotted to determine the best cut-off ranges with relevant sensitivities and specificities for OSCC group compared with controls screening for each single value or ratio [52]. Area under the curve (AUC) analysis was determined for quality measurement of the classifier. The

cut-off point was determined as the value corresponding with the highest diagnostic average of sensitivity and specificity (highest diagnostic accuracy/Youden's index). These values were graphically displayed in an interactive dot diagram to study the accuracy of each diagnostic test. Based on resulting sensitivity and specificity the likelihood ratios (LRs) were calculated +LR = sensitivity/(1 − specificity) and −LR = (1 − sensitivity)/specificity. LRs were used to assess how good the single values and ratios are to determine OSCC disease. LR is the ratio between the probability of a positive test (positive likelihood ratio, +LR) or a negative test (negative likelihood ratio, −LR) result given the presence of OSCC disease and the probability of a positive or negative test result given the absence of OSCC disease.

For all data, the Kolmogorov–Smirnov test was applied to test for a normal distribution. Chi square test (χ^2) and Fisher's exact test were used to investigate the relation between two categorical variables. Data were analyzed using the Student's t test when means of two groups were compared. Correlation between two variables was judged by the Pearson's correlation (Rr) coefficient test. The trend lines of the correlation analysis were displayed in a scatter diagram and plotted by local regression smoothing (LOESS). Regression analysis was used to describe the relationship between two variables and to predict one variable from another. The coefficient of determination R^2 is the proportion of the variation in the dependent variable explained by the regression model. It can range from 0 to 1 and is a measure of the goodness of fit of the model.

Mean values, median values, and ROC analysis results were given with 95 % confidence intervals (CI). All p values presented were two-sided and $p < 0.05$ was considered statistically significant.

Results

Evaluation of pretreatment single laboratory tests and calculated ratios for screening OSCC disease compared with controls

ROC analysis determined cut-off values for single laboratory tests and calculated ratios in OSCC compared with controls. A significant cut-off value has been determined for CRP value, WBC count, monocyte count, lymphocyte count, neutrophil count, NLR, dNLR, LMR, and PLR. The cut-off points that gave the best sensitivity and specificity for the diagnosis of OSCC were evaluated using AUC analysis (Fig. 1). Additionally, values were graphically displayed in an Interactive dot diagram to show and to control the highest diagnostic accuracy of each diagnostic test (Fig. 2). A comparison of variables, calculated sensitivities and specificities between the parameters is presented in Table 2.

Table 3 shows mean values of pretreatment single laboratory tests and calculated ratios for screening of OSCC disease compared with controls.

Association of clinicopathological characteristics with single laboratory tests and calculated ratios in patients with OSCC

Older patients (≥64 years) were significantly associated with decreased lymphocyte count (≤1.41 × 10^3/µl; $p = 0.0029$) and increased NLR (>2.68; $p = 0.0063$).

Males had a significantly increased monocyte count (>0.42 × 10^3/µl; $p = 0.0026$), NLR (>2.68; $p = 0.0049$), dNLR (>1.99; $p = 0.0251$), and a decreased LMR (≤4.1; $p = 0.0009$).

Patients with alveolar ridge carcinoma and section overlapping tumors had a significantly decreased lymphocyte count (≤1.41 × 10^3/µl; $p = 0.0085$) compared with patients with carcinoma of the palate and floor of the mouth.

Advanced tumor stages UICC III/IV were significantly associated with increased WBC count (>8.5 × 10^3/µl; $p = 0.0370$) and neutrophil count (>5.36 × 10^3/µl; $p = 0.0008$).

Nicotine abuse was significantly associated with males ($p = 0.0001$), increased WBC count (>8.5 × 10^3/µl; $p < 0.0001$), neutrophil count (>5.36 × 10^3/µl; $p = 0.0008$), alcohol intake ($p = 0.0001$), and weakly significant associated with increased monocyte count (>0.42 × 10^3/µl; $p = 0.0710$), NLR (>2.68; $p = 0.0795$), and decreased LMR (≤4.1; $p = 0.0970$) but not with elevated CRP values (>1.5 mg/l; $p = 0.1943$).

Alcohol abuse was significantly associated with cervical lymph node metastasis pN+ ($p = 0.0206$), advanced tumor stages UICC III/IV ($p = 0.0388$), and weakly significant associated with advanced tumor size pT3/4 ($p = 0.0826$) but not with elevated CRP values (>1.5 mg/l; $p = 0.3359$).

Synchronous nicotine and alcohol abuse were significantly associated with males ($p = 0.0298$), advanced tumor size pT3/4 ($p = 0.0258$), advanced tumor stages UICC III/IV ($p = 0.0214$), weakly significant associated with advanced grading (G3/4 = 0.0997), and cervical lymph node metastasis pN+ ($p = 0.0732$).

To assess a trend of clinicopathological characteristics with single laboratory tests and calculated ratios weak associations ($p = 0.05$–0.1) were further described as the following: advanced grading (G3/4) was weakly associated with increased WBC count (>8.5 × 10^3/µl; $p = 0.0997$) and advanced tumor stages UICC III/IV have been weakly associated with increased monocyte count (>0.42 ×

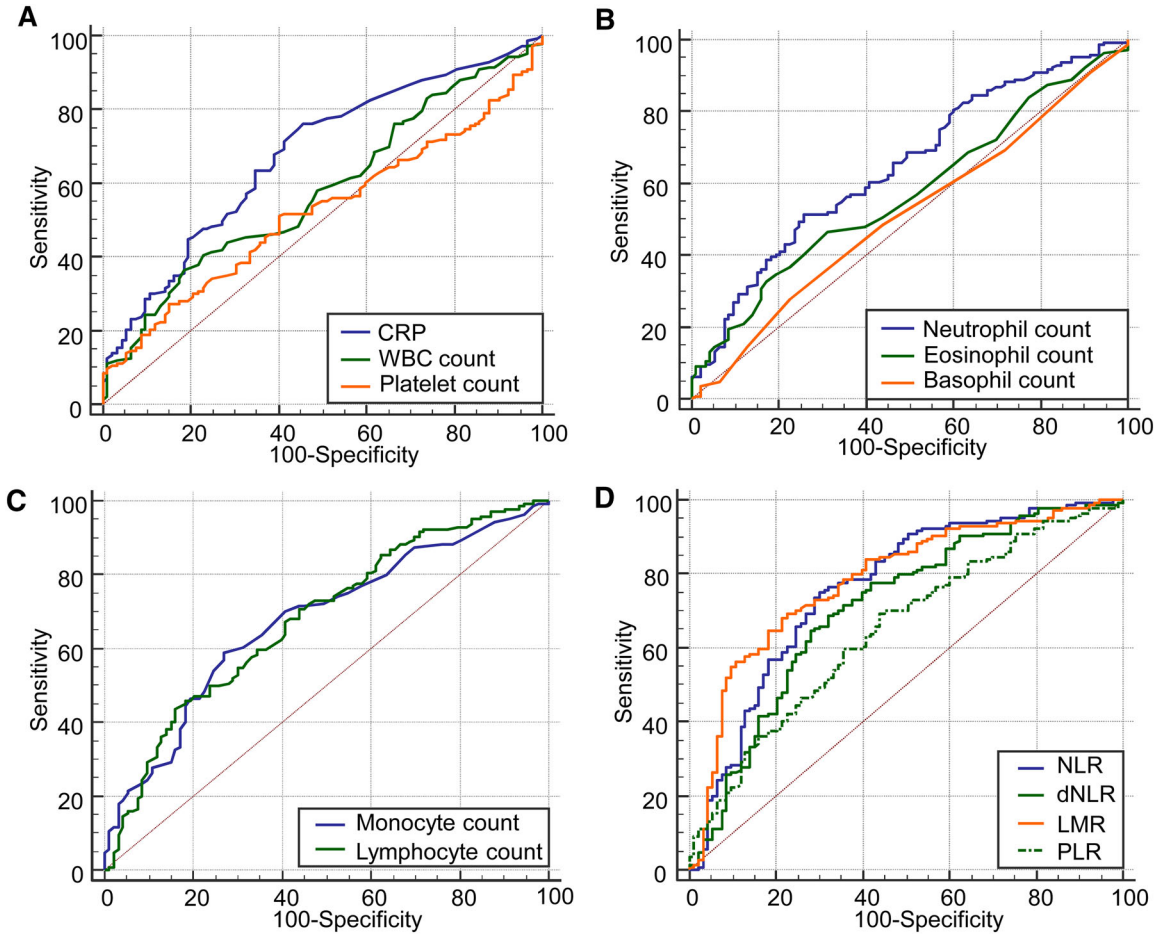

Fig. 1 Receiver operating characteristic curves (ROC) of single laboratory tests (CRP, WBC count, platelet count **a**; neutrophil count, basophil count, eosinophil count **b**; monocyte count and lymphocyte count **c**) and calculated ratios (NLR, dNLR, LMR, PLR, **d**). The true positive rates (sensitivity) are plotted in function of the false positive rate (100-specificity) analyzing different cut-off points with highest diagnostic accuracy to distinguish controls ($n = 93$) from OSCC patients ($n = 146$). The corresponding test characteristics sensitivity and specificity are given in Table 2

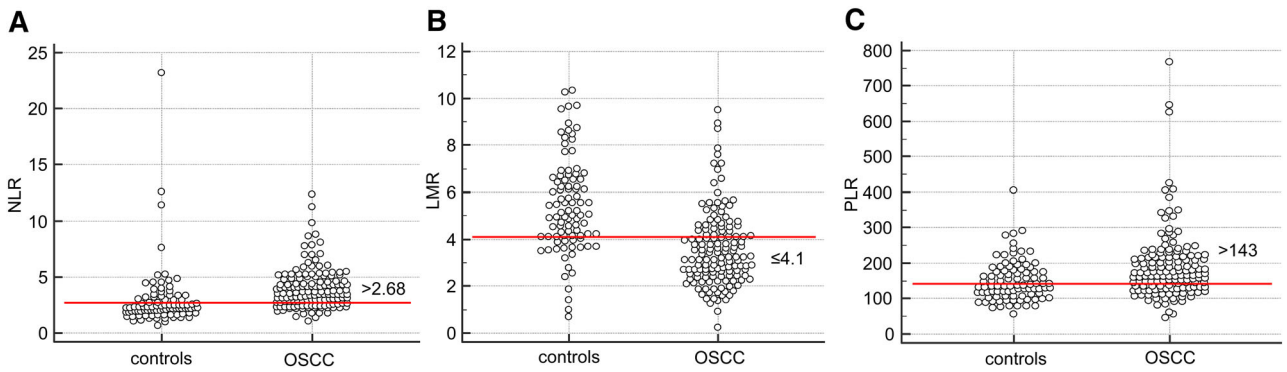

Fig. 2 Interactive dot diagrams (part of ROC curve analysis, Fig. 1) of calculated ratios (NLR **a**, LMR **b**, PLR, **c**) of controls ($n = 93$) and OSCC group ($n = 146$) are displayed as *dots* on *two vertical axes*. The *red horizontal lines* indicate the cut-off points with the best separation/highest accuracy (minimal false negative and false positive results) between controls and OSCC group. The corresponding test characteristics sensitivity and specificity are given in Table 2

10^3/µl; $p = 0.0915$). Cervical lymph node metastasis (cN+) was weakly associated with increased neutrophil count (>5.36 × 10^3/µl; $p = 0.0836$) and PLR (>143; $p = 0.0623$). An increased neutrophil count (>5.36 × 10^3/µl) has been weakly associated with males ($p = 0.0888$) and advanced tumor size (pT3/4; $p = 0.0565$).

Table 2 Comparison of calculated sensitivities, specificities, positive likelihood ratios, and negative likelihood ratios of single laboratory tests and calculated ratios in OSCC ($n = 146$) compared with controls ($n = 93$)

Parameter	Cut-off value[a]	Sensitivity (95 % CI)	Specificity (95 % CI)	+LR (95 % CI)	−LR (95 % CI)	AUC (95 % CI)	*p* value**
CRP (mg/dl)	>1.5	76.22 (68.4–82.9)	54.34 (43.6–64.8)	1.67 (1.3–2.1)	0.44 (0.3–0.6)	0.676 (0.612–0.735)	**<0.0001**
WBC count ($\times 10^3$/μl)	>8.5	36.11 (28.3–44.5)	81.72 (72.4–89.0)	1.98 (1.2–3.2)	0.78 (0.7–0.9)	0.581 (0.515–0.644)	**0.0303**
Monocyte count ($\times 10^3$/μl)	>0.42	59.03 (50.5–67.1)	73.12 (62.9–81.8)	2.20 (1.5–3.2)	0.56 (0.4–0.7)	0.675 (0.611–0.734)	**<0.0001**
Lymphocyte count ($\times 10^3$/μl)	≤1.41	43.75 (35.5–52.3)	83.87 (74.8–90.7)	2.71 (1.6–4.5)	0.67 (0.6–0.8)	0.679 (0.615–0.738)	**<0.0001**
Neutrophil count ($\times 10^3$/μl)	>5.36	51.39 (42.9–59.8)	74.19 (64.1–82.7)	1.99 (1.4–2.9)	0.66 (0.5–0.8)	0.648 (0.583–0.708)	**<0.0001**
Basophil count ($\times 10^3$/μl)	>0.04	27.78 (20.6–35.8)	77.42 (67.6–85.4)	1.23 (0.8–1.9)	0.93 (0.8–1.1)	0.513 (0.447–0.578)	0.7369
Eosinophil count ($\times 10^3$/μl)	>0.18	32.64 (25.1–40.9)	82.80 (73.6–89.8)	1.90 (1.1–3.1)	0.81 (0.7–0.9)	0.572 (0.506–0.636)	0.0534
Platelet count ($\times 10^3$/μl)	≤213	27.08 (20.0–35.1)	84.95 (76.0–91.5)	1.80 (1.0–3.1)	0.86 (0.8–1.0)	0.524 (0.459–0.590)	0.5124
NLR	>2.68	75.00 (67.1–81.8)	69.89 (59.5–79.0)	2.49 (1.8–3.4)	0,36 (0.3–0.5)	0.762 (0.703–0.815)	**<0.0001**
dNLR	>1.99	64.58 (56.2–72.4)	72.04 (61.8–80.9)	2.31 (1.6–3.3)	0.49 (0.4–0.6)	0.711 (0.648–0.768)	**<0.0001**
LMR	≤4.1	69.44 (61.2–76.8)	77.42 (67.6–85.4)	3.08 (2.1–4.5)	0.39 (0.3–0.5)	0.784 (0.727–0.835)	**<0.0001**
PLR	>143	69.44 (61.2–76.8)	55.91 (45.2–66.2)	1.58 (1.2–2.0)	0.55 (0.4–0.7)	0.649 (0.584–0.709)	**<0.0001**

Bold values represent that *p* values were two-sided and $p < 0.05$ was statistically significant

+LR positive likelihood ratio, *−LR* negative likelihood ratio, *CI* confidence interval, *AUC* area under the ROC curve

** The *p* value is the probability that the observed sample area under the ROC curve is found when in fact, the true (population) area under the ROC curve is 0.5 (null hypothesis: area = 0.5). If *p* is low ($p < 0.05$) then it can be concluded that the Area under the ROC curve is significantly different from 0.5 and that therefore there is evidence that the single laboratory test or the ratio does have an ability to distinguish between the two groups (OSCC group vs. control group)

[a] Value with highest diagnostic accuracy

Table 3 Comparison of mean values of single laboratory tests and calculated ratios in OSCC ($n = 146$) and controls ($n = 93$)

Parameter	Mean values[a]			*p* value
	Normal values	Controls	OSCC	
CRP value	<5 mg/dl	3.13 (2.23–4.02)	9.52 (6.31–12.74)	**0.0002**
WBC count	4–10 × 10^3/μl	7.35 (6.98–7.73)	8.09 (7.68–8.50)	**0.0089**
Monocyte count	0.08–0.540 × 10^3/μl	0.38 (0.35–0.41)	0.49 (0.45–0.52)	**<0.0001**
Lymphocyte count	1–3.6 × 10^3/μl	1.96 (1.82–2.10)	1.57 (1.48–1.66)	**<0.0001**
Neutrophil count	2.2–6.3 × 10^3/μl	4.67 (4.34–5.00)	5.68 (5.33–6.02)	**<0.0001**
Basophil count	0.015–0.05 × 10^3/μl	0.056 (0.014–0.099)	0.036 (0.033–0.039)	0.3286
Eosinophil count	0.05–0.250 × 10^3/μl	0.127 (0.111–0.144)	0.167 (0.144–0.189)	**0.0051**
Platelet count	150–400 × 10^3/μl	270.27 (257.55–283.00)	272.46 (255.90–289.02)	0.8360
NLR	<2.68	2.90 (2.33–3.47)	3.94 (3.63–4.24)	**0.0017**
dNLR	<1.99	1.87 (1.69–2.06)	2.42 (2.26–2.58)	**<0.0001**
LMR	≥4.1	5.43 (5.01–5.84)	3.57 (3.30–3.83)	**<0.0001**
PLR	>143	149.61 (137.84–161.38)	190.76 (174.18–207.33)	**0.0001**

Bold values represent that *p* values were two-sided and $p < 0.05$ was statistically significant

[a] Mean values are given with 95 % confidence intervals (in brackets)

Table 4 Association of pretreatment single laboratory tests in OSCC ($n = 146$)

	Parameter														
	CRP value (>1.5 mg/l)			WBC count (>8.5 × 10^3/μl)			Monocyte count (>0.42 × 10^3/μl)			Lymphocyte count (≤1.41 × 10^3/μl)			Neutrophil count (>5.36 × 10^3/μl)		
	Low	High	*p* value	Low	High	*p* value	Low	High	*p* value	Low	High	*p* value	Low	High	*p* value
Parameter															
CRP value (>1.5 mg/l)			–			**0.0475**			0.1827			0.1266			**0.0363**
Low	–	–		29	8		19	42		12	52		24	48	
High	–	–		65	44		18	67		25	57		13	61	
WBC count (>8.5 × 10^3/μl)			–			–			**<0.0001**			**<0.0001**			**<0.0001**
Low	–	–		–	–		53	41		51	43		72	22	
High	–	–		–	–		8	44		13	39		0	52	
Monocyte count (>0.42 × 10^3/μl)			–			–			–			0.3118			**<0.0001**
Low	–	–		–	–		–	–		30	31		45	16	
High	–	–		–	–		–	–		34	51		27	58	
Lymphocyte count (≤1.41 × 10^3/μl)			–			–			–			–			0.3170
Low	–	–		–	–		–	–		–	–		35	29	
High	–	–		–	–		–	–		–	–		37	45	

Bold values represent that *p* values were two-sided and $p < 0.05$ was statistically significant

Association of pretreatment single laboratory tests and calculated ratios in OSCC

Significantly associated parameters of pretreatment laboratory tests and calculated ratios (Tables 4, 5, 6) were correlated and graphical displayed for a trend (Fig. 3).

NLR was significant directly correlated with WBC count (Rr = 0.3764, $p < 0.0001$; $R^2 = 0.1417$; $p < 0.0001$), neutrophil count (Rr = 0.5740, $p < 0.0001$; $R^2 = 0.3295$; $p < 0.0001$), and monocyte count (Rr = 0.2085, $p = 0.0121$; $R^2 = 0.0434$; $p = 0.0121$), but significant inversely correlated with lymphocyte count (Rr = −0.5345, $p < 0.0001$; $R^2 = 0.2857$; $p < 0.0001$). LMR was significant inversely correlated with neutrophil count (Rr = −0.2522, $p = 0.0023$; $R^2 = 0.0636$; $p = 0.0023$) and monocyte count (Rr = −0.5668, $p < 0.0001$; $R^2 = 0.3213$; $p < 0.0001$).

LMR was significant directly correlated with lymphocyte count (Rr = 0.5470, $p < 0.0001$; $R^2 = 0.2992$; $p < 0.0001$). PLR was significant inversely correlated with lymphocyte count (Rr = −0.5205, $p < 0.0001$; $R^2 = 0.2709$; $p < 0.0001$) (Fig. 2).

NLR was significant directly correlated with PLR (Rr = 0.5552, $p < 0.0001$; $R^2 = 0.3082$; $p < 0.0001$). LMR was significant inversely correlated with NLR (Rr = −0.5996, $p < 0.0001$; $R^2 = 0.3595$; $p < 0.0001$) and PLR (Rr = −0.3903, $p < 0.0001$; $R^2 = 0.1523$; $p < 0.0001$) (Fig. 2).

A combination of the ratios or a combination with CRP using a combinatory index was not beneficial for improved association with any clinicopathological characteristics (data not shown).

Immunohistochemistry of CRP expression in the carcinogenesis of OSCC

Liver tissues stained positive for CRP. However, CRP expression was not found in normal oral mucosa, oral precursor lesions, or OSCC specimen.

Discussion

This is the first study focusing on the development for cut-off values based on single laboratory results and calculated ratios that might serve as an extension to clinicopathological parameters for prognosis, treatment, and monitoring [35] of patients with OSCC. Moreover, the calculated baselines can be used as a goal for successful immunotherapies in the future.

Evidence for the use of inflammatory hematologic markers as predictors of clinical outcome in OSCC and head and neck squamous-cell carcinoma (HNSCC) are available. Increased CRP values [11, 14], leucocytosis [11, 15], monocytosis [16], lymphopenia [29], neutrophilia [20,

Table 5 Association of pretreatment laboratory tests with calculated ratios in OSCC ($n = 146$)

	Parameter														
	CRP value (>1.5 mg/l)			WBC count (>8.5 × 10^3/μl)			Monocyte count (>0.42 × 10^3/μl)			Lymphocyte count (≤1.41 × 10^3/μl)			Neutrophil count (>5.36 × 10^3/μl)		
	Low	High	*p* value	Low	High	*p* value	Low	High	*p* value	Low	High	*p* value	Low	High	*p* value
Parameter															
NLR (>2.68)			0.1924			**0.0001**			**0.0077**			**<0.0001**			**<0.0001**
Low	13	25		34	4		23	15		6	32		33	5	
High	24	84		60	48		38	70		58	50		39	69	
dNLR (>1.99)			0.2741			**0.0143**			**0.0171**			**0.0429**			**0.0006**
Low	4	6		10	9		8	2		1	9		10	0	
High	33	103		84	52		53	83		63	73		62	74	
LMR (≤4.1)			0.8382			0.5748			**<0.0001**			**0.0001**			**0.0195**
Low	25	76		63	38		26	75		55	46		43	58	
High	12	33		31	14		35	10		9	36		29	16	
PLR (>143)			0.0663			1.0000			0.1863			**0.0001**			0.1069
Low	16	29		29	16		20	19		9	36		27	18	
High	21	80		65	36		41	66		55	46		45	56	

Bold values represent that *p* values were two-sided and $p < 0.05$ was statistically significant

Table 6 Association of pretreatment calculated ratios in OSCC ($n = 146$)

	Parameter											
	NLR (>2.68)			dNLR (>1.99)			LMR (≤4.1)			PLR (>143)		
	Low	High	*p* value	Low	High	*p* value	Low	High	*p* value	Low	High	*p* value
Parameter												
NLR (>2.68)			–			**<0.0001**			**<0.0001**			**0.0141**
Low	–	–		10	28		15	23		18	20	
High	–	–		0	108		86	22		27	81	
dNLR (>1.99)			–			–			**0.0001**			**0.0099**
Low	–	–		–	–		1	9		7	3	
High	–	–		–	–		100	36		38	98	
LMR (≤4.1)			–			–			–			**0.0207**
Low	–	–		–	–		–	–		25	76	
High	–	–		–	–		–	–		20	25	

Bold values represent that *p* values were two-sided and $p < 0.05$ was statistically significant

21], and elevated NLR [14, 29] have been associated with reduced tumor-specific survival in OSCC. However, no data regarding OSCC survival are available for decreased LMR and increased PLR but have been described in the pathogenesis of HNSCC [19, 30, 35, 41]. Lymphopenia [29] was demonstrated by our previous data [18] in OSCC patients and was confirmed in this study. Our results showed that older patients (≥64 years) were more frequently associated with lymphopenia, which is in accordance with previous published data in tumor patients [53], including HNSCC [17]. Therefore, the issue of immunosuppression seems to be important for the carcinogenesis of OSCC. As a consequence of an inadequate immunologic reaction with subsequent weakened defense against cancer interleukin-2 (IL-2) cytokine-based immunotherapies designed to increase the number and biological activity of circulating lymphocytes were introduced in renal cancer [54], metastatic melanoma [55], and have been suggested for OSCC [56]. Lymphocytes are very important cellular components of the immune system that are crucial for activation of an effective antitumor response [57], the destruction of residual tumor cells, as well as related micrometastasis [58, 59]. Due to sustained activation of T cells in cancer patients [60] tumor-infiltrating T

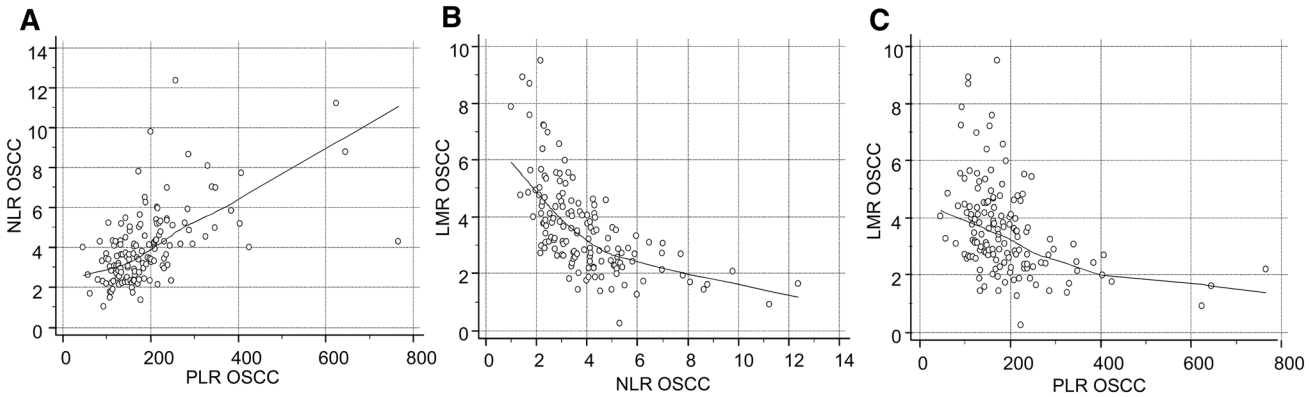

Fig. 3 Correlation analysis of calculated ratios (**a**–**c**) in OSCC patients ($n = 146$) with trend lines. NLR is significant directly correlated with PLR (**a**). LMR is significant inversely correlated with NLR (**b**) and PLR (**c**). The trend lines are plotted by local regression smoothing (LOESS). The degree of smoothing is controlled by the span (80 %) which is the proportion (expressed as a percentage) of the total number of points that contribute to each local fitted value

lymphocytes could help to drive tumor cells towards apoptosis and by presenting tumor-associated antigens to immune cells lymphocytes lead to death of cancer cells in response to chemoradiation (CRT) [12, 61]. Hence, lymphocytes are very crucial for improvement of adjuvant therapies and preventing tumor recurrence.

For the first time, similar to the survival data we defined cut-off values for all single laboratory results and calculated ratios compared with controls. These data are strongly needed for a follow-up of cancer patients, which were measured for each parameter by ROC analysis and have not been determined for all cancer diseases at all. Indeed, significant cut-off values for single laboratory parameters (CRP, WBCs, monocytes, lymphocytes, neutrophils) and calculated ratios (NLR, dNLR, LMR, PLR) were found in OSCC patients compared with controls. In the clinical course of carcinogenesis the relation of calculated ratios could be useful for predicting tumor recurrence and probably tumor disease. Compared with single laboratory tests calculated ratios were superior in measuring sensitivity and specificity of OSCC disease. In our study, we found that NLR was significant directly associated and correlated with PLR. Additionally, LMR was significant inversely associated and correlated with NLR and PLR. This link might be helpful to evaluate the clinical course (follow-up/monitoring [35, 45]) of OSCC patients. However, compared with others [14, 35] a combination of the ratios or a combination with CRP using a combinatory index was not beneficial for improved association with any clinicopathological characteristics.

In patients with breast cancer NLR was shown to be better than dNLR in terms of predicting prognosis [62], which confirms our results of NLR to be a more sensitive and specific marker for OSCC than dNLR.

Various outcome thresholds for these ratios were identified analyzing the relative risk of OSCC and HNSCC tumor recurrence: NLR >1.99 [29] (OSCC), >2.44 [14] (OSCC); LMR <5.07 [19] (HNSCC), <5.2 [41] (HNSCC); PLR >167 [30] (HNSCC), >170 [35] (HNSCC). In our case control study, we found NLR (>2.68), LMR (≤4.1), and PLR (>143) values comparable with previous published results of survival data in OSCC and HNSCC. However, this is the first study describing LMR and PLR cut-off values in OSCC compared with controls. To the best of our knowledge, neither data regarding survival nor data comparing tumor patients with controls are available for OSCC to date. Drawing a conclusion for this question of different cut-off values in the follow-up period (monitoring [35, 45]) the clinical course of ratios (relation of NLR, LMR, PLR) indicated by the scatter plots will be crucial, whereby clinicians are principally involved. For example, in cases of tumor recurrence an increase of NLR with PLR in parallel to a decrease of LMR would be expected. Using NLR and PLR the data published by Rassouli et al. [35] were at least as good as TNM staging system in predicting survival of HNSCC patients. Moreover, predicting response to CRT is an important issue in the treatment of cancer. The results showed by Lin et al. [19] demonstrated that increased LMR is associated with better prognosis in patients with newly diagnosed metastatic nasopharyngeal carcinoma receiving chemotherapy. He et al. [39] reported that pretreatment NLR, percentages of lymphocytes and neutrophils are independent prognostic factors for survival for HNSCC patients undergoing radiation or chemoradiation. Similar to this, An et al. [40] demonstrated NLR as a significant predictor of both survival and response to CRT in nasopharyngeal carcinoma. In patients with gastric cancer the normalization of NLR

and PLR after chemotherapy was found to be associated with significant improvement in progression free survival [45]. The pretreatment NLR and PLR represented significant prognostic indicators of survival in patients treated for early-stage non-small-cell lung carcinoma with stereotactic radiation [63]. Determined by NLR dominant pro-tumor activities of neutrophils or reduced anti-tumor immune response by lymphocytes may have an impact on poor tumor response to preoperative CRT and unfavorable prognosis in rectal cancer patients [64]. The results published by Huang et al. demonstrated that neutrophils and monocytes appear to have a strong impact on radiation outcome in HNSCC [17]. Thus, as a supplement using systemic inflammatory markers as single laboratory parameters or as calculated ratios provide a clear rationale for predicting surgical outcome and/or CRT response. Our results strengthen the evidence suggesting that neutrophils, monocytes, or lymphocytes of the systemic inflammatory response and anti-tumor response represent targets for novel therapeutic strategies. The utility of NLR, LMR, and PLR in the posttreatment management (monitoring [35, 45]) of OSCC patients including surgical outcome and/or CRT response have currently not been determined.

Several explanations for the relation of increased NLR, PLR and decreased LMR with poor prognosis in cancer are available. Neutrophils contain and secrete various cytokines including IL-8, vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), matrix metalloproteinases (MMPs), and elastases [17, 20, 21, 65–68]. These cytokines create a microenvironment for tumor cell dissociation, endothelial cell migration, and extracellular matrix remodelling [17, 20, 21, 65–68]. Subsequently, the cytolytic activity of activated lymphocytes (T cells) and natural killer cells (NK) is suppressed and the degree of suppression is closely associated with the number of neutrophils [69, 70]. Therefore, neutrophilia may cause immunosuppression and can sustain tumor growth. On the other hand, increased NLR could result from lymphopenia, which impair the role of cell-mediated immunity and its function of host cancer cell destruction [71]. Finally, our data analyzed in OSCC patients confirmed the findings of other tumor entities demonstrating neutrophilia, lymphopenia, and increased NLR with strong prognostic impact on survival or treatment outcome results.

In OSCC conflicting data are available concerning thrombocytosis [27, 28], which has been associated with higher levels of IL-6 as one major cytokine of inflammation [72]. This cytokine was linked with various tumor-promoting activities in the tumor microenvironment, such as angiogenesis and tumor cell proliferation [73]. Hence, in our study, no indication of thrombocytosis has been found. Conversely, increased PLR could result from lymphopenia. Therefore, the significant increased PLR is rather the consequence of lymphopenia than the result of thrombocytosis in OSCC patients.

Beside lymphopenia the decreased LMR can be attributed to a significant monocytosis found in OSCC patients. The study conducted by Tsai et al. [16] showed that leucocytosis, monocytosis, and neutrophilia were associated with advanced OSCC tumor stages and poor tumor differentiation. The monocyte count was also increased in those patients with lymph node metastasis. Moreover, the pretreatment circulating monocyte count was an independent prognostic factor for worse OSCC specific survival [16]. This is in accordance with our findings showing leucocytosis, neutrophilia, and monocytosis to be associated with advanced tumor stages. Additionally, neutrophilia was associated with advanced tumor size and cervical lymph node metastasis, and both, neutrophilia and monocytosis with male gender. In the context of carcinogenesis monocytes (and tumor-associated macrophages, M2 [74]) secrete several proinflammatory cytokines such as IL-1, IL-6, tumor necrosis factor-alpha (TNF-α) [75], and immunosuppressive anti-inflammatory IL-10 [74, 76], which have been associated with poor clinical outcome in malignancies [75–78]. Therefore, neutrophils and monocytes acting the opposite role as lymphocytes and seem to promote carcinogenesis.

The increased NLR and decreased LMR in male gender is attributed to the detected neutrophilia and monocytosis, which have been both associated with nicotine abuse. Therefore, smoking may contribute to a systemic inflammatory reaction supporting tumor progression. In general, social habits like nicotine and alcohol abuse were associated with advanced clinicopathological characteristics, e.g. advanced tumor stages and confirm previous published data [79]. Smoking is associated with a broad range of alterations in systemic immune and inflammation marker levels including accelerated erythropoiesis, thrombocytosis, leukocytosis [80] specifically with chronic neutrophilia [81], and elevation of CRP levels [82]. Beside platelets (secreting IL-6) and neutrophils (secreting IL-8), which are attracted by the inflammatory microenvironment to the tumor, cancer cells including OSCC have been shown to secrete IL-6 [83] and IL-8 [84] and in turn induce the production of CRP [85, 86]. These mechanisms imply that increased CRP levels are a response to the neoplastic process and/or the systemic inflammatory response of leucocytes (e.g. neutrophils) to the tumor. Measurement of both, IL-6 and IL-8 have been associated with reduced tumor-specific survival in OSCC [87] and are worth for standardized analysis in further surveys. Although some tumors have been shown to express CRP [88, 89] our data didńt reveal tumor cells or precancerous lesions as a potential source of elevated

CRP expression in OSCC patients. Our data support the suggestion of CRP as an important marker for monitoring of the disease although the cut-off value has been determined at a low level but confirm our previous published results [11]. Increased serum CRP levels were significantly associated and correlated with elevated blood leucocytes/neutrophils but they were not associated with calculated ratios (NLR, dNLR, LMR, PLR). Therefore, increasing numbers of leucocytes and more specific neutrophils together with serum CRP reflect a systemic inflammatory response, which could be useful predicting tumor disease and probably tumor recurrence after excluding other criteria (e.g. bacterial infection) in the after-care. Elevated CRP values were not associated with social habits (nicotine and alcohol abuse) and therefore they could be attributed as a consequence to the inflammatory cancer microenvironment.

Basophils [22] and eosinophils [22–25] in the peripheral blood have not been determined for OSCC as yet. The clinical relationship between eosinophilia and basophilia with patients' outcome is not really clear. In patients of myelodysplastic syndromes eosinophilia and basophilia have been significantly associated with reduced survival [22]. In our study, basophils and eosinophils were not associated with any clinicopathological characteristics, single laboratory results, or calculated ratios. Therefore, these parameters seem to be not relevant for monitoring of OSCC patients.

Conclusions

This study highlights the first analysis for cut-off values of pretreatment single laboratory tests and calculated ratios, which are strongly needed for a follow-up of cancer patients. Additionally, the calculated baselines can be used as a goal for successful immunotherapies in the future. The links between NLR, LMR, and PLR might be helpful for the clinical course (monitoring [35, 45]) of cancer patients and have been first described for OSCC in this study. Taken together, analyzing these data provides an additional practical guideline of further postoperative OSCC management.

Acknowledgments The authors thank the assistance of Dr. Julia Grimm for her technical support.

Compliance with ethical standards

Informed consent and research involving human participants Written informed consent to participate was obtained from all patients (Ethics Committee Tuebingen, Germany, approval number: 562-2013BO2).

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Grimm M (2012) Prognostic value of clinicopathological parameters and outcome in 484 patients with oral squamous cell carcinoma: microvascular invasion (V+) is an independent prognostic factor for OSCC. Clin Transl Oncol 14(11):870–880. doi:10.1007/s12094-012-0867-2
2. Kreppel M, Eich HT, Kubler A, Zoller JE, Scheer M (2010) Prognostic value of the sixth edition of the UICC's TNM classification and stage grouping for oral cancer. J Surg Oncol 102(5):443–449. doi:10.1002/jso.21547
3. Grandi C, Alloisio M, Moglia D, Podrecca S, Sala L, Salvatori P et al (1985) Prognostic significance of lymphatic spread in head and neck carcinomas: therapeutic implications. Head Neck Surg 8(2):67–73
4. Greenberg JS, Fowler R, Gomez J, Mo V, Roberts D, El Naggar AK et al (2003) Extent of extracapsular spread: a critical prognosticator in oral tongue cancer. Cancer 97(6):1464–1470. doi:10.1002/cncr.11202
5. Sano D, Myers JN (2007) Metastasis of squamous cell carcinoma of the oral tongue. Cancer Metastasis Rev 26(3–4):645–662. doi:10.1007/s10555-007-9082-y
6. Coussens LM, Werb Z (2002) Inflammation and cancer. Nature 420(6917):860–867. doi:10.1038/nature01322
7. Balkwill F, Mantovani A (2001) Inflammation and cancer: back to Virchow? Lancet 357(9255):539–545
8. Balkwill F, Charles KA, Mantovani A (2005) Smoldering and polarized inflammation in the initiation and promotion of malignant disease. Cancer Cell 7(3):211–217. doi:10.1016/j.ccr.2005.02.013
9. Balkwill F, Coussens LM (2004) Cancer: an inflammatory link. Nature 431(7007):405–406
10. Choi S, Myers JN (2008) Molecular pathogenesis of oral squamous cell carcinoma: implications for therapy. J Dent Res 87(1):14–32 **(87/1/14 [pii])**
11. Grimm M, Lazariotou M (2012) Clinical relevance of a new pretreatment laboratory prognostic index in patients with oral squamous cell carcinoma. Med Oncol 29(3):1435–1447. doi:10.1007/s12032-011-0045-3
12. Apetoh L, Tesniere A, Ghiringhelli F, Kroemer G, Zitvogel L (2008) Molecular interactions between dying tumor cells and the innate immune system determine the efficacy of conventional anticancer therapies. Cancer Res 68(11):4026–4030. doi:10.1158/0008-5472.CAN-08-0427
13. Greten FR, Eckmann L, Greten TF, Park JM, Li ZW, Egan LJ et al (2004) IKKbeta links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. Cell 118(3):285–296. doi:10.1016/j.cell.2004.07.013S0092867404006713
14. Fang HY, Huang XY, Chien HT, Chang JT, Liao CT, Huang JJ et al (2013) Refining the role of preoperative C-reactive protein by neutrophil/lymphocyte ratio in oral cavity squamous cell carcinoma. Laryngoscope 123(11):2690–2699. doi:10.1002/lary.24105
15. Chen YW, Chen IL, Lin IC, Kao SY (2014) Prognostic value of hypercalcaemia and leucocytosis in resected oral squamous cell carcinoma. Brit J Oral Maxillofac Surg 52(5):425–431. doi:10.1016/j.bjoms.2014.02.014
16. Tsai YD, Wang CP, Chen CY, Lin LW, Hwang TZ, Lu LF et al (2014) Pretreatment circulating monocyte count associated with poor prognosis in patients with oral cavity cancer. Head Neck 36(7):947–953. doi:10.1002/hed.23400
17. Huang SH, Waldron JN, Milosevic M, Shen X, Ringash J, Su J et al (2015) Prognostic value of pretreatment circulating neutrophils, monocytes, and lymphocytes in oropharyngeal cancer stratified by human papillomavirus status. Cancer 121(4):545–555. doi:10.1002/cncr.29100

18. Grimm M, Feyen O, Hofmann H, Teriete P, Biegner T, Munz A et al (2015) Immunophenotyping of patients with oral squamous cell carcinoma in peripheral blood and associated tumor tissue. Tumour Biol. doi:10.1007/s13277-015-4224-2
19. Lin GN, Peng JW, Liu DY, Xiao JJ, Chen YQ, Chen XQ (2014) Increased lymphocyte to monocyte ratio is associated with better prognosis in patients with newly diagnosed metastatic nasopharyngeal carcinoma receiving chemotherapy. Tumour Biol 35(11):10849–10854. doi:10.1007/s13277-014-2362-6
20. Glogauer JE, Sun CX, Bradley G, Magalhaes MA (2015) Neutrophils increase oral squamous cell carcinoma invasion through an invadopodia-dependent pathway. Cancer Immunol Res. doi:10.1158/2326-6066.CIR-15-0017
21. Magalhaes MA, Glogauer JE, Glogauer M (2014) Neutrophils and oral squamous cell carcinoma: lessons learned and future directions. J Leukoc Biol 96(5):695–702. doi:10.1189/jlb.4RU0614-294R
22. Wimazal F, Germing U, Kundi M, Noesslinger T, Blum S, Geissler P et al (2010) Evaluation of the prognostic significance of eosinophilia and basophilia in a larger cohort of patients with myelodysplastic syndromes. Cancer 116(10):2372–2381. doi:10.1002/cncr.25036
23. Jain M, Kasetty S, Khan S, Jain NK (2014) Tissue eosinophilia in head and neck squamous neoplasia: an update. Exp Oncol 36(3):157–161
24. Jain M, Kasetty S, Sudheendra US, Tijare M, Khan S, Desai A (2014) Assessment of tissue eosinophilia as a prognosticator in oral epithelial dysplasia and oral squamous cell carcinoma-an image analysis study. Pathol Res Int 2014:507512. doi:10.1155/2014/507512
25. Pereira MC, Oliveira DT, Kowalski LP (2011) The role of eosinophils and eosinophil cationic protein in oral cancer: a review. Arch Oral Biol 56(4):353–358. doi:10.1016/j.archoralbio.2010.10.015
26. Buergy D, Wenz F, Groden C, Brockmann MA (2012) Tumor–platelet interaction in solid tumors. Int J Cancer 130(12):2747–2760. doi:10.1002/ijc.27441
27. Lu CC, Chang KW, Chou FC, Cheng CY, Liu CJ (2007) Association of pretreatment thrombocytosis with disease progression and survival in oral squamous cell carcinoma. Oral Oncol 43(3):283–288. doi:10.1016/j.oraloncology.2006.03.010
28. Kargus S, Weber FE, Luebbers HT, Zemann W, Graetz KW, Kruse AL (2012) Pretreatment thrombocytosis: a prognostic marker for oral squamous cell carcinoma? Oral Maxillofac Surg 16(2):197–200. doi:10.1007/s10006-011-0305-6
29. Perisanidis C, Kornek G, Poschl PW, Holzinger D, Pirklbauer K, Schopper C et al (2013) High neutrophil-to-lymphocyte ratio is an independent marker of poor disease-specific survival in patients with oral cancer. Med Oncol 30(1):334. doi:10.1007/s12032-012-0334-5
30. Sun W, Zhang L, Luo M, Hu G, Mei Q, Liu D et al (2015) Pretreatment hematologic markers as prognostic factors in patients with nasopharyngeal carcinoma: neutrophil-lymphocyte ratio and platelet–lymphocyte ratio. Head Neck. doi:10.1002/hed.24224
31. Rachidi S, Wallace K, Wrangle JM, Day TA, Alberg AJ, Li Z (2015) Neutrophil-to-lymphocyte ratio and overall survival in all sites of head and neck squamous cell carcinoma. Head Neck. doi:10.1002/hed.24159
32. Feng JF, Huang Y, Chen QX (2014) Preoperative platelet lymphocyte ratio (PLR) is superior to neutrophil lymphocyte ratio (NLR) as a predictive factor in patients with esophageal squamous cell carcinoma. World J Surg Oncol 12:58. doi:10.1186/1477-7819-12-58
33. Feng JF, Huang Y, Zhao Q, Chen QX (2013) Clinical significance of preoperative neutrophil lymphocyte ratio versus platelet lymphocyte ratio in patients with small cell carcinoma of the esophagus. Sci World J 2013:504365. doi:10.1155/2013/504365
34. Millrud CR, Mansson Kvarnhammar A, Uddman R, Bjornsson S, Riesbeck K, Cardell LO (2012) The activation pattern of blood leukocytes in head and neck squamous cell carcinoma is correlated to survival. PLoS One 7(12):e51120. doi:10.1371/journal.pone.0051120
35. Rassouli A, Saliba J, Castano R, Hier M, Zeitouni AG (2015) Systemic inflammatory markers as independent prognosticators of head and neck squamous cell carcinoma. Head Neck 37(1):103–110. doi:10.1002/hed.23567
36. Deng Q, He B, Liu X, Yue J, Ying H, Pan Y et al (2015) Prognostic value of pre-operative inflammatory response biomarkers in gastric cancer patients and the construction of a predictive model. J Transl Med 13:66. doi:10.1186/s12967-015-0409-0
37. Tu XP, Qiu QH, Chen LS, Luo XN, Lu ZM, Zhang SY et al (2015) Preoperative neutrophil-to-lymphocyte ratio is an independent prognostic marker in patients with laryngeal squamous cell carcinoma. BMC Cancer 15:743. doi:10.1186/s12885-015-1727-6
38. Templeton AJ, McNamara MG, Seruga B, Vera-Badillo FE, Aneja P, Ocana A et al (2014) Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. J Natl Cancer Inst 106(6):dju124. doi:10.1093/jnci/dju124
39. He JR, Shen GP, Ren ZF, Qin H, Cui C, Zhang Y et al (2012) Pretreatment levels of peripheral neutrophils and lymphocytes as independent prognostic factors in patients with nasopharyngeal carcinoma. Head Neck 34(12):1769–1776. doi:10.1002/hed.22008
40. An X, Ding PR, Wang FH, Jiang WQ, Li YH (2011) Elevated neutrophil to lymphocyte ratio predicts poor prognosis in nasopharyngeal carcinoma. Tumour Biol 32(2):317–324. doi:10.1007/s13277-010-0124-7
41. Li J, Jiang R, Liu WS, Liu Q, Xu M, Feng QS et al (2013) A large cohort study reveals the association of elevated peripheral blood lymphocyte-to-monocyte ratio with favorable prognosis in nasopharyngeal carcinoma. PLoS One 8(12):e83069. doi:10.1371/journal.pone.0083069
42. Nishijima TF, Muss HB, Shachar SS, Tamura K, Takamatsu Y (2015) Prognostic value of lymphocyte-to-monocyte ratio in patients with solid tumors: a systematic review and meta-analysis. Cancer Treat Rev. doi:10.1016/j.ctrv.2015.10.003
43. Templeton AJ, Ace O, McNamara MG, Al-Mubarak M, Vera-Badillo FE, Hermanns T et al (2014) Prognostic role of platelet to lymphocyte ratio in solid tumors: a systematic review and meta-analysis. Cancer Epidemiol Biomarkers Prev 23(7):1204–1212. doi:10.1158/1055-9965.EPI-14-0146
44. Zhou X, Du Y, Huang Z, Xu J, Qiu T, Wang J et al (2014) Prognostic value of PLR in various cancers: a meta-analysis. PLoS One 9(6):e101119. doi:10.1371/journal.pone.0101119
45. Lee S, Oh SY, Kim SH, Lee JH, Kim MC, Kim KH et al (2013) Prognostic significance of neutrophil lymphocyte ratio and platelet lymphocyte ratio in advanced gastric cancer patients treated with FOLFOX chemotherapy. BMC Cancer 13:350. doi:10.1186/1471-2407-13-350
46. Grimm M, Calgeer B, Teriete P, Biegner T, Munz A, Reinert S (2015) Targeting thiamine-dependent enzymes for metabolic therapies in oral squamous cell carcinoma? Clin Transl Oncol. doi:10.1007/s12094-015-1352-5
47. Driemel O, Hertel K, Reichert TE, Kosmehl H (2006) Current classification of precursor lesions of oral squamous cell carcinoma principles of the WHO classification 2005. Mund Kiefer Gesichtschir 10(2):89–93. doi:10.1007/s10006-006-0675-3
48. Sobin LH, Wittekind CH (2010) UICC. TNM classification of malignant tumors, 7th edn. Springer, Berlin
49. Hamilton SR, Aaltonen LA (2000) Pathology and genetics. Tumours of the digestive system, 3rd edn. IARC Press, Lyon

50. Grimm M, Cetindis M, Lehmann M, Biegner T, Munz A, Teriete P et al (2014) Association of cancer metabolism-related proteins with oral carcinogenesis-indications for chemoprevention and metabolic sensitizing of oral squamous cell carcinoma? J Transl Med 12:208. doi:10.1186/1479-5876-12-208
51. Walker RA (2006) Quantification of immunohistochemistry-issues concerning methods, utility and semiquantitative assessment I. Histopathology 49(4):406–410. doi:10.1111/j.1365-2559.2006.02514.x
52. Zweig MH, Campbell G (1993) Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem 39(4):561–577
53. Mehrazin R, Uzzo RG, Kutikov A, Ruth K, Tomaszewski JJ, Dulaimi E et al (2015) Lymphopenia is an independent predictor of inferior outcome in papillary renal cell carcinoma. Urol Oncol 33(9):388 e319–388-e325. doi:10.1016/j.urolonc.2014.06.004
54. Thomas JS, Kabbinavar F (2015) Metastatic clear cell renal cell carcinoma: a review of current therapies and novel immunotherapies. Crit Rev Oncol Hematol. doi:10.1016/j.critrevonc.2015.07.009
55. Hughes T, Klairmont M, Sharfman WH, Kaufman HL (2015) Interleukin-2, Ipilimumab, and Anti-PD-1: clinical management and the evolving role of immunotherapy for the treatment of patients with metastatic melanoma. Cancer Biol Ther. doi:10.1080/15384047.2015.1095401
56. Cheriyan VT, Thomas C, Balaram P (2011) Augmentation of T-cell immune responses and signal transduction proteins in oral cancer patients: potential for IL-2-mediated immunotherapy. J Cancer Res Clin Oncol 137(10):1435–1444. doi:10.1007/s00432-011-1012-2
57. Rabinowich H, Cohen R, Bruderman I, Steiner Z, Klajman A (1987) Functional analysis of mononuclear cells infiltrating into tumors: lysis of autologous human tumor cells by cultured infiltrating lymphocytes. Cancer Res 47(1):173–177
58. Fogar P, Sperti C, Basso D, Sanzari MC, Greco E, Davoli C et al (2006) Decreased total lymphocyte counts in pancreatic cancer: an index of adverse outcome. Pancreas 32(1):22–28 **(00006676-200601000-00004 [pii])**
59. Sarraf KM, Belcher E, Raevsky E, Nicholson AG, Goldstraw P, Lim E (2009) Neutrophil/lymphocyte ratio and its association with survival after complete resection in non-small cell lung cancer. J Thorac Cardiovasc Surg 137(2):425–428. doi:10.1016/j.jtcvs.2008.05.046
60. Dworacki G, Meidenbauer N, Kuss I, Hoffmann TK, Gooding W, Lotze M et al (2001) Decreased zeta chain expression and apoptosis in CD3+ peripheral blood T lymphocytes of patients with melanoma. Clin Cancer Res 7(3 Suppl):947s–957s
61. Youn JI, Collazo M, Shalova IN, Biswas SK, Gabrilovich DI (2012) Characterization of the nature of granulocytic myeloid-derived suppressor cells in tumor-bearing mice. J Leukoc Biol 91(1):167–181. doi:10.1189/jlb.0311177
62. Dirican A, Kucukzeybek BB, Alacacioglu A, Kucukzeybek Y, Erten C, Varol U et al (2015) Do the derived neutrophil to lymphocyte ratio and the neutrophil to lymphocyte ratio predict prognosis in breast cancer? Int J Clin Oncol 20(1):70–81. doi:10.1007/s10147-014-0672-8
63. Cannon NA, Meyer J, Iyengar P, Ahn C, Westover KD, Choy H et al (2015) Neutrophil-lymphocyte and platelet-lymphocyte ratios as prognostic factors after stereotactic radiation therapy for early-stage non-small-cell lung cancer. J Thorac Oncol 10(2):280–285. doi:10.1097/JTO.0000000000000399
64. Kim IY, You SH, Kim YW (2014) Neutrophil-lymphocyte ratio predicts pathologic tumor response and survival after preoperative chemoradiation for rectal cancer. BMC Surg 14:94. doi:10.1186/1471-2482-14-94
65. Schaider H, Oka M, Bogenrieder T, Nesbit M, Satyamoorthy K, Berking C et al (2003) Differential response of primary and metastatic melanomas to neutrophils attracted by IL-8. Int J Cancer 103(3):335–343. doi:10.1002/ijc.10775
66. Di Carlo E, Forni G, Musiani P (2003) Neutrophils in the antitumoral immune response. Chem Immunol Allergy 83:182–203
67. Scapini P, Nesi L, Morini M, Tanghetti E, Belleri M, Noonan D et al (2002) Generation of biologically active angiostatin kringle 1–3 by activated human neutrophils. J Immunol 168(11):5798–5804
68. De Larco JE, Wuertz BR, Furcht LT (2004) The potential role of neutrophils in promoting the metastatic phenotype of tumors releasing interleukin-8. Clin Cancer Res 10(15):4895–4900. doi:10.1158/1078-0432.CCR-03-0760
69. Petrie HT, Klassen LW, Kay HD (1985) Inhibition of human cytotoxic T lymphocyte activity in vitro by autologous peripheral blood granulocytes. J Immunol 134(1):230–234
70. Kay HD, Smith DL (1983) Regulation of human lymphocyte-mediated natural killer (NK) cell activity. I. Inhibition in vitro by peripheral blood granulocytes. J Immunol 130(1):475–483
71. Lissoni P, Brivio F, Fumagalli L, Messina G, Ghezzi V, Frontini L et al (2004) Efficacy of cancer chemotherapy in relation to the pretreatment number of lymphocytes in patients with metastatic solid tumors. Int J Biol Markers 19(2):135–140
72. Estrov Z, Talpaz M, Mavligit G, Pazdur R, Harris D, Greenberg SM et al (1995) Elevated plasma thrombopoietic activity in patients with metastatic cancer-related thrombocytosis. Am J Med 98(6):551–558
73. Naugler WE, Karin M (2008) The wolf in sheep's clothing: the role of interleukin-6 in immunity, inflammation and cancer. Trends Mol Med 14(3):109–119. doi:10.1016/j.molmed.2007.12.007
74. Fujita Y, Okamoto M, Goda H, Tano T, Nakashiro K, Sugita A et al (2014) Prognostic significance of interleukin-8 and CD163-positive cell-infiltration in tumor tissues in patients with oral squamous cell carcinoma. PLoS One 9(12):e110378. doi:10.1371/journal.pone.0110378
75. Anand M, Chodda SK, Parikh PM, Nadkarni JS (1998) Abnormal levels of proinflammatory cytokines in serum and monocyte cultures from patients with chronic myeloid leukemia in different stages, and their role in prognosis. Hematol Oncol 16(4):143–154
76. Torisu-Itakura H, Lee JH, Huynh Y, Ye X, Essner R, Morton DL (2007) Monocyte-derived IL-10 expression predicts prognosis of stage IV melanoma patients. J Immunother 30(8):831–838. doi:10.1097/CJI.0b013e318158795b
77. Pollard JW (2004) Tumour-educated macrophages promote tumour progression and metastasis. Nat Rev Cancer 4(1):71–78. doi:10.1038/nrc1256
78. Grivennikov SI, Greten FR, Karin M (2010) Immunity, inflammation, and cancer. Cell 140(6):883–899. doi:10.1016/j.cell.2010.01.025
79. Zygogianni AG, Kyrgias G, Karakitsos P, Psyrri A, Kouvaris J, Kelekis N et al (2011) Oral squamous cell cancer: early detection and the role of alcohol and smoking. Head Neck Oncol 3:2. doi:10.1186/1758-3284-3-2
80. Hasselbalch HC (2015) Smoking as a contributing factor for development of polycythemia vera and related neoplasms. Leuk Res. doi:10.1016/j.leukres.2015.09.002
81. Weir AB, Lewis JB Jr, Arteta-Bulos R (2011) Chronic idiopathic neutrophilia: experience and recommendations. South Med J 104(7):499–504. doi:10.1097/SMJ.0b013e31821ec7cc
82. Shiels MS, Katki HA, Freedman ND, Purdue MP, Wentzensen N, Trabert B et al (2014) Cigarette smoking and variations in systemic immune and inflammation markers. J Natl Cancer Inst. doi:10.1093/jnci/dju294
83. Jinno T, Kawano S, Maruse Y, Matsubara R, Goto Y, Sakamoto T et al (2015) Increased expression of interleukin-6 predicts poor response to chemoradiotherapy and unfavorable prognosis in oral

squamous cell carcinoma. Oncol Rep 33(5):2161–2168. doi:10.3892/or.2015.3838
84. Watanabe H, Iwase M, Ohashi M, Nagumo M (2002) Role of interleukin-8 secreted from human oral squamous cell carcinoma cell lines. Oral Oncol 38(7):670–679
85. O'Riordain MG, Falconer JS, Maingay J, Fearon KC, Ross JA (1999) Peripheral blood cells from weight-losing cancer patients control the hepatic acute phase response by a primarily interleukin-6 dependent mechanism. Int J Oncol 15(4):823–827
86. Wigmore SJ, Fearon KC, Sangster K, Maingay JP, Garden OJ, Ross JA (2002) Cytokine regulation of constitutive production of interleukin-8 and -6 by human pancreatic cancer cell lines and serum cytokine concentrations in patients with pancreatic cancer. Int J Oncol 21(4):881–886
87. St John MA, Li Y, Zhou X, Denny P, Ho CM, Montemagno C et al (2004) Interleukin 6 and interleukin 8 as potential biomarkers for oral cavity and oropharyngeal squamous cell carcinoma. Arch Otolaryngol Head Neck Surg 130(8):929–935. doi:10.1001/archotol.130.8.929
88. Nozoe T, Korenaga D, Futatsugi M, Saeki H, Maehara Y, Sugimachi K (2003) Immunohistochemical expression of C-reactive protein in squamous cell carcinoma of the esophagus—significance as a tumor marker. Cancer Lett 192(1):89–95 **(S0304383502006304 [pii])**
89. Jabs WJ, Busse M, Kruger S, Jocham D, Steinhoff J, Doehn C (2005) Expression of C-reactive protein by renal cell carcinomas and unaffected surrounding renal tissue. Kidney Int 68(5):2103–2110. doi:10.1111/j.1523-1755.2005.00666.x