Prognostic significance of preoperative anemia on occurrence of regional metastases and other primary tumors in patients with early stage oral squamous cell carcinoma

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LIST OF ABBREVIATIONS

American Joint Committee on Cancer	(AJCC)
Angiopoietin-2	(Ang-2)
B-cell lymphoma	(bcl)
Bone/cartilage	(B/C)
Basic fibroblast growth factor	(bFGF)
Cyclin-dependent kinase inhibitor 2a	(CDKN2A)
Extranodal extension (or extracapsular extension)	(ENE)
Epidermal growth factor receptor	(EGFR)
fantiliter	(fL)
Head and neck carcinomas	(HNCs)
Head and neck squamous cell carcinoma	(HNSCC)
Hemoglobin	(Hb)
Hypoxia inducible factor	(HIF-1α)
Glutathione S-transferase M1	(GSTM1)
Human papilloma virus	(HPV)
International Classification of Diseases and Related Health Problems	(ICDRHP)
Lymph node metastases	(LNM)
Mean cell volume	(MCV)
Metastases-associated protein 1	(MTA1)
Matrix metalloproteinases	(MMPs)
Parathyroid adenomatosis 1	(PRAD-1)
Plummer-Vinson syndrome	(PVS)
Oral cancer	(OC)
Monocyte chemoattractant protein 1	(MCP-1)
Oral squamous cell carcinoma	(OSCC)
Retinoblastoma	(Rb)
Retinoblastoma protein	(pRb)
Royal College of Pathologists	(RCPath)
Red blood cell	(RBC)
Socioeconomic status	(SES)
Second primary tumors	(SPT)

Selective neck dissection	(SND)
Vascular endothelial growth factor	(VEGF)
Signal transducer and activator of transcription 3	(STAT3)
Surveillance, Epidemiology, and End Results	(SEER)
Transforming growth factor alpha	(TGF-alfa)
World Health Organization	(WHO)

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1. INTRODUCTION

1.1 Oral squamous cell carcinoma (OSCC)

Squamous cell carcinoma (SCC) is the most common type of oral cancers, accounting for 90% of all oral cancers. SCC is particularly common in the developing world, mostly in older males (1-4). There is concern about an ongoing increase in younger patients and in women. The etiology appears to be multifactorial and strongly related to lifestyle, mostly habits and diet (particularly tobacco and alcohol consumption), although other factors, such as infective agents, may also be implicated (5,6). OSCC portends high rates of mortality and cervical lymph node metastasis (7). Treatment of OSCC includes surgical resection of the primary tumor with appropriate neck dissection, followed by postoperative radiotherapy/chemotherapy, depending on the histopathologic features of the tumor (8).

Epidemiology

Incidence

According to the International Classification of Diseases and Related Health Problems (ICDRHP), oral cancer and pharyngeal cancer is the 8th most common cancer in Europe and the 11th leading cause of cancer-related mortality (9). Oral cancer accounts for over 300,000 annually new diagnosed cases. There are annually 100,000 new cases of oral and pharyngeal cancer in Europe (10). The global incidence of oral cavity cancer per 100,000 person-years was 5.5 in males and 2.5 in females (2012), while the incidence was higher in Europe at 7.7 in males and 2.5 in females. When oral and pharyngeal cancer are considered together, the annual incidence goes up to 18.2 in males and 4.9 in females (11).

Among the states of Southern Europe, the highest incidence of oral cancer (all sub-sites (C00-14)) is observed in Portugal (15.4), Croatia (12), and Serbia (11.7). The incidence by gender by all sub-sites C00-14, is as follows: in men, the highest incidence is observed in Portugal (27.5), Croatia (20.2), and Serbia (18.8), while in women, the highest incidence is observed in Montenegro (5.3), Malta (5.3), and Serbia (5.2). These data are GLOBALCAN 2012 projections.

The incidence sub-classified to ICD-10 sub-sites according to EUREG database are as follows (Table 1):

Table 1. Latest incidence rates in Southern Europe.[a]

	C00-14			C00		C01-2		C03-6		C07-8		C09		C10	
Countries	Total number (ASR-W)	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Albania	211(7.2)	9.4	5.3	-	-	-	-	-	-	-	-	-	-	-	-
Bosnia-Herzegovina	290(6.3)	9.4	3.7	-	-	-	-	-	-	-	-	-	-	-	-
Croatia	685(12)	20.2	5.1	1.7	0.5	4.7	0.7	5.1	0.6	1.1	1.0	1.8	0.2	1.4	0.1
Cyprus	32(2.7)	4.2	1.3	0.9	0.1	0.3	0.2	1.8	1.0	0.7	0.9	0.2	0.1	0.1	0.0
Greece	570(3.5)	5.5	1.8	-	-	-	-	-	-	-	-	-	-	-	-
Italy	5835(6.8)	9.8	4.0	0.8	0. 1	2.9	1.3	3.0	1.1	1.1	0.6	1	0.3	0.6	0.1
Macedonia	69(3.3)	2.9		-	-	-	-	-	-	-	-	-	-	-	-
Malta	52 (9.9)	14.9	5.3	1.1	0.1	2.8	1.1	2.6	1.3	1.7	1.2	1.4	0.3	0.3	0.1
Montenegro	67(9.7)	14.4	5.3	-	-	-	-	-	-	-	-	-	-	-	-
Portugal	2082(15.4)	27.5	4.7	6.2	0.5	3.9	0.9	3.6	1.3	0.8	0.9	5.1	0.2	1.3	0.0
Serbia	1311(11.7)	18.8	5.2	1.9	0.7	1.9	0.4	1.9	0.7	1.1	0.6	0.6	0.2	0.4	0.1
Slovenia	326 (12.6)	20.6	5.2	1.2	0.4	3.9	0.3	4.8	2.1	0.7	0.3	1.9	0.4	2.7	0.3
Spain	5978(10.1)	16.8	4.2	3.6	0.4	4.1	1.3	4.6	1.1	1.0	0.5	1.0	0.2	1	0.1

ASR-W: world age-standardized rate per 100,000.
[a]Data from EUCAN 2012 and CI5X

Lip cancer (C00) is most common in Spain (2.3), then Serbia (1.6) followed by Croatia (1.5).

Cancer of the tongue (C01-02) has its highest incidence in Spain (2.6), Croatia (2.6), Portugal (2.4), and Italy (2.1). The incidence of tongue cancer by gender: in men, the highest incidence is observed in Croatia (4.7), while in women the highest incidence is observed in Italy (1.3) and Spain (1.3).

Cancer in the C03-06 sub-sites has the highest incidence in Slovenia (3.4), Spain (2.8), and Croatia (2.7). By gender, the highest incidence in men is observed in Croatia (4.7), Italy (1.3), and Spain (1.3).

For cancer in C(09-14) sub-sites (cancer in pharynx), the highest incidence is observed in Slovenia (6.5), Spain (5.6), and Croatia (5.2)(12).

Mortality

Oral cancer accounts for over 140,000 deaths annually across the world. Among the states of Southern Europe, the highest rate of mortality, from cancer in the C01-06 sub-sites, is observed in Croatia (1.8), followed by Portugal (1.4), and Spain (1.4).

These are data retrievable from EUREG (Table 2).

Among the states of Southern Europe, the highest rate of mortality of patients with all sub-types (C00-14) of oral and pharyngeal cancer, is observed in Croatia (6.5), Portugal (5.6), and Serbia (5.3). The mortality rate in women is the highest in Serbia (2.0), Montenegro (1.7), and Italy (1.5). These are projections from GLOBOCAN.

These are online data from common population-based registries, which are regularly updated and adapted to ICD-10. These are European Cancer Observatory (EUCAN) and the Association of Nordic Cancer Registries.

According to Diz et al., socio-economic and cultural characteristics findings, higher incidence of oral cancer in Danish women attributed to smoking as risk factor, alcohol in Lithuanian man, tobacco and alcohol increasing rate of incidence in United Kingdom young people and human papilloma virus (HPV) as risk factor in increasing incidence in Denmark and Scotland young population (12).

Table 2. Latest mortality rates in Southern Europe. [a]

	C00-14		
Countries	Total number (ASR-W)	Male	Female
Albania	114 (3.9)	5.1	2.9
Bosnia-Herzegovina	81 (1.7)	2.5	1.0
Croatia	373 (6.5)	12.6	1.3
Cyprus	10 (0.9)	1.4	0.3
Greece	302 (1.8)	2.8	0.8
Italy	2699 (2.9)	4.5	1.5
Macedonia	58 (2.6)	4.0	1.3
Malta	20 (3.5)	5.9	1.5
Montenegro	28 (4.0)	6.6	1.7
Portugal	751 (5.5)	10.0	1.4
Serbia	672 (5.9)	10.2	2.0
Slovenia	146 (5.4)	9.8	1.6
Spain	2070 (3.4)	5.8	1.3

[a] Data from EUCAN 2012

Prognostic, predictive and risk factors of oral squamous cell carcinoma (OSCC)

Age

The OSCC incidence has increased in recent years, especially among younger adults and women. The incidence rate is marked in every age, even though it appears mostly in elderly males (most commonly in their late sixties) (13). Survival rate decreases significantly in patients with OSCC older than 65 years of age. The worse survival rate in this group of patients is attributed to multifactorial aspects including comorbidity (chronic disease) and the overall health condition. This makes them more susceptible in developing cancer as well as treatment effectiveness by itself, compared to younger patients (14).

Young adults (<45) have an increased incidence of tongue SCC but their survival rate is better compared to older patients. Increased incidence in young population explanations remains unclear, but infective agents may be implicated (15).

In industrialized countries, men are affected two times as often as women, largely due to higher use of alcohol and tobacco, and the mean onset of oral cancer in men is 7 years earlier than in women. Young adult (patients under the age of 40 years) are mainly males (16,17).

Some studies seem to suggest that mortality is also increasing at a more rapid rate in younger adults. A study reports a 2-fold rise in mortality from cancer of the tongue in the US during the period 1950-82 for those aged 10-29 years, with slightly lower increases among those aged 30-39, but no increase reported in older age- groups (18).

Gender

Differences between men and women seem to be narrowing over time, even though men are more likely to develop OSCC than women. According to descriptive epidemiology data, male to female ratio is lower in young adults than in older patients.

The overall OSCC incidence male-female ratio is 1.8, with range variating from 8.3 male - female ratio in lower lip SCC, down to 0.9 for the upper alveolar ridge SCC (19).

The mortality rate in men with OSCC over the last decade has declined in Western European countries, including France (8.6/100,000), Spain (6.0/100,000), Germany (5.7/100,000), and Italy (4.3/100,000). In the same period, in Eastern European countries (including Hungary, Belarus, Lithuania, and Romania), mortality in women with OSCC has continued to rise persistently.

These divergences in both genders are attributed to different lifestyles, socioeconomic circumstances and habits, especially in tobacco consumption and alcohol intake.

This decline in male mortality rate in Western European countries is attributed to low tobacco consumption over the last few decades by men. Controversially, in Eastern European women, tobacco consumption has increased.

This effect of declined mortality rate in men years later after the vulnerable data show the decreased number of people who smoke, is because the risk persists to remain high many years afterwards (20,21).

Alcohol consumption has its own role in mortality rate, which rises persistently in Eastern countries. It depends on overall quantity, pattern, and type of alcohol consumed. Despite that, in these countries (Hungary, Slovakia, Moldova, Lithuania, Croatia, Romania), the homemade alcohol is widespread, which means that high levels of acetaldehyde can act as carcinogens (22).

Race/Ethnicity

The US National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) most recent available data show that in people before the age of 55, oral and pharyngeal cancer is the fourth most common in Black men and sixth most common cancer in White men (23).

In the United States of America, incidence rates in Black Americans approached 25 per 100,000, compared to an average of 11 per 100,000 in the White American population, of the estimated 30,000 newly diagnosed cases in 2004 (23).

Differences in alcohol and tobacco consumption amongst the Black subjects, socioeconomic factors and nutrition (a lower consumption of fruits and vegetables among blacks), could primarily be related to differences in oral cancer between Black and White people (24).

American Indians have the lowest rates (4/100,000), whereas a low increase is seen in Asian, Pacific Islander countries men and those of Hispanic origin, with similar rates (5/100,000), lower than the rates of Black and White people (25).

Socioeconomic status(SES)

Socioeconomic status (SES) has also been implicated as a factor in the incidence, stage at diagnosis, and prognosis of OSCC patients. Lower socioeconomic status (SES) is known to be associated with low survival and increased incidence of cancer.

It can impact health outcomes and is dependent on many variables, such as low income, housing, educational attainment, employment, family structure, wealth, health insurance coverage, and disability benefits (26).

Socioeconomic circumstances should be considered as risk factors, the same as genetic factors that have a prospect potential to influence gene expression and their interaction with the environment, so it may be possible to influence the upstream socioeconomic factors.

Diagnosis at the early stage strongly influences cancer-specific survival rate. Early stage OSCC detection among men and women is decreased in deprived neighborhoods. This is attributed to dental visit absence as well as to oral cancer screening services absence, which may result in poor and delayed diagnosis and low survival rate (27).

A study shows that oral cancers diagnosed by a dentist had 3.44 times the odds of being at a later stage than other head and neck carcinomas (HNCs) (CI 1.01-11.96), but oral cancers diagnosed by other means had 11.42 times the odds of being at a later stage than other HNCs (28).

Patients with lower SES have low survival rate, due to absence of medical insurance, absence of surgical treatment, poor communication, and lack of patient navigation facilities (28).

Tobacco and alcohol

Incidence of OSCC in people who use tobacco is 5-9 times higher in comparison with people who do not, and it goes up to 17 times higher in heavy smokers (80 or more cigarettes per day). Patients who continue smoking after treatment have 2-6 times greater risk of developing a second malignancy of upper aerodigestive tract than those who stop using tobacco.

At early stage OSCC, the expected incidence of second primary tumors (SPT) is about 4%, as a consequence of field cancerization of upper aerodigestive tract. This field cancerization is thought to be a consequence of chronic exposure to tobacco and alcohol.

People who consume alcohol have 3-9 greater risk of OSCC incidence. Extremely heavy drinkers (greater than 100 grams of alcohol per day) have 30 times greater risk in comparison with people who do not use alcohol. Synergistic effect of alcohol and smoking exists, so patients who are both heavy smokers and heavy drinkers have over 100 times greater risk on OSCC occurrence (29,30).

Human papilloma virus (HPV)

OSCC can be caused by an infective agent such as human papilloma virus (HPV), mostly by high risk type of HPV, predominantly HPV type 16 (HPV-16), which can be detected in tumor cells. For malignant behavior of these tumors, the responsible is expression of viral E6 and E7 oncoprotein that inactivates the tumor-suppressor p53 and the retinoblastoma protein (pRb) (31-33).

OSCC that includes the floor of the mouth, gum, cheeks and ventrolateral tongue, is associated with tobacco and alcohol consumption rather than HPV, whereas OSCC that includes the base of the tongue, tonsils and oropharynx, is strongly related to HPV infection and has shown a high incidence in the last decade (34).

These high incidence rates for OSCC in sites related to HPV infections, such as the base of the tongue, oropharynx, and tonsils, are increased in young adults in the Western Countries (USA and Europe) which is hypothesized to be in part due to changes in oral sexual behavior (35).

Among patients with HPV-positive tumors, there seems to be a less frequent presence of second primary tumors, which are largely related to smoking habits (31).

It was found that among patients with HPV-positive tumors, the 5-year survival rates is approximately 75 to 80%, whereas in patients with HPV-negative tumors, it is approximately 45 to 50% (31).

TNM staging

TNM-staging classifies tumors according to tumor size (T; T0-T4), lymph node metastasis (N; N0-N3), and distant metastasis (M; M0-M1). The treatment strategy and the prognosis of patients are strongly correlated with TNM stage of the tumors. The individual T, N, and M values classify the tumor to either of the four stages (stage I-IV), where the early stage is associated with better prognosis and the higher stage is strongly associated with worse prognosis. Patients with cervical lymph node metastasis (N+) are classified as stage III. Regional lymph node metastasis is one of the major prognostic factors, where patients with positive involvement of regional lymphatic nodes display worse prognosis. According to the American Joint Committee on Cancer (AJCC), there are 2 methods of categorizing N in the TNM system: clinical and pathological. Clinical N is used for patients who have not received a neck dissection (cTNM). Pathological N is used for those who have had a lymph node dissection (pTNM). The disease is classified as stage IV when distant

metastases (M+) are found, which reduce the 5-year survival rate from approximately 45% to 20%. A common problem is that patients with tumors of the same stage often respond differently to the same treatment. This might in part be explained by the molecular heterogeneity of these tumors, hence better prognostic markers are needed, especially biomarkers predicting invasive and metastatic tumor behavior (36-40).

Tx		The primary tumor cannot be evaluated	
Tis		Carcinoma in situ	Cancer cells are found only in 1 layer of tissue
T1		The tumor is 2 cm or less at its greatest dimension.	The tumor has invaded nearby tissues to a depth of 5 mm or less; this is called depth of invasion
T2		The tumor is 2cm or smaller, and the depth of invasion is between 5-10 mm	Or, the tumor is larger than 2 cm, but not larger than 4 cm, and the depth of invasion is 10 mm or less.
T3		The tumor is larger than 4 cm	Or, it is any tumor with the depth of invasion more than 10 mm
T4	T4a (lip)	Tumor began on the lip but has invaded bone or spread to the inferior alveolar nerve in the mouth, the floor of the mouth, or the skin of the face	
	T4a (oral cavity)	The tumor has invaded nearby structures in the mouth, such as jaw, sinuses, or the skin of the face	
	T4b	The tumor has invaded the muscles and bones that form the mouth or the base of the skull, and/or it encases the internal arteries	

Figure1. AJCC oral cavity TNM classification (T - Primary tumor)(36).

NX	The regional lymph nodes cannot be evaluated
N0	There is no evidence of cancer in regional lymph nodes
N1	The cancer has spread to a single lymph node on the same side as the primary tumor, and the cancer found in the node is 3 cm or smaller. There is no ENE.

N2a	The cancer has spread to a single lymph node on the same side as the primary tumor, and is larger than 3 cm but not larger than 6 cm. There is no ENE.
N2b	The cancer has spread to more than 1 lymph node on the same side as the primary tumor, and none measures larger than 6 cm. There is no ENE.
N2c	The cancer has spread to more than 1 lymph node on the either side of the body, and none measure larger than 6 cm. There is no ENE.
N3a	The cancer is found in the lymph node and is larger than 6 cm. There is no ENE.
N3b	There is ENE in any lymph node.

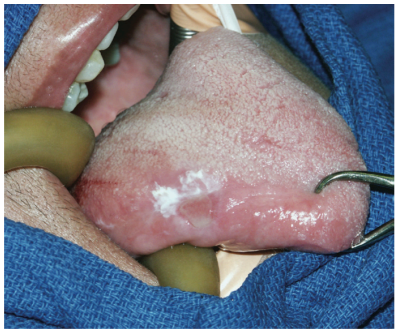
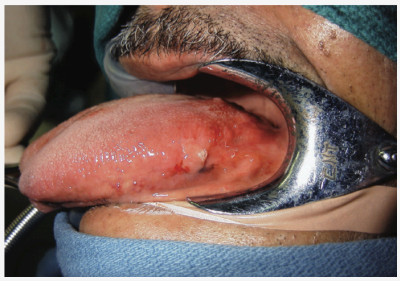
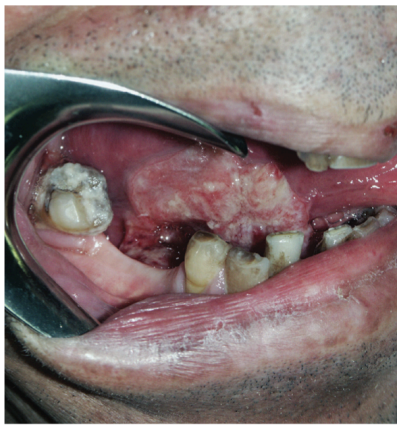
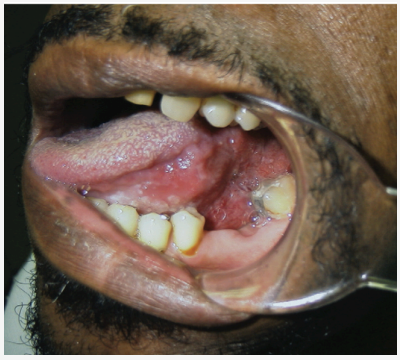
Figure 2. AJCC oral cavity TNM classification (N - Regional lymph nodes) - Clinical (36)

NX	The regional lymph node cannot be evaluated
N0	There is no evidence of cancer in regional lymph nodes
N1	The cancer has spread to a single lymph node on the same side as the primary tumor, and the cancer found in the node is 3 cm or smaller. There is no ENE.
N2a	The cancer has spread to one lymph node and is 3cm or smaller, but there is no ENE. Or, the cancer has spread to a single lymph node on the same side as the primary tumor, and is larger than 3 cm but not larger than 6 cm, and there is no ENE.
N2b	The cancer has spread to more than one lymph node on the same side as the primary tumor, and none measures larger than 6 cm. There is no ENE
N2c	The cancer has spread to more than one lymph node on the either side of the body, and none measures larger than 6 cm. There is no ENE.
N3a	The cancer is found in the lymph node and is larger than 6 cm. There is no ENE.
N3b	There is ENE in a single lymph node on the same side as the primary tumor, and it is larger than 3 cm. Or, cancer has spread to many lymph nodes, and at least 1 has ENE. Or, there is ENE in a single lymph node on the opposite side of the primary tumor that is 3 cm or smaller.

Figure 3. AJCC oral cavity TNM classification (N - Regional lymph nodes) - Pathological (36)

M0 (M plus 0)	Cancer has not spread to other parts of the		
M1	Cancer has spread to other parts of the body		

Figure 4. AJCC oral cavity TNM classification (M - Distant metastasis) (36)

Stage 0	Tis N0 M0		
Stage I	T1 N0 M0	Tumor is 2 cm or smaller and the depth of invasion is 5 mm or less. The cancer has not spread to lymph nodes or other parts of the body.	
Stage II	T2 N0 M0	The tumor is 2 cm or smaller, and the depth of invasion is between 5-10mm. Or, the tumor is larger than 2 cm but no larger than 4 cm and the depth of invasion is 10 mm or less. The cancer has not spread to lymph nodes or other parts of the body.	
Stage III	T3 N0 M0	The tumor is larger than 4 cm, or it is any tumor with the depth of invasion greater than 10mm. The cancer has not spread to lymph nodes or other parts of the body.	
	T1/T2/T3 N1 M0	The tumor is of any size, but it has not invaded the nearby structures, of the oral cavity. There is cancer in a single lymph node, on the same side as the primary tumor and the cancer is 3 cm or smaller. There is no ENE. The cancer has not spread to other parts of the body.	

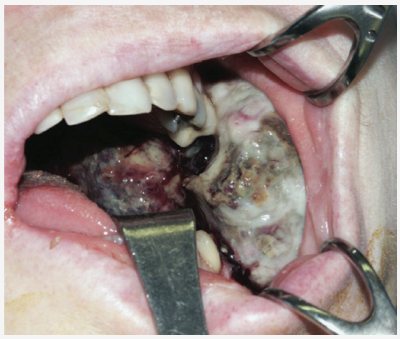
Stage IV A	T4a, N0 or N1, M0	Tumor has invaded nearby structures in the mouth, such as jaw, sinuses or skin of the face. If cancer has spread to a lymph node, it is only to 1 node on the same side as the primary tumor, and the cancer is 3cm or smaller, with no ENE. The cancer has not spread to other parts of the body.	
	T1/T2/ T3/T4 N2 M0	The tumor may be small or it may have invaded nearby structures. The cancer has spread to 1 or more lymph nodes, but none is larger than 6 cm. There is no ENE. The cancer has not spread to other parts of the body.	
Stage IV B	Any T N3 M0	The tumor is of any size. The cancer is found in a lymph node and is larger than 6 cm, but there is no ENE; or there is ENE in any lymph node. The cancer has not spread to other parts of the body.	
	T4b AnyN M0	The tumor has invaded the muscles and bones that form the mouth or the base of the skull, and/r it encases the internal arteries. The cancer may involve the lymph nodes, but it has not spread to other parts of the body.	
Stage IV C	Any T Any N M1	The cancer has spread to other parts of the body.	

Figure 5. AJCC oral cavity anatomic stage/prognostic groups (36)

Histopathologic grade

OSCC histopathologic grading depends on the subjective assessment of the mitotic activity, cellular and nuclear pleomorphism and degree of keratinization (37). According to the degree of differentiation, the OSCC histopathologically may be: Gx-grade cannot be assessed. In well differentiated tumors (grade 1: pG1), tumor cells are arranged in an orderly stratification, resembling normal epithelial cells. Heavy keratinization can usually be found in pearl formations. In moderately differentiated tumors (grade 2; pG2), the cells are less keratinized, less stratified, and contain prickle cells. Poorly differentiated cell tumors (grade 3; pG3) can still be identifiable as squamous cell carcinoma. In undifferentiated tumors (grade4; pG4), cells cannot be identifiable. More than 50% of OSCC are moderately differentiated.

Histopathologic grading is considered as a poor indicator to predict the outcome and response to treatment. It is significantly related to nodal disease at the time of diagnosis. However, a combination of histopathologic staging with tumor growth pattern improves the prognostic value of histologic staging (38-42).

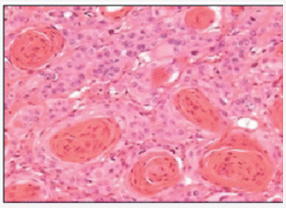
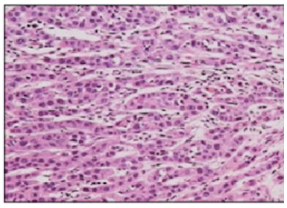
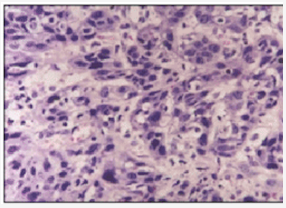
Grade x	Cannot be assessed		
Grade 1	Well differentiated		
Grade 2	Moderate differentiation		
Grade 3	Poor differentiation		
Grade 4	Undifferentiated		

Figure 6. AJCC histopathologic grade - G (36)

Treatment of OSCC

Several factors determine the treatment strategy of patients with OSCC, such as primary tumor size and location, lymph node status, distant metastasis presence, and the patient's overall health condition, and each of them may act as a prognostic factor in survival rate (43). Treatment of OSCC includes surgery, radiation therapy, and chemotherapy, but the most common treatment is the combination of radiation therapy and surgery, as it is most often used for advanced stages of the disease (43,44).

Even though that long-term risk factors for OSCC patients have been well established, efforts at prevention, early detection, and treatment are still of concern.

Recent advances in genetic predisposition, biomarkers, photodiagnostic imaging, and differentiation therapy may soon offer improved outcomes (45).

Primary site of tumor location

The anterior border of the oral cavity is the junction of the skin and vermilion border of the lip. The posterior border is formed superiorly by the junction of the hard and soft palates, inferiorly by the circumvallate papillae, and the anterior tonsillar pillars laterally. The various sites within the oral cavity include the lip, floor of the mouth, anterior two-thirds of tongue, gingival, hard palate, buccal mucosa and retromolar trigone. The soft tissue mucosa is squamous cell epithelium, although the extent of keratinization varies throughout the oral cavity (46).

Primary site of OSCC more often is in the floor of the mouth and lateral borders of the tongue. Despite that, in the European and American populations, the most common site for OSCC is the tongue (40-50%), in Asian populations (mainly in Sri Lanka and India), buccal mucosa is the most affected site (40%) and this is attributed to betel quid/tobacco chewing habits (47).

Posteriorly located tumors have a significantly reduced five-year survival compared to anteriorly located tumors (oropharyngeal vs. oral) (48). Tumor's site influence in regional lymph node metastases and low accessibility for the surgeon due to anatomic structure restrictions on resection with clear margins of tumors, make the survival rate reduced in people with OSCC.

The anatomic sites within the oral cavity exhibit variations in histological features, vascular supply, and lymphatic network.

OSCC which occurred on the buccal mucosa tends to be well differentiated, whereas cancers of the palate, tongue and floor of the mouth are poorly differentiated (49).

Table 3. Overall 5-year survival rates based on the location of OSCC.

Location		Authors	Sample size	Overall 5-year survival rate
Lip		Han et al.	15,832	69.9%
		Cabello et al.	74	73%
		Ozturk et al.	101	82.1%
		Schüller et al.	105 (lower lip OSCC)	61.2%
Buccal mucosa		Iqbal et al.	63	30%
Tongue and floor of the mouth		Kelner et al.	222	70-77%
		Lopez-Cedrün and de Llano	64 (advanced cases)	34.4%
Floor of the mouth		Alvarez et al.	63	63.1%
Gingiva	Mandibular	Niu et al.	207	71.8%
	Maxillar	Yang et al.	31	49.2%
Maxillary alveolus and hard palate		Givi et al.	199	86% (with elective lymph node dissection); 62% (without elective lymph node dissection)
Hard palate		Yang et al.	31	66.3%

OSCC: Oral squamous cell carcinoma (49).

Tumor thickness and depth of invasion

The tumor size, thickness, and the depth of invasion usually affect the choice and outcome of treatment. Assessing margins from the resection specimen rather than the tumor bed consistently predicts local control (50).

Increased tumor size has been linked to cervical involvement, high recurrence rate, and poor prognosis.

The association of tumor thickness with lymph node metastasis is reflected in the aggressiveness of tumor growth (51).

Tumor thickness of >7 mm was found to be predictive of lymph node metastasis and a thickness of >10 mm showed poorer disease-free survival in the cases of early SCC of the tongue (50).

The correlation between tumor thickness at two different mucosal sites (tongue and floor of the mouth) and their propensity for nodal metastasis showed that the critical tumor thickness value (which represents the thickness at which the probability of nodal metastases exceeds 20%) for SCC involving the floor of the mouth was 1–2 mm, whereas for SCC of the tongue it was approximately 4mm (50).

There is a tumor thickness and ipsilateral cervical metastasis relationship. The relationship between thickness of the primary tumor and occurrence of contralateral cervical metastasis was reported to increase by 5% in T1/T2 SCC of the oral tongue. It is now widely accepted that thickness is a more

accurate predictor of subclinical nodal metastasis, local recurrence, and survival compared with tumor size (52-55).

Tumor invasion

The tumor cells at the invading site are relatively more proliferative as compared with superficial part. The correlation between survival and invasive front revealed that a higher invasive front grading (tumor islands with >15 and <15 tumor cells) is associated with overall poor survival (56-59).

Lymphovascular and nerve invasion

Vascular invasion means neoplastic cells presence within the lumen or in the wall of lymphatics and blood vessels, while in perineural invasion malignant cells invade within any of the tree layers of nerve sheaths or tumor foci outside of the nerve with involvement of at least 33% of the nerve's circumference. Various studies have suggested that both perineural and vascular invasion are the known predictor of poor outcome in OSCC patients (60).

Lymphovascular and peri-/endoneural invasion show a significant association with tumor size, histological grading, invasive front, nodal involvement, status of the surgical margins, overall prognosis and survival (60).

Lymphovascular invasion implies a considerable number of tumor cells are entering the vascular compartment, which increases the likelihood of regional and distant metastases.

A recent study reported that a weak or limited lymphocyte response at the tumor/host interface is strongly associated with local recurrence and death (61).

It has been proposed that tumor emboli are more difficult to form in the small-caliber lymphatics of superficial areas than in the wider lymphatics of deep tissue, hence the tumor thickness may play a vital role in lymphovascular invasion (61,62).

Bone/cartilage (B/C) invasion

It's a prognostic significance in OSCC to have a grouped pattern of bone invasion into cortical/ medullary or erosive/infiltrative patterns. The mandibular medullary invasion could be an independent prognostic factor rather than merely the mandibular cortical invasion in OSCC patients (63,64).

There are two principal reasons why cancers invading bone and cartilage are considered unsuitable for treatment with radiation therapy. First, these cancers does not respond to radiotherapy like tumors in soft tissues. This because of poor results obtained when patients with T4 OSCC are treated with radiotherapy as the primary modality. The second reason for preferring surgical treatment for advanced cancers of the head and neck that invade bony and cartilaginous structures is a concern for a higher incidence of radiation-induced complications in these patients (65).

Bone and cartilage invasions affect the prognosis and this usually influences the type and extent of treatment. Studies have suggested that T4N0 has a better prognosis than the other stage IV categories (66,67).

Tumor clearance

The UK Royal College of Pathologists (RCPath) classifies the surgical margin as clear (when the distance of 5 mm or more is evident from the invasive tumor cells), close (1–5 mm), and positive or involved (<1mm) (68).

This usually ignores the formalin-shrinkage effect which can be at least 30%. Therefore, in order to achieve a 5-mm pathological clearance, an 8-10-mm *in situ* surgical margin needs to be taken (69).

Positive or close margins are associated with increase in local recurrence and have a negative effect on survival. Furthermore, several studies have shown that local recurrence and overall survival benefit from achieving negative resection margins (70-72).

Surgery

Surgery continues to be the gold standard as initial definitive treatment for majority of patients with OSCC (73). Primary tumor resection, when indicated, is associated with dissection/removal of cervical lymphatic nodes. Defect is reconstructed with regional flaps or distant free tissue transfer, which in combination with radiotherapy has improved survival from 40% up to 70% (74).

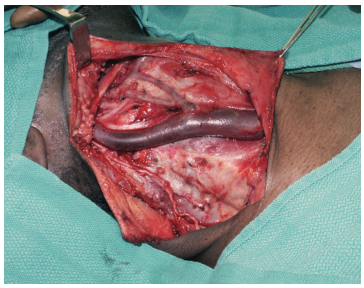
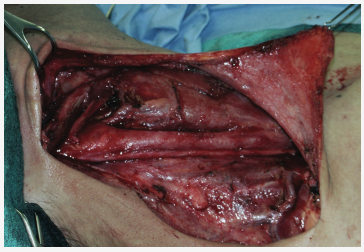
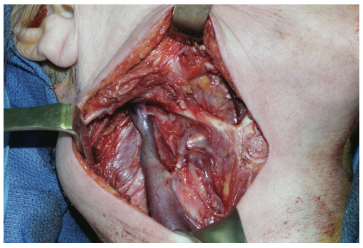
For patients with extracapsular spread, aggressive adjuvant therapy has been recommended.

Most of T1 lesions can be treated by simple excision with scalpel, laser or photodynamic therapy. Neck management depends on tumor thickness, which is the most significant predictor of regional lymph nodes metastasis. According to this, neck lymph nodes metastasis is at increased risk when cut-off is at about 4-6 mm of tumor thickness. This serves as an indicator and is the base for identifying T1, T2N0M0 OSCC patients who benefit from elective neck dissection (75,76).

Most of T2 thin lesions are resected safely, until the increase of tumor thickness appears, which then needs to advocate the neck dissection (77,78).

Elective neck dissection may be both therapeutic and diagnostic. While therapeutic neck dissection is of high benefit in patients with regional metastasis, the diagnostic elective neck dissection helps in detecting the involvement of cervical lymph nodes and removing the non-detectable lymphatic nodes. It is employed when the risk of regional metastasis exceeds 15-20% (79,80).

Studies have shown that in OSCC patients with cancer located in the tongue, and with positive cervical lymph nodes, disease-free survival for those who underwent prophylactic neck dissection was twice as high compared to patients undergoing therapeutic neck dissection (57% vs. 28%). Metastasis in contralateral lymphatic nodes of the neck has been identified as a significant factor in neck failures in OSCC patients undergoing simultaneous prophylactic neck dissection (81,82)

Radical neck dissection	Radical neck dissection is considered to be the standard basic procedure for cervical lymph node removal. All other procedures represents alterations of this procedure. Removal of all ipsilateral cervial lymph node groups extending from the inferior border of mandible superiorly, to the clavicle inferiorly, medially from the lateral border of the sternohyoid muscle, hyoid bone, and contralateral anterior belly of the digastric muscle; and laterally to anterior border of the trapezius muscle, is referred to radical neck dissection. Radical neck dissection does not include removal of the suboccipital nodes, periparotid nodes (except infraparotid nodes located in the posterior aspect of the submandibular triangle), retropharyngeal nodes, buccinator nodes, and midline visceral (central compartment) nodes.	
Modified radical neck dissection	Modified radical neck dissection refers to the removal of all lymph nodes routinely removed by the radical neck dissection, with preservation any of non-lymphatic structures: i.e., spinal accessory nerve (SAN), internal jugular vein (IJV), and sternocleidomastoid muscle (SCM). The structure(s) which is preserved should be specifically named—e.g., “modified radical neck dissection with preservation of the spinal accessory nerve”.	
Selective neck dissection	Selective neck dissection (SND) refers to a cervical lymphadenectomy in which there is removal of one or more of the lymph node groups that are routinely removed in the radical neck dissection. The lymph node groups removed are based on the patterns of metastases that are predictable relative to the primary site of disease. For oral cavity cancers, the lymph nodes located in Levels I, II, III, and upper IV, are at great risk. In the Levels, II, III and IV are located the lymph nodes at greatest risk for oropharyngeal, hypopharyngeal, and laryngeal cancers; for thyroid cancer, they are located in Level VI.	

	Specific variations of the selective neck dissection are: - Anterior neck dissection - Includes Level VI lymph node removal - Supraomohyoid neck dissection - Includes Levels IA & IB, Level IIA or Levels IIA & IIB, and Level III lymph node removal - Lateral neck dissection - Includes Level IIA or Levels IIA & IIB, Level III, and Level IV lymph node removal - Posterolateral neck dissection - Includes Levels II, III, IV, & V lymph node removal lt is recommended to use the term "selective neck dissection" or "SND," followed by the levels and/or sublevels in which the lymph node are removed - e.g., SND (IB, IIA, and III), since there is variation of levels and sublevels associated with the names given to the various types of selective neck dissection.	

Figure 7. Radical neck dissection, modified radical neck dissection, and selective neck dissection (83).

Radiotherapy and chemotherapy

Early stage and locally advanced OSCC can be managed by radiotherapy alone or combined with surgery and chemotherapy (84). Positive tumor margins are locally controlled postoperatively due to postoperative radiation effect (85).

Chemotherapy has its own role in the advanced OSCC, with the favored regimens such as cisplatin, infusional 5-fluorouracil, and docetaxel (86).

Clinical trials recently showed a clear survival rate up to 11% in patients using concurrent single chemotherapy agent (cisplatin).

In some cases, in early stage OSCC surgically directed radiotherapy can be used in the form of brachytherapy, and this is recommended in cases where surgical sequelae outweigh the disadvantage of normal tissue radiation. The brachytherapy availability is limited because the patient is inducted in isolation for a period, and this is done for radiation protection purposes.

Electroporation to the margin can also be used in some selected cases (84,87).

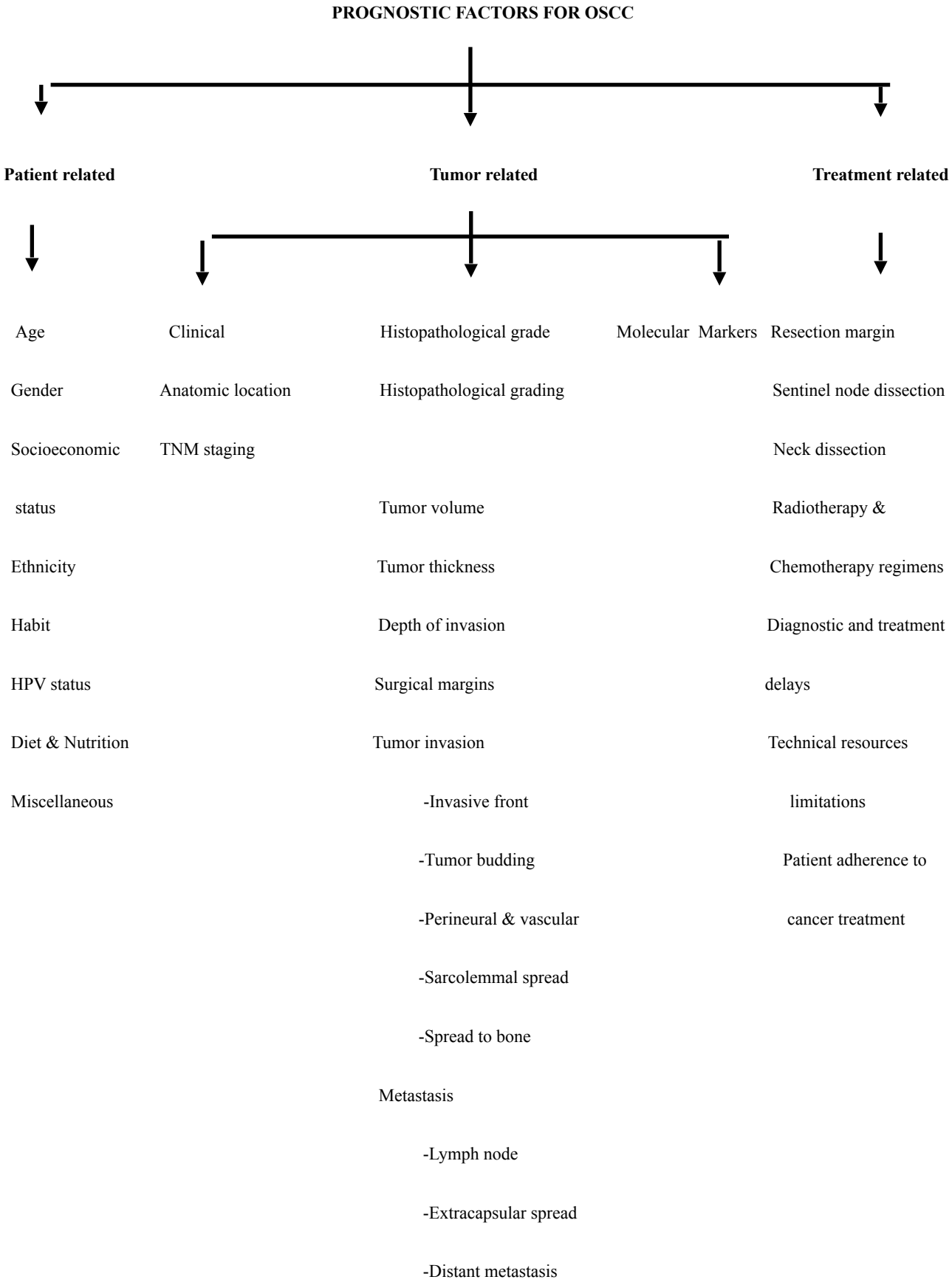


Figure 8. Working classification of prognostic determinants of OSCC (49).

1.2 Anemia

Anemia is strictly defined as a decrease in red blood cell (RBC) mass. The function of the RBC is to deliver oxygen from the lungs to the tissues accomplished by using hemoglobin (89). Anemia as a chronic disease that more often can be seen in cancer is a subject of discussion by many authors. A large cohort study by "The European Cancer Anemia Survey" (ECAS) revealed that 39.3% of all cancer patients were anemic, while in tumors of the head and neck region only 24.9% of patients were diagnosed with anemia (88).

There are three main types of anemia due to: blood loss (trauma and gastrointestinal bleeding among others), decreased red blood cell production (iron deficiency, lack of vitamin B12, thalassemia), and increased red blood cell breakdown (sickle cell anemia, infections like malaria and some autoimmune diseases) (89).

It can also be classified based on the amount of hemoglobin (Hb) in each cell (in men – less than 13 to 14 g/dL, in women – less than 12 to 13 g/dL) and mean cell volume (MCV) (microcytic anemia <80 fL, macrocytic anemia >100 fL, and normocytic anemia 80-100 fL) (90).

The red cell mass is the volume of red cells in the circulation. The normal range in women is 23–29 mL/kg, whereas in men it is 26–32 mL/kg.

- Microcytic anemia – iron deficiency anemia, thalassemia, sideropenic anemia, anemia of chronic disease.
- Normocytic anemia – an acute blood loss, a decreased production of normal blood cells (such as anemia of chronic disease, anaplastic anemia), an increased destruction of blood cells (such as hemolysis, posthemorrhagic anemia, hypersplenism), vitamin B2 and vitamin B6 deficiency.
- Macrocytic anemia – megaloblastic anemia, non-megaloblastic anemia (red cell membrane disorders producing codocytes, alcohol, association with rapid red cell turnover and reticulocytosis (90-92).

In addition, patients presenting with anemia are also at a higher risk of metastasis and worst outcome. There are several studies that have shown the effects of hemoglobin (Hb) level on patients' outcome (93-97).

Hypoxia seems to be an influencing factor for oral squamous cell carcinomas (SCC), and it is well known that to kill hypoxic cells radiation doses needed are approximately 2-3 times higher than those needed to destroy well-oxygenated cells (98).

This radiation resistance is due to the Hb level, which presumably mediates tumor response to radiation through the delivery of oxygen to the tumor (99). There is increasing evidence that low Hb levels are associated with poor tumor oxygenation.

Also, it has been found that a low Hb concentration and cigarette smoking together contribute to inadequate oxygenation of SCC of the head and neck and thus to increased radio-resistance (99).

Studies have shown that in patients with advanced head and neck carcinoma, oxygenation prior to radiotherapy has shown an effect on increasing pO2 of primary tumor more than in lymph node metastasis (100).

There are several studies about the impact of preoperative hemoglobin level on tumor control and outcome also in different primary sites of SCC, such as vulvar SSC, and these have significantly proven the preoperative level of hemoglobin as an independent prognostic factor in the inguinofemoral and distant metastases (101,102).

No study has evaluated yet the effect of hypoxia in esophageal cancer (studies suggest that metastases-associated protein 1 (MTA1) plays an important role in lymphangiogenesis and lymph node metastases (LNM) by stabilizing hypoxia inducible factor HIF-1α in esophageal cancer), in gastric cancer (studies have shown that hypoxia promotes metastasis in human gastric cancer by up-regulating the 67-kDa laminin receptor), and colon cancer (studies have shown that hypoxia enhances colon cancer migration and invasion through promotion of epithelial-mesenchymal transition) (103-107). Anemia is often considered a side effect of cancer therapy in oncology patients; however, it may occur before any antineoplastic treatment (cancer-related anemia). There is a study which has evaluated anemia in colorectal cancer, a study using electronic primary care records of patients, and it has found that low hemoglobin level and features of iron deficiency are important prognostic factors for outcome, particularly for men over 60 years (108). There are studies that have evaluated the preoperative anemia effect in patients with early stage of cervical cancer, and they have concluded that preoperative anemia is not an independent prognostic factor for survival in patients with early cervical cancer, but it is associated with poor prognostic factors (109). Some studies have evaluated the effect of preoperative anemia in advanced stage of the head and neck squamous cell carcinoma (these have shown that Hb level is an important prognostic factor for both loco-regional control and overall survival among patients with SCC head and neck

treated with surgery and postoperative radiotherapy) (110), but so far anemia/low Hb level has not been evaluated as a risk factor for regional metastases/second primary tumor occurrence in patients with early stage (T1-T2N0M0) of oral SCC.

1. Fanconi anemia	Lesion of oral mucosa like generalized erythroleukoplakia with focal ulcerations, disease of the periodontium and dental anomalies. Fanconi anemia patients are at high risk for OSCC.
2. Iron deficiency anemia	Oral manifestations include angular cheilitis, atrophic glossitis, and general mucosal atrophy.
3. Megaloblastic anemia	Oral manifestation of painful atrophy of the entire oral mucous membranes and tongue (glossitis), stomatitis as well as mucosal ulceration (recurrent aphthous ulcers).
4. Congenital hypoplastic anemia (Diamond-Blackfan anemia)	Oral manifestations of severe gingivitis, multiple carious lesions, and poor healing of recent extraction sites.
5. Sickle cell anemia	Midface overgrowth attributable to bone marrow hyperplasia, mandibular infarction that may follow over by mandibular osteosclerosis, osteomyelitis, paresthesia or anesthesia of mental nerve, asymptomatic pulpal necrosis, orofacial pain, enamel hypomineralization and diastema.
6. Thalassemia	Orofacial manifestations as ‘chipmunk face’: bossing of the skull, enlargement of the maxilla, and prominent molar eminences.
7. Aplastic anemia	Oral manifestations with multiple hemorrhages, which most often develop in patients with platelet count $<25x10^9/L$.
8. Minkovski-Chauffard hemolytic anemia	Oral manifestations of paleness of the oral mucous membrane is especially obvious at the level of soft palate, tongue, and sublingual tissues.

Figure 9. Orofacial manifestations associated with different types of anemia (111).

Hypoxia and angiogenesis

Hypoxia means a reduction in the physiological oxygen level, which leads to an angiogenic induction, resulting in growth and new blood vessel formation. It occurs typically in two ways:

- vasculogenesis, which is defined as the differentiation of precursor cells (angioblasts) into the endothelial cells and the *de novo* formation of a primitive vascular network (*in situ* formation of a new blood vessel); and
- angiogenesis, which is defined as a growth of new capillaries from pre-existing blood vessels (112).

Angiogenic factors are triggered by relative hypoxia, in cases where tissue enlargement goes beyond physiological oxygen diffusion limit. Among those angiogenic factors, the most important one is considered to be vascular endothelial growth factor (VEGF), which together with other angiogenic factors (angiopoietin-2/angiopoietin-1, Tie2, PFGF, basic fibroblast growth factor - bFGF and monocyte chemoattractant protein 1 - MCP-1) is responsible for vascular permeability, endothelial sprouting, maintenance, differentiation and remodelling, same as for cell proliferation, migration, endothelial assembly enhancement and lumen formation (113).

Understanding the angiogenic process, and which one is influenced by microenvironment and modulated by a multitude of pro- and anti-angiogenic factors, may lead to therapeutic modality of patients with ischemic vascular disease on one side, and disease characterized by excessive angiogenesis on other side (i.e. tumors). Angiogenesis is a response to hypoxia, which seems to be an inducer of VEGF via HIF-1-related transcription (114,115).

Anemia and tumor hypoxia

Anemia plays a role in tumor hypoxia, which can be defined as a low oxygen tension (pO2) in the tumor compared to the surrounding tissue of $pO2<2.5$ mmHg, but is most commonly defined as $pO2>10$ mmHg (116).

Oxygen content within tissues is dependent on a number of complex factors, such as the physical presence of oxygen, the programming of the tumor and stroma cells whether to utilize the available oxygen, vasculature, perfusion and diffusion distance that may be influenced by systemic factors such as anemia and chronic obstructive lung disease (117).

Postoperative anemia ($Hb<12$ g/dl) is an independent prognostic factor for local recurrences and free survival.

Preoperative anemia plays a significant role in overall survival. Low Hb levels may impair survival by impairing tissue and possibly tumor oxygenation causing hypoxia, and thereby reducing the effectiveness of chemotherapy and radiotherapy.

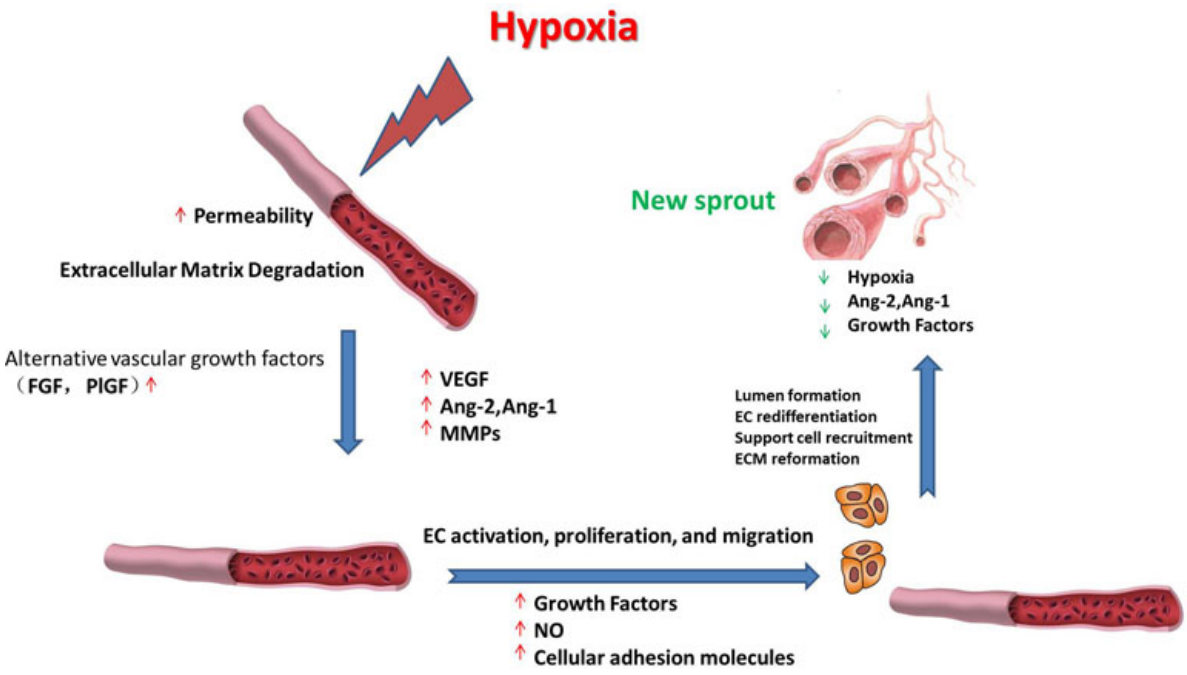


Figure 10. Schematic representation of the roles of vascular endothelial growth factor (VEGF) (118)

1.3 VEGF - Vascular endothelial growth factor

VEGF is a signal protein produced by cells (tumor cells, macrophages, platelets, keratinocytes, and renal mesangial cells), normal functions of which are to create new blood vessels (vasculogenesis and angiogenesis) during embryonic development, new blood vessels after injury, muscles after exercises, and new vessels to bypass blocked vessels, but its role is also in bone formation, hematopoiesis, wound healing and development.

VEGF is a sub-family of growth factors, the platelet-derived growth factor family of cysteine-knot growth factor (119-121)

Solid cancers cannot grow beyond a limited size without an adequate blood supply; cancers that can express VEGF are able to grow and give metastases. Vascular endothelial growth factor-C can stimulate lymph-angiogenesis (via VEGFR3) and angiogenesis via VEGFR2.

Cancer treatment strategies were designed in causing neovascular inhabitation through targeting the proangiogenic function of VEGF. This anti-VEGF therapy may play a dual role because of paracrine and autocrine VEGF loops, which exist within tumors. Chemo/radiotherapy increases the VEGF within tumors, which may protect cells from apoptosis, and this increases their resistance to conventional chemo/radiotherapy. With these anti-VEGF therapies, only the angiogenic potential of VEGF is not affected but also the the anti-apoptic function of VEGF. Thus, in many types of tumors this combination of therapies (anti-VEGF and chemo/radiotherapy) is effective (122).

Several authors have detected that low hemoglobin level is associated with an increased level of vascular endothelial growth factor (VEGF) in cancer patients, which may therefore be an indicator for the angiogenic potential and biological aggressiveness of a tumor (123).

1.4 Second primary tumors and oral field cancerization

Second primary tumors are the tumors that develop in the oral cavity in succession to the primary malignant tumors, which occurrence might vary in duration ranging from a few months to years. The concept of oral field cancerization explains the mechanisms by which second primary tumors (SPT) develop. Patients at a higher risk of SPT are those with early stage of head and neck cancer. According to ‘classical view’ explanation of SPT occurrence, large areas of the aerodigestive tissue are affected by long-term exposure to carcinogens. In this preconditioned epithelium, multifocal carcinomas can develop as a result of independent mutations, and thus would not be genetically related (124,125).

Oral carcinogenesis is a progressive disease, a multifactorial process and highly complex that occurs when epithelial cells are affected by various genetic alterations (126). The most important genetic alterations are TP53, NOTCH1 (Notch homolog 1 genes are translocation-associated [Drosophila]), EGFR (epidermal growth factor receptor), CDKN2A (cyclin-dependent kinase inhibitor 2a), STAT3 (signal transducer and activator of transcription 3), Cyclin D1, Rb (retinoblastoma) (127).

Oral carcinogenesis starts from the transformation of a number of normal keratinocytes, into an unstable keratinocyte up to a precancerization field and leading to malignant neoplastic changes, which can be transmissible to their clones. The processes involved in this transformation can be expressed via cytogenetic changes and epigenetic processes that modify the progression of cell cycle, DNA repair mechanisms, cell differentiation and apoptosis, which may be caused by random mutation, by exposure to a variety of biological factors, carcinogens or errors in the DNA repair process (128).

Genetic susceptibility

Genetic predisposition is one of the established factors in the development of oral cancer. Several studies have shown familial clustering of OC (oral cancer), such as in a group of Ashkenazi group in Israel compared to others in that country (129). Another study, which followed up first-degree relatives of 105 head and neck cancer patients, found that 31 of those subjects developed cancers of respiratory tract and upper aerodigestive tract (130). However, population-based studies to determine genetic or familial disposition to oral cancers are limited by the co-existing risk factors like smoking and alcohol. Intensive research has been done in specific genetic polymorphism in key genes involved in oral carcinogenesis.

Glutathione S-transferase M1 (GSTM1) null genotype increased significantly the risk for HNSCC (head and neck squamous cell carcinoma), found in a meta-analysis by Tripathy and Roy (131). Furthermore, the CYP1A1 polymorphysm is another susceptibility marker with 35% increased risk (132).

Genetic alterations

Genetical alterations, such as point mutations, amplifications, rearrangements and deletions, define the molecular basis of carcinogenesis (133). Several oncogenes are related to oral carcinogenesis.

Oral cancer development has been reported in aberrant expression of epidermal growth factor, K-ras, c-myc, int-2, parathyroid adenomatosis 1 (PRAD-1) and B-cell lymphoma (bcl) like oncogenes, as well as transforming growth factor alpha (TGF-alfa), which is known to promote neovascularization and mitogenesis (134).

Homozygous deletion at chromosome 9p21 is the most frequent genetic change identified in head and neck cancers (135). This can lead to genomic instability, such as loss of heterozygosity and microsatellite instability, frequently observed in oral cancers (136).

SPT – epidemiological and clinical data

Many studies have shown that nearly half of the patients are diagnosed with early stage (stage I/II) disease, at the first visit. Although treatment given at this stage is relatively successful, local recurrences and second primary cancers are counted as most common failure.

Four percent of patients with T1/T2N0M0 OSCC will develop the second primary tumor and the expected incidence of second primaries varies by head and neck site (137).

The interval between the initial disease and the SPT occurrence usually ranges from 2 to 4 years, mostly in younger patients, who underwent surgical resection of early stage OSCC presented in favorable location for treatment (138).

Tumor topography of primary tumor location (oropharynx, nasopharynx, hypopharynx, larynx, and major salivary glands) and age have been considered risk factors for SPT development (138).

Fresh fruits and vegetable intake might be effective in preventing oral cancer. Combining this with the fact that the greater cancer risk following oral cancer is localized in the oral cavity itself, diet offers a potential for some level of SPT control (139).

On the other hand, there is a little concern about radiotherapy effect, especially when the tumor is located in the oral cavity (effect on oral mucosa), where a higher risk for head and neck carcinomas (HNC) has been observed after a 10-year latency period. This may improve the treatment strategy in the future, in patents with OSCC who underwent radiotherapy treatment (140).

Consistent with this, some studies report that predictors of second primary tumors development include both continued smoking and alcohol intake, after the primary tumor resection (141,142).

On the other hand, some studies have shown that anemia (especially iron deficiency anemia) is associated with oral manifestation like burning sensation of oral mucosa, lingual varicosity, dry mouth, oral lichen planus, and atrophic glossitis (143). It is even associated with an increased risk

of oral carcinoma in patients with Plummer-Vinson syndrome (PVS) (144). No study has evaluated yet the effect of anemia independently in second primary tumor occurrence in patients with surgically treated early stage OSCC (T1/T2N0M0).

2. HYPOTHESIS

Preoperative anemia in patients with surgically treated early stage OSCC (T1-T2N0M0) is independently associated with an increased risk of regional metastases or a second primary tumor.

3. AIMS OF THE STUDY

3.1 GENERAL AIM

To evaluate the effects of preoperative reduced blood oxygen carrying capacity (anemia and low hemoglobin) on the risk of regional metastases and second primary tumor occurrence in patients with early stage (T1-T2N0M0) OSCC after primary surgical treatment.

3.2 SPECIFIC AIMS

1. To evaluate whether anemia at presentation is independently associated with the risk of regional metastases and second primary tumor occurrence in patients with early stage (T1-T2N0M0) OSCC after primary surgical treatment;

2. To evaluate whether severity of anemia is associated with the risk of regional metastases and second primary tumor occurrence in patients with early stage (T1-T2N0M0) OSCC after primary surgical treatment;

3. To evaluate whether the type of anemia (normocytic vs. microcytic/macrocytic) is associated with the risk of regional metastases and second primary tumor occurrence in patients with early stage (T1-T2N0M0) OSCC after primary surgical treatment.

4. MATERIALS AND METHODS

4.1 Subjects

This was a retrospective cohort study based on hospital databases at the University Hospital Dubrava, Zagreb, Croatia, and University Clinical Center of Kosovo, Pristina, Kosovo.

Candidates for inclusion were consecutive patients with OSCC referred to the respective institutions between 1 January 2000 and 31 December 2010, meeting the following criteria: 1) adults >18 years of age; 2) verified T1-T2N0M0 stage; 3) available data on clinical and laboratory work-up allowing for assessment of demographics, life-style/habits, anemia, and comorbidity. Patients with concurrent malignancy (such as patients with sarcoma of the soft tissue, malignant melanoma, and carcinomas of minor salivary glands) were not included.

4.2 Clinical, radiological and histopathological data

Evaluation of patient records was performed by the end of 2015, and they were assessed for occurrence of regional metastases/second primary tumors. The inclusion time-frame allowed for the longest potential censored observation of 15 years and the shortest censored observation of 5 years (patients treated by the end of 2010).

Occurrence of regional metastases/second primary tumors were considered verified based on clinical, radiological and histopathological data.

The outcome of interest was time since surgery until occurrence of regional metastases or second primary tumor, whichever occurred first ("event"). Patients who died during the observational period but without "events" or those who completed the entire follow-up without an "event" were considered censored.

4.3. Anemia definition and classification

Anemia is defined by hemoglobin levels of ≤130 g/L for males and ≤120 g/L for females. Based on anemia severity, patients are classified into three groups: "mild" (Hb level: 95-110 g/L), "moderate" (Hb level: 80-95 g/L), and "severe" (Hb level: < 80 g/L). In addition, based on mean cell volume (MCV) anemic patients are categorized into three types: normocytic – MCV=80-100 fL, microcytic –MCV<80 fL, and macrocytic – MCV>100 fL).

4.4 Statistical analysis

Continuous variables are expressed as mean+SD, and categorical (ordinal) variables are expressed as numbers (percentages). For comparisons between continuous variables, we used unpaired Student t test, and for comparisons between categorical variables, we have use chi-square test. Binary logistic regression analysis was used to investigate the effect of anemia type on regional metastases and alcohol in second primary tumor, by fitting several models.

In the regional metastases analysis, microcytic anemia was used as a dependent variable, and several risk factors and patient demographic and clinical data as covariates. In second primary tumor analysis, alcohol consumption was used as a dependent variable; age, gender, and tobacco use were used as covariates. The analyses were repeated separately for regional metastases and for a new (second) primary tumor. We used SPSS Statistics 22.0 software for data analysis.

4.5 Ethics

This study was conducted according to all currently valid and applied guidelines whose purpose was to assure proper conduction and protection of persons included in this research as examinees, including the Basics of Good Clinical Practice and Helsinki Declaration, Health Protection Law of the Republic of Croatia (NN 121/03), and Patient's Rights Law of the Republic of Croatia (NN169/04).

All collected data, including the identity of patients, and examinees, remained confidential and protected.

Prior to starting the investigation, the project proposal was submitted to the Ethics Committee of Clinical Hospital Dubrava, University of Zagreb School of Medicine.

A written approval for these documents was obtained.

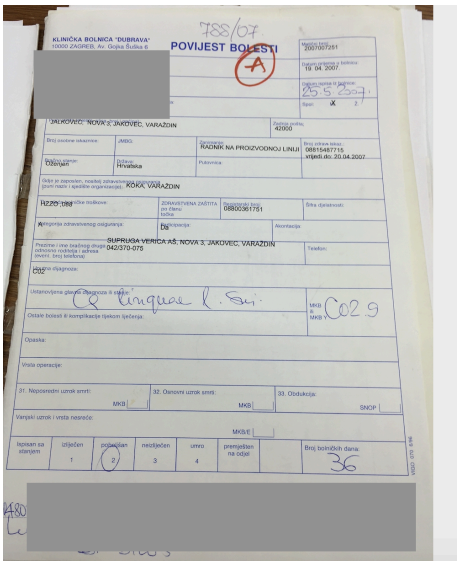
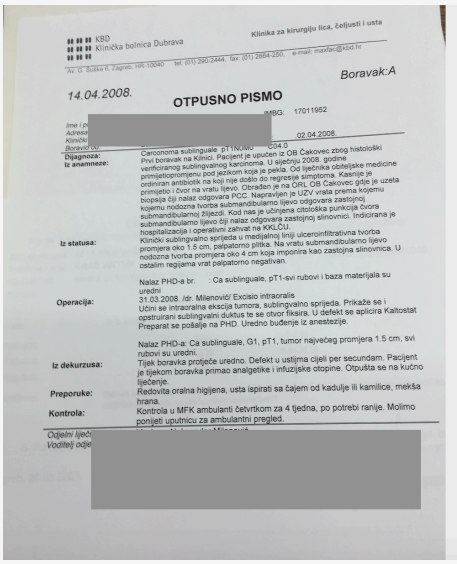
4.6 Samples and demonstration

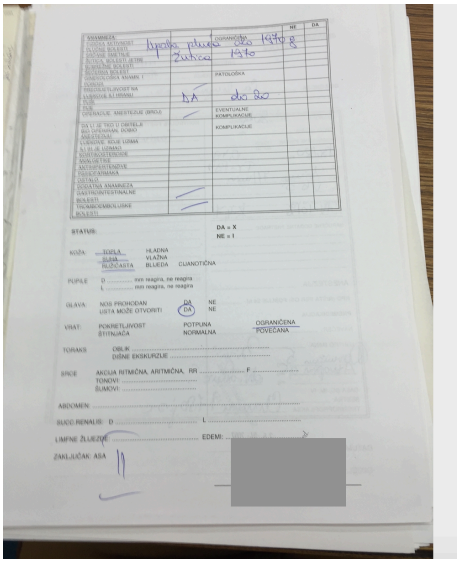
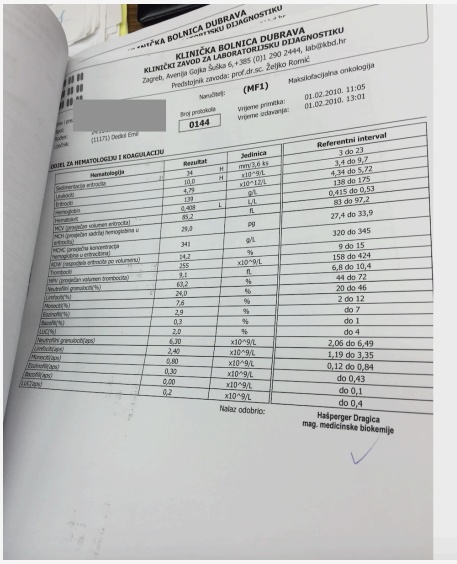
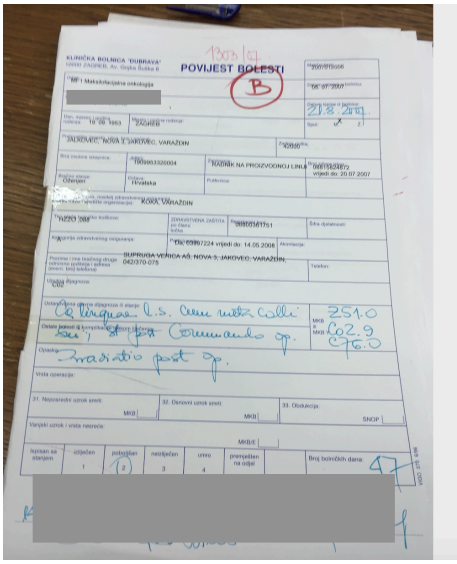
Patient samples were collected in Clinical Hospital Dubrava and University Clinical Center of Kosovo.

We obtained access to the University Hospital of Dubrava databases, and from more than 1,200 oncologic patients, we selected more than 1,100 patient histories.

From those, more than 400 hundred were with complete “main data”, meaning patients with T1/ T2N0M0 stage, data about preoperative Hb and MCV, later regional metastasis, and later second primary tumor occurrence.

From those, there were patient histories that had “main data” but missing “accessory data”, such as tobacco and alcohol consumption, comorbidity, and tumor location.

First hospitalization of the patient with early stage T1/T2 N0 M0	Usually signed with an ‘A’	
First hospitalization of the patient	We collected data such as: Age (56 years) Sex (male) First hospitalization (2008) Location of the tumor (Ca linguae lat. dex.) Stage (T1 N0 M0) Histopathologic diagnosis (Carcinoma squamosum Gr 2)	

First hospitalization of the patient	We collected data such as: -Tobacco consumption Number of years he/she smoked (20 years) Number of cigarettes per day (more than 20) -Alcohol consumption Type of alcohol (beer and wine) Daily intake amount (0.5 l beer, and 2 glasses of wine) Number of years he/she consumed alcohol (more than 15 years) -Comorbidity We saw patients with a chronic disease and to check if those had any relationship with data of interest to us (patients suffering from Dg. Gastritis chronica).	
First patient hospitalization	Complete blood count preoperative data. Here we checked hemoglobin (Hb) level and mean cell volume (MCV) range In this patient: Hb level is 139 g/L MCV range is 85.2 fL	
Second patient history	Usually signed with ‘B’	

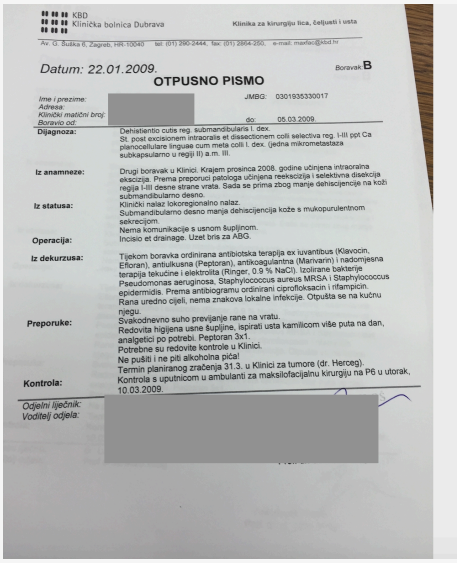
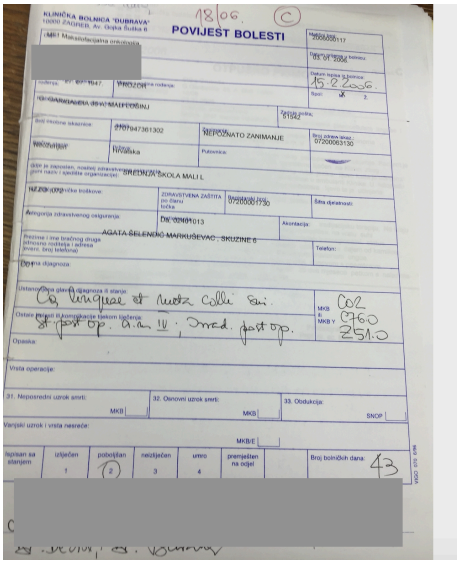
Second patient hospitalization	Here we collected data for: Regional metastasis (the year patient visited after the first hospitalization) SPT (second primary tumors). In this patient, regional metastasis appeared 2 years after the primary surgery.	
Third patient history	Usually signed with C. It may be continued with D, E, F. We even had a history signed with M. Here we checked if the patient had: Regional metastasis (the year patient visited after the first hospitalization) SPT (second primary tumors) Second primary tumor appeared in this patient 3 years after the first surgery.	

Figure 11. An example of an early stage OSCC patient parameters who had regional metastasis and SP.

5. RESULTS

Clinical data

In this study, we included 304 patients with OSCC stage T1-T2N0M0 (mean age 59.5±11.0, 79% males). Of those, 32.9% (n=100) of patients had regional metastases, and 13.2% (n=40) had second primary tumors; 10.2% (n=31) of patients had anemia (Table 4); 49.3% (n=150) of patients consumed tobacco, and 26.3 (n=80) consumed alcohol. Most of the patients' data were collected in Zagreb (89%) and only 11% in Pristina (Table 4). OSCC was observed in different locations, most commonly in sublingual (n=143), lingual (n=141), and retromolar (n=61) region, followed by mandibular gingiva (n=37), maxillary gingiva (n=16), and buccal mucosa (n=24) (Figure 12). All of the continuous variables were normally distributed (Figure 13).

Table 4. Patients' clinical and demographic data

		n=304 (%)	Missing, n (%)
Age, mean (SD)		59.5±11.0	0
Sex			
	female	63 (20.7)	0
	male	241 (79.3)	
Regional metastases			0
	yes	100 (32.9)	
	no	204 (67.1)	
Tobacco			49 (16.1)
	yes	150 (49.3)	
	no	105 (34.5)	
Alcohol			57 (18.8)
	yes	80 (26.3)	
	no	167 (54.9)	
Center			0
	Zagreb	274 (89)	
	Pristina	30 (11)	

Hemoglobin, mean (SD)		140.1±16.2	0
MCV, mean (SD)		93.8+6.4	0
Anemia			
	yes	31 (10.2)	0
	no	273 (89.8)	
Microcytic			0
	yes	14 (4.6)	
	no	290 (95.4)	
Macrocytic			0
	yes	5 (1.6)	
	no	299 (98.4)	
Normocytic			0
	yes	12 (3.9)	
	no	292 (96.1)	

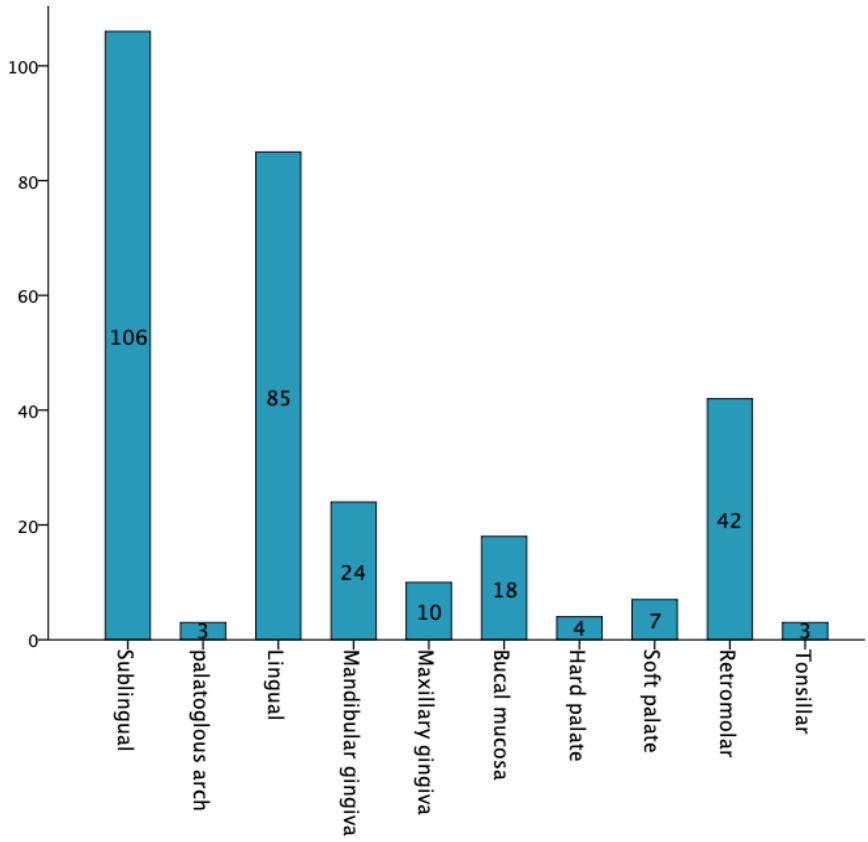


Figure 12. Frequency of OSCC in different regions.

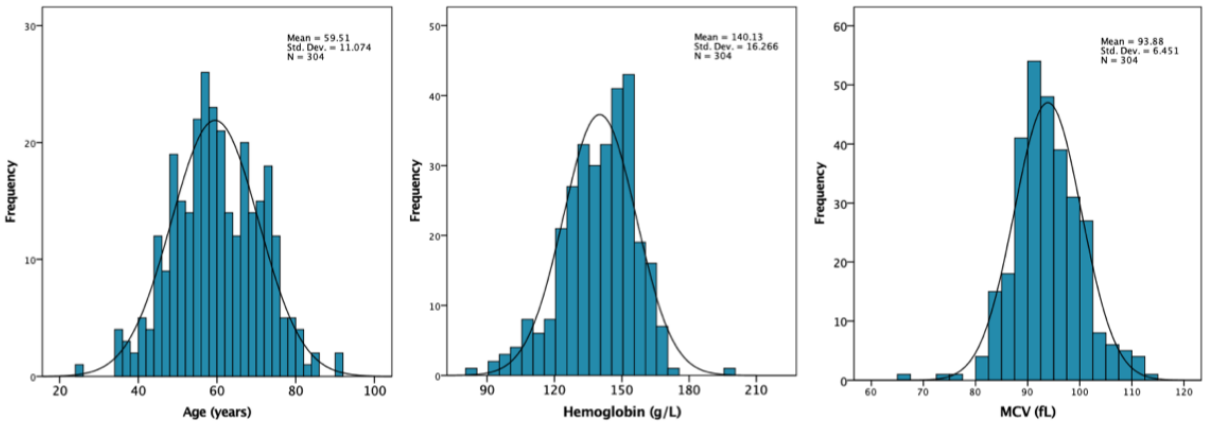


Figure 13. Distributions of continuous variables.

Regional metastases

Regional metastases were present in 100 patients. Mean age of patients with regional metastases was 58.8±10.3, and most of them were males (n=75).

Regional metastases were significantly more frequent in patients with microcytic anemia (71.4%) compared to those without microcytic anemia (31.0%), p=0.002 (Table 5, Figure 14). MCV (as a continuous variable) was lower in patients with regional metastases (92.8±8.5 vs. 94.3+-5.7, p=0.04). Even though hemoglobin values were lower in patients with regional metastases, they were not significantly different (138±16.8 vs. 140±15.9, p=0.23) (Table 5, Figure 14).

Table 5. Comparison between different variables and regional metastases in patients presenting with T1-T2N0M0 oral squamous cell carcinoma (OSCC). Regional metastases were significantly more frequent in patients with microcytic anemia. A lower MCV (as a continuous variable) was also significantly associated with regional metastases.

Variables		**Regional metastases, n (%)**		**p**
		yes	**no**	
Age, mean ± SD		58.8±10.3	59.8±11.4	0.16*
Sex				
	female	25 (39.7)	38 (60.3)	0.128

	male	75 (31.1)	166 (68.9)	0.128
Smoking				
	yes	48 (32)	102 (68)	0.935
	no	35 (33.3)	70 (66.6)	
Alcohol				
	yes	30 (37.5)	50 (62.5)	0.54
	no	51 (30.5)	116 (69.4)	
Anemia				
	yes	13 (41.93)	18 (58.0)	0.176
	no	87 (31.8)	186 (68.1)	
Microcytic anemia				
	yes	10 (71.4)	4 (28.5)	**0.002**
	no	90 (31.0)	200 (68.9)	
Macrocytic anemia				
	yes	1 (20)	4 (80)	0.53
	no	99 (33.1)	200 (66.8)	
Normocytic anemia				
	yes	2 (16.6)	10 (83.3)	0.22
	no	98 (33.5)	194 (66.4)	
MCV, mean ± SD		92.8±8.5	94.3±5.7	**0.04***
Hemoglobin, mean ± SD		138±16.8	140±15.9	0.23*

**Analyses were performed using independent sample Students t test. In other analysis we used chi-square test.*

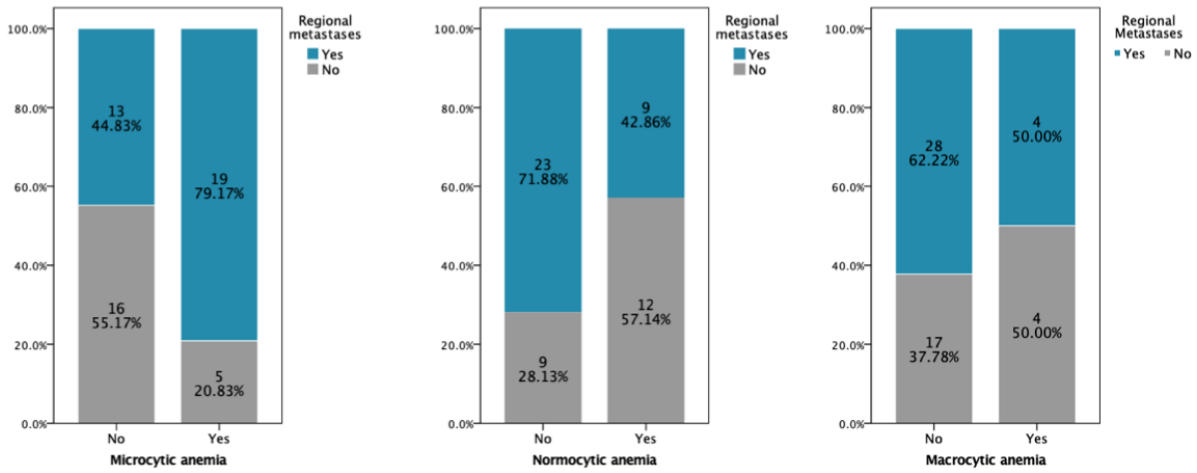


Figure 14. Regional metastases were more frequent in patients with microcytic anemia.

We used binary logistic regression analysis, which showed that microcytic anemia was associated with regional metastases with OR 5.55 (95% CI 1.6 to 18.1), p=0.005. Even after adjusting for several other risk factors, age and sex, microcytic anemia remained significantly associated with an increased risk of regional metastases with OR of 5.18 (95% CI 1.52 to 17.2), p=0.008. MCV (as a continuous variable) was also significantly associated with an increased risk of regional metastases (Table 6, Figure 15).

Regional metastases occurred most frequently in cases when tumor was located in the lingual region (63%), maxillary gingiva (56%), and soft palate (63%). They were less commonly present in hard palate (20%), palatoglossus arcus (40%), and tonsillar (40%) locations. In general, however, the frequency of regional metastases was not significantly related to tumor location (Figure 16).

Table 6. Binary logistic regression analysis of different risk factors and microcytic anemia at the time of diagnosis associated with regional metastases in patients presenting as T1-T21N0M0 at the time of diagnosis. Microcytic anemia and MCV were the only variables associated with regional metastases as an outcome. Microcytic anemia remained significantly associated with regional metastases even after adjusting for different risk factors.

Model 1: Microcytic anemia adjusted for age;

Model 2: Microcytic anemia adjusted for age and sex;

Model 3. Microcytic anemia adjusted for age, sex, tobacco use and alcohol consumption.

Variable	OR	95% confidence interval	p
Microcytic anemia	5.55	(1.6-18.1)	**0.005**
Tobacco	1.06	(0.62-1.8)	0.82
Alcohol consumption	0.73	(0.41-1.28)	0.27
Age	1.01	(0.98-1.03)	0.48
Sex (male)	1.22	(0.66-2.25)	0.51
Hemoglobin	1.01	(0.99-1.02)	0.22
MCV	1.008	(1.005-1.01)	**0.001**
Microcytic anemia (model 1)	5.58	(1.70-18.30)	**0.005**
Microcytic anemia (model 2)	5.102	(1.5-17.2)	**0.009**
Microcytic anemia (model 3)	5.18	(1.52-17.2)	**0.008**

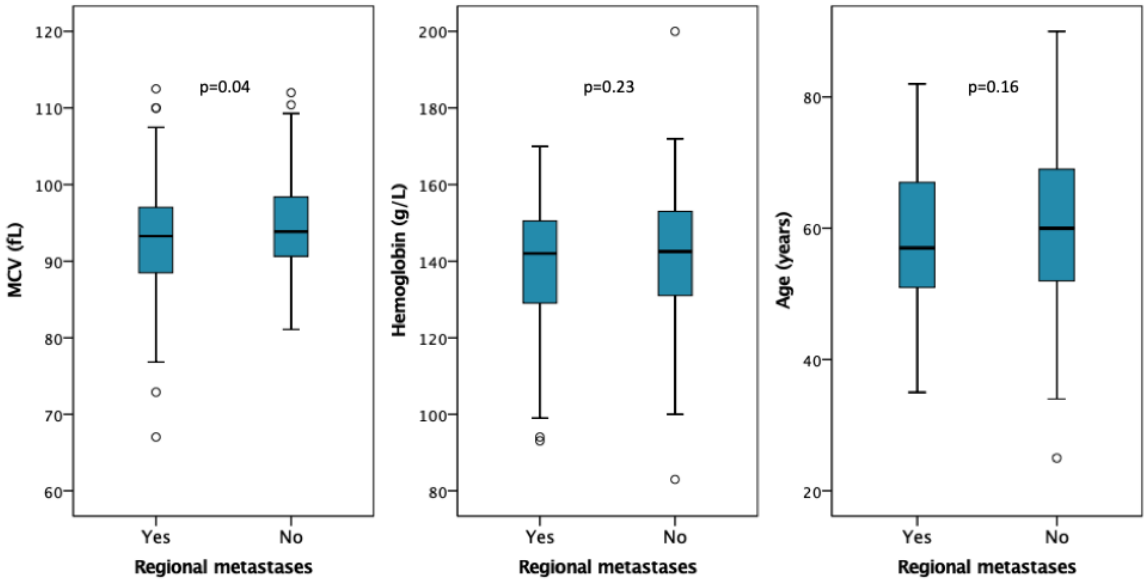


Figure 15. Comparison of MCV, hemoglobin level, and age between patients with and without regional metastases.

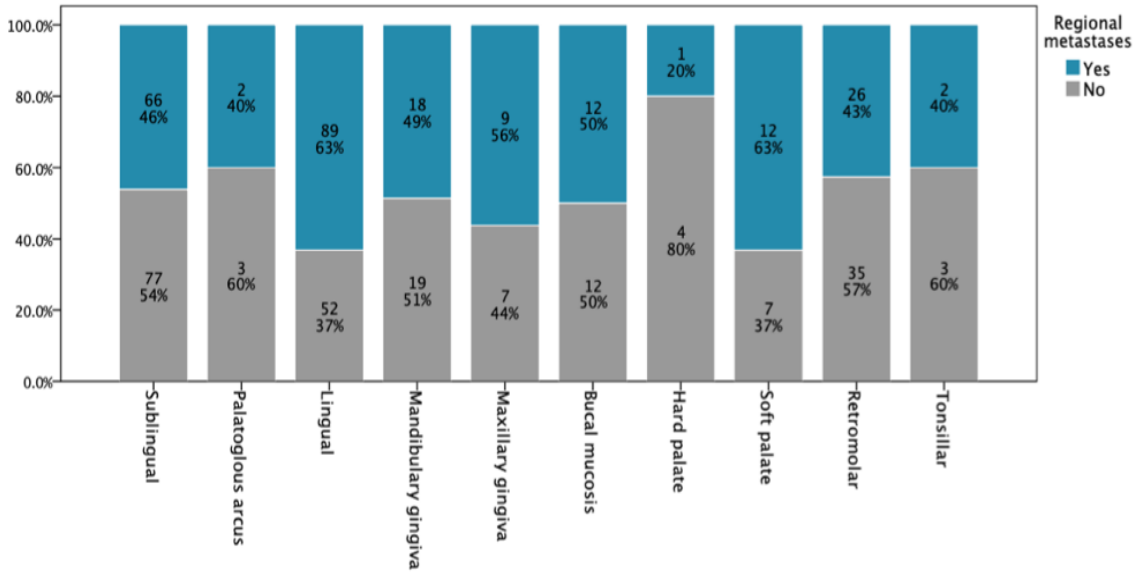


Figure 16. Frequency of regional metastases in OSCC different locations.

Second primary tumor

Second primary tumor was present in 40 patients. Mean age of patients with second primary tumor was 62.7±12.0, significantly greater compared to patients without second primary tumor 59.0±10.8 (p=0.04). Most patients with second primary tumors were males (n=30) (Table 7). Alcohol consumption was associated with an increased risk of second primary tumor occurrence (Table 7). Although hemoglobin was lower in patients with second primary tumor (137.7±16.1 vs. 140.5±16.2), the difference was not significant, p=0.31.

Table 7. Comparison between different variables and second primary tumor in patients presenting with T1/T2N0M0 OSCC. Second primary tumors were significantly more frequent in older age and alcohol consumption patients.

Variables	Second primary tumor, n (%)		p
	yes	no	
Age, mean ± SD	62.7±12.0	59.0±10.8	**0.04***
Sex			
female	10 (25)	53 (20.1)	0.47
male	30 (75)	211 (79.9)	
Smoking			
yes	19 (67.9)	131 (57.7)	0.303
no	9 (32.1)	96 (42.3)	
Alcohol			
yes	14 (51.9)	66 (30.0)	**0.02**
no	13 (48.1)	154 (70.0)	
Anemia			
yes	4 (10.0)	27 (10.2)	0.61
no	36 (90.0)	273 (89.8)	
Microcytic anemia			
yes	3 (7.5)	11 (4.2)	0.38
no	37 (92.5)	253 (95.8)	
Macrocytic anemia			
yes	0 (0)	5 (1.9)	0.53
no	40 (100)	259 (98.1)	
Normocytic anemia			
yes	1 (2.5)	11 (4.2)	0.51
no	39 (97.5)	253 (95.8)	
Anemia severity			
mild	36 (90)	273 (89.8)	0.95
moderate	3 (7.5)	22 (8.3)	
severe	1 (2.5)	5 (1.9)	
MCV, mean ± SD	94.7±6.8	93.7±6.3	0.35*
Hemoglobin, mean ± SD	137.7±16.1	140.5±16.2	0.31*

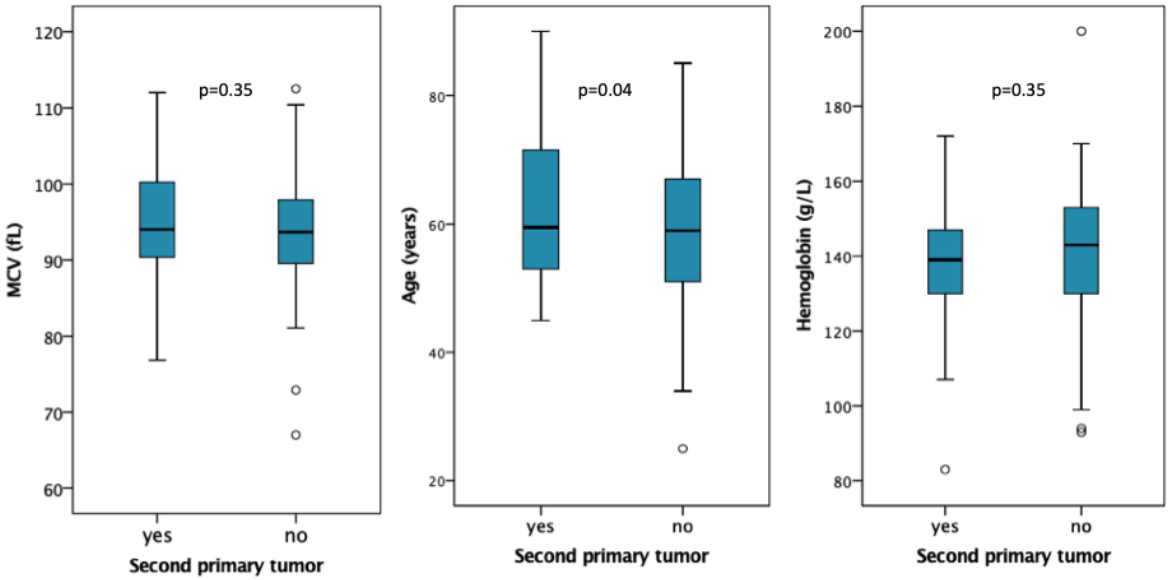


Figure 17. Comparison of MCV, hemoglobin level, and age between patients with and without second primary tumors.

In a binary logistic regression analysis, including alcohol consumption as a dependent variable and age, sex and tobacco as covariates, alcohol consumption was independently associated with higher frequency of metastases with OR of 5.18 (95% CI 1.52-17.2), p=0.008 (Table 8).

Table 8. Binary logistic regression analysis of anemia and risk factors at the time of diagnosis associated with second primary tumor occurrence in patients presenting with OSCC at T1-T2NM0. Alcohol consumption and older age were significantly associated with regional metastases as an outcome. Alcohol consumption remained significantly associated with regional metastases even after adjusting for other risk factors.

Model 1: Microcytic anemia adjusted for age;

Model 2: Microcytic anemia adjusted for age and sex;

Model 3. Microcytic anemia adjusted for age, sex and tobacco use.

Variable	OR	95% confidence interval	p
Alcohol consumption	2.51	(1.12-5.63)	**0.025**
Age	1.03	(1.02-1.04)	0.012

Tobacco	1.54	(0.67-3.56)	0.30
Anemia	1.02	(0.33-3.10)	0.96
Sex (male)	1.32	(0.61-2.88)	0.47
Hemoglobin	1.01	(0.99-1.03)	0.22
MCV	0.97	(0.92-1.02)	**0.009**
Alcohol consumption (model 1)	2.57	(1.14-5.80)	**0.023**
Alcohol consumption (model 2)	2.73	(1.19-6.27)	**0.018**
Alcohol consumption (model 3)	2.78	(1.01-7.63)	**0.047**

Second primary tumors occurred most frequently in cases when the tumor was located in soft palate (43%), buccal mucosa (28%) and hard palate (25%). They were less commonly observed in patients with the tumor located in lingual (12%), sublingual (8%), and tonsillar (0%) regions. In general, however, the frequency of second primary tumor was not significantly related to tumor location.

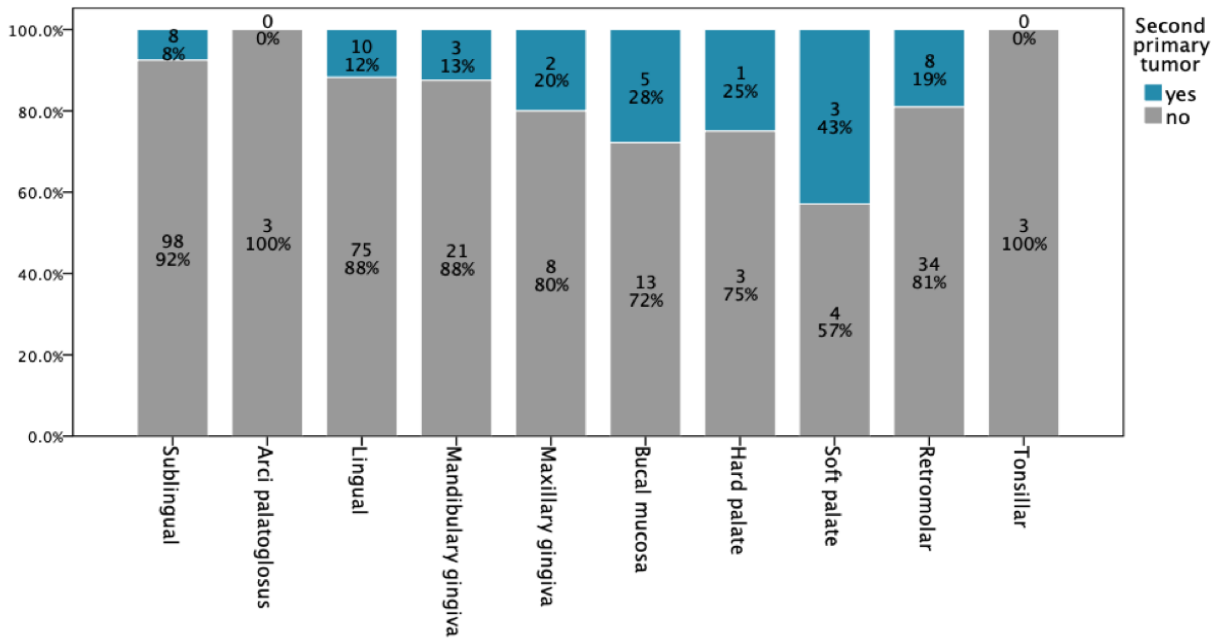


Figure 18. Frequency of second primary tumors in different locations.

Two centers

Patients for the present study were selected from two different centers, in Zagreb (n=274) and Pristina (n=30). Patients selected in Zagreb were more frequently associated with second primary tumors and patients selected in Pristina had more frequently regional metastases. However, the comparison for these variables between two centers was not significantly different (Figure 19).

Patients selected in Zagreb were more frequently alcohol consumers, and those selected in Pristina were more tobacco users. Anemic patients were proportionally distributed between the two centers (Figure 20).

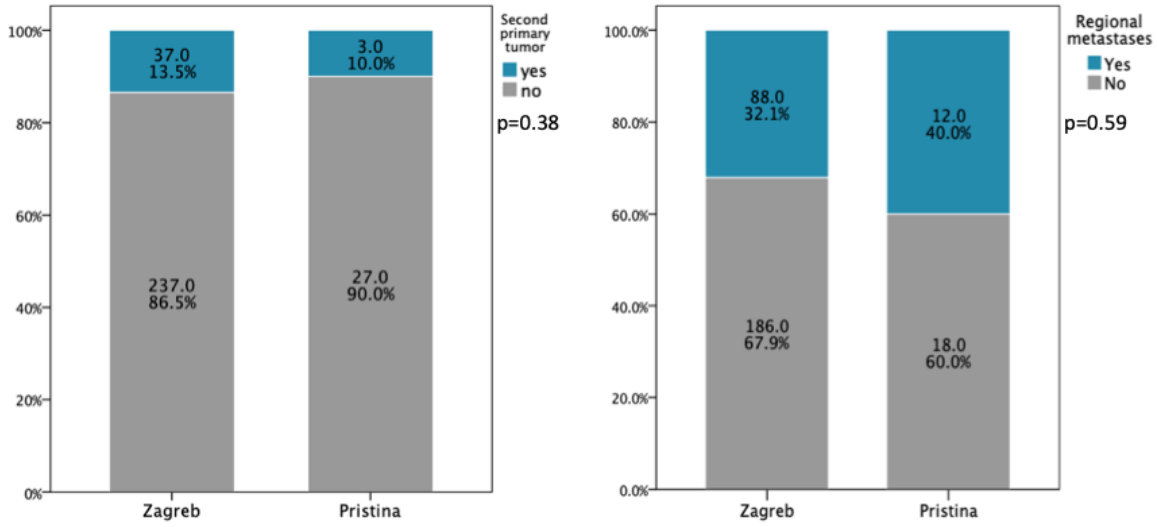


Figure 19. Comparison of the regional metastases and second primary tumors between the two centers, in Zagreb and Pristina.

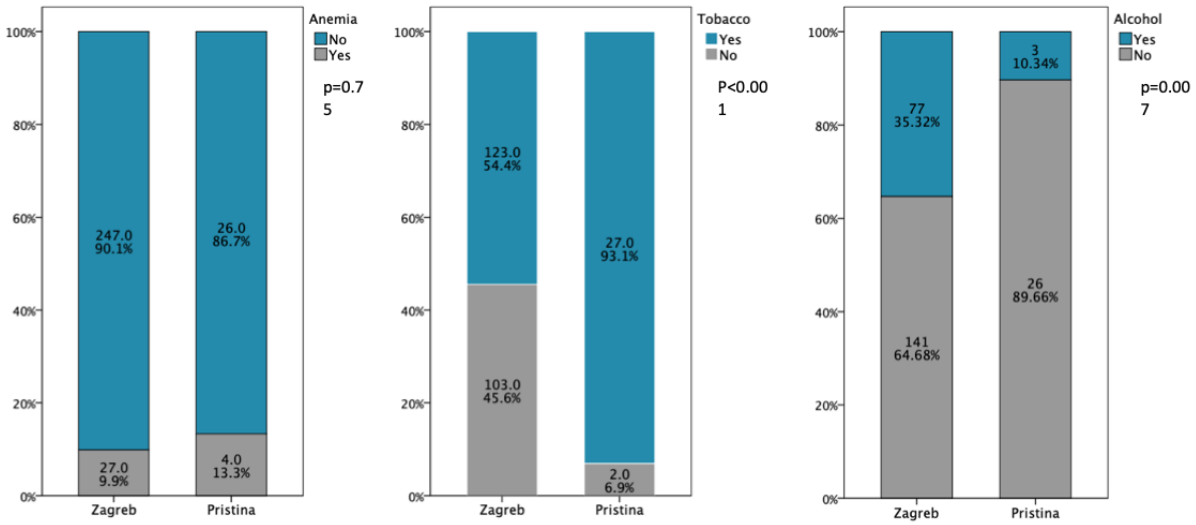


Figure 20. Anemia, tobacco and alcohol consumption comparison between the two centers.

6. DISCUSSION

Our findings

The present study shows that in patients with OSCC presenting at early stages (T1-T2N0M0), microcytic anemia and lower MCV values, but not anemia and hemoglobin levels, were associated with an increased frequency of regional metastases. The association between microcytic anemia and regional metastases remained significant even after adjusting for age, sex, tobacco and alcohol consumption. Older age and alcohol consumption were significantly associated with second primary tumor occurrence. Second primary tumors occurred almost three times more frequently in alcoholic patients with T1-T2N0M0 OSCC in a model adjusting for several risk factors.

Data interpretation

There are controversial data on the effect of anemia on the outcome in patients with different types of cancer. Hefler et al. have evaluated the effect of anemia in vulvar cancer patients, and in univariate analysis, anemia was not associated with a shortened disease-free, but with a shortened overall survival of patients with vulvar cancer. The multivariate analysis showed, however, that Hb pretreatment did not give any prognostic information on disease-free and overall survival (145).

In addition, there is conflicted evidence on the effect of blood transfusion prior to surgical intervention and radiotherapy. In the recent years, there has been a growing evidence that low hemoglobin level is associated with poor tumor oxygenation, and increasing hemoglobin concentrations are correlated with lower hypoxic tissue fractions. Therefore, several authors advocate blood transfusions before radiotherapy for anemic patients (146).

There are several pathophysiological mechanisms intended to explain the effect of anemia on the outcome of patients with cancer. An important factor to consider in the therapy and progression of OSCC is the correlation between the number of blood vessels and OSCC regional metastases. Solid cancer cannot grow beyond a limited size without an adequate blood supply, so angiogenesis is a crucial factor in successful tumor growth and metastasis. On the other hand, lymphangiogenesis correlates with regional lymph node metastasis (147-150).

A common feature of the tumor is the HIF-1 transcriptional changing during hypoxia. This HIF-1 activation as a response by the neoplastic cell to adapt to the hypoxic environment, results in dimerization of beta subunit (HIF-1 beta), which plays a critical role in tumor growth and angiogenesis by inducing VEGF and lymphangiogenesis by inducing VEGFc via VEGF3R receptors (150).

The role of HIF-1 alfa expression in the malignant progression of OSCC through the facilitation of the process of malignant cell adaptation in hypoxic microenvironment, invasive properties, cell survival and angiogenesis, has been discussed by many studies.

Ribeiro et al. have evaluated 93 patients with early stage OSCC, analyzing their clinicopathologic data and correlating them with distinct OSCC subtypes (superficial OSCC, conventional OSCC and basaloid OSCC) after HIF-1 expression. According to their study, no expression of HIF-1 is detected in oral mucosa, and most neoplastic cells showing HIF-1 alfa expression were in superficial OSCC, and less in conventional OSCC and basaloid OSCC, suggesting a distinct biology among tumor subtypes. In addition, in nodal involvement immunohistochemistry analysis, the expression of HIF-1 alfa was high as in the corresponding primary tumors. These data were significant on superficial OSCC, suggesting its role in its growth pattern in the early development (151).

Liang et al. have evaluated the correlation between VEGFc, HIF-1, and nodal involvement in 63 patients with radical resection surgery without preoperative chemotherapy. Through immunohistochemistry, they detected the presence of HIF-1 expression in the nucleus and the cytoplasm of OSCC cell and VEGFc homogeneously distributed into the OSCC cell cytoplasm and some stromal cells but not in normal mucosa of the oral cavity. According to their results, high expression of HIF-1 alfa and VEGFc was correlated with the presence of lymph node metastasis and also with TNM stage (152).

A retrospective study by Yovino et al., on patients with early stage non-small cell carcinoma of the lung (NSCLC), analyzed the prognostic factors associated with survival in patients treated with surgery alone. The anemic group Stage I NSCLC and presenting with Hb<12 mg/dl, predicted a worse outcome. According to the authors, this could explain poorer outcomes of anemic patients with solid tumors who undergo radiotherapy (153).

In patients with SCC of oropharynx, Aebersold et al. have shown that HIF-1 was overexpressed in most of those patients, and the degree of HIF-1α immunoreactivity had a strong predictive and prognostic significance in patients with radiotherapy treatment. However, the extent of this

hypoxia-treatment failure relationship remains to be clarified via HIF-1α expression and tumor oxygenation (154).

By contrast, some authors have shown that HIF-1α overexpression is associated with good patient outcome. Santos et al. showed significant relationship between strong HIF1α expression and lower disease relapse and increased local disease-free survival, suggesting that weak HIF-1α expression is an independent risk factor for local disease relapse in patients with early stage OSCC. However, in 'surgery only' cases, there was no correlation between HIF1α expression and disease-free survival or local disease-free survival; in contrast, there were correlations between HIF1α expression and disease-free survival and local disease free-survival for patients with postoperative radiotherapy, suggesting an interaction between tumor vascularization and radiotherapy response (155).

Becker et al. have concluded that primary tumors and their lymph node metastasis in advanced SCCHH show different patterns of oxygenation regarding the proportion of hypoxic measurements and median pO2. According to them, in patients with such carcinomas, the oxygenation data taken from one site are related with tumor oxygenation data from the other site, so the measurements in any anatomic site would be sufficient to estimate the tumors oxygenation status, and the correlation between pO2 and hemoglobin level requires further investigation (156).

There are also clinical studies that have evaluated the effect of anemia/low hemoglobin (Hb) level, in oral squamous cell carcinomas. A study by Cordella et al., from the University Hospital of Zurich, found that patients with OSCC (early stage and advanced stage) presenting with low hemoglobin level were found to be associated with the development of lymph node metastases as well as with the development of local recurrence, but no association with initial T status was found (157).

Tumor stage and tumor thrombocytosis were significantly associated with a shortened disease-free and overall survival in a univariate analysis. Tumor anemia is associated with a shortened overall survival of patients with vulvar cancer, but not with a shortened disease-free. A multivariate Cox regression model considering tumor anemia, tumor stage and tumor thrombocytosis showed that pretreatment Hb and platelet count did not confer additional prognostic information to that already obtained by the established prognosticator tumor stage on overall survival and disease free, in patients presenting with vulvar cancer (145).

A study by Haremza et al. has shown that over 25% of patients with OSCC presented with second primary tumors, with poor prognosis, regardless of oral tumor locations (158). In our study,

metastases occurred in 32.9% of the patients, and second primary tumors were reported in 10.2% of cases with OSCC.

A study by Schwarts et al. study has confirmed a high rate of second primary tumors in patients with initial head and neck malignancies. Their study shows 19% of SPT from the total number of patients in a 5-year follow-up. The probability of developing second primary tumor 5 year after surgically removed primary tumors was 22%. The incidence based on location was 46% in the tongue, and the difference between smokers and non-smokers was 3% vs. 26%, and between people who consumed alcohol was 5% for non-drinkers vs. 32% for drinkers. Anatomic site of the primary tumor and the age of patients were two independent variables that influenced the occurrence of the second primary tumor, and the survival rate after second primary tumor occurrence was influenced significantly by the location of the second primary tumor (159).

Katada et al. evaluated the association between multiple areas of dysplastic squamous epithelium with tobacco and alcohol, in the development of SCC of the esophagus or head and neck cancer. Alcohol abstinence decreased the risk of secondary malignancies in the esophagus, whereas smoking abstinence did not (160). Likewise, our study showed that alcohol consumption, but not tobacco use, was independently associated with an increased risk of second primary tumor occurrence.

The strength of this study lies in the long-term follow-up period of patients (minimum five years). In addition, we included patients with the very early stage of OSCC (T1-T2N0M0). Compared to other studies that showed an association between anemia and low hemoglobin level (161) in our study it was only microcytic anemia and low MCV levels that were associated with an increased risk of regional metastases.

Study limitations

This was a retrospective study, a type known for its limitations. Information on patients' clinical data and tumor information was acquired from patients' medical records, saved as a hard copy in patients' folders, and this represents another limitation. However, we attempted to minimize this limitation by taking a double check of the documents for all the information included in the analysis.

Some data on tobacco and alcohol consumption were not completed for all patients. In addition, in the analysis, we did not include other comorbidities such as cardiovascular, cerebrovascular, and

respiratory diseases that could aggravate systemic and tissue hypoxia and could influence the final results.

7. CONCLUSION

In patients presenting with early stage OSCC, microcytic anemia at presentation was independently associated with an increased risk for regional metastases, and alcohol consumption was associated with an increased risk for second primary tumors. Knowing that microcytic anemia is most frequently associated with iron deficiency, iron supplementation or even blood transfusion before operation and on the following days might improve patient outcome. However, such an implication can be justified once our results are reproduced prospectively in a larger patient cohort.

8. SAŽETAK (ABSTRACT IN CROATIAN)

Cilj ovog istraživanja bio je procijeniti značaj smanjenja prijeoperativnog kapaciteta oksigenacije krvi (anemija) na pojavu regionalnih metastaza i drugih primarnih tumora u bolesnika s ranim stadijem planocelularnog karcinoma usne šupljine nakon primarnog kirurškog liječenja.

Kandidati za uključivanje u istraživanje bili su konsekutivni bolesnici s planocelularnim karcinomom usne šupljine iz dvije institucije (KB Dubrava i KBC Kosovo), koji su bili liječeni u razdoblju od 1. siječnja 2000. do 31. prosinca 2010. i koji su zadovoljavali sljedeće kriterije: 1. stariji od 18 godina; 2. dokazani T1-T2N0M0 stadiji bolesti; 3. prisutni podaci o kliničkim i laboratorijskim parametrima koji omogućuju procjenu, kao i demografski podaci o stilu života i navikama, anemiji i komorbiditetu. Bolesnici s multiplim primarnim tumorima i bolesnici koji su ranije liječeni zbog tumora u području glave i vrata bili su isključeni iz istraživanja. Vrijeme praćenja bolesnika uključivalo je vremenski okvir od najdužeg potencijalnog cenzuriranog promatranja od 15 godina do najkraćeg cenzuriranog promatranja od 5 godina (bolesnici liječeni do kraja 2010. godine).

Pojava regionalne metastaze ili drugog primarnog tumora bila je dokazana na temelju kliničkog pregleda, radiološki i patohistološki. Binarna logistička regresija bila je upotrijebljena da se istraži učinak anemije i tipova anemije nakon prilagođavanja za druge faktore rizika.

Rezultati: u ovoj studiji uključili smo 304 pacijenata koji su bili u ranom stadiju OSCC, srednje dobi 59,5±11,0 godina, a 79% pacijenata bilo je muškog spola. Regionalne metastaze bile su identificirane u 100 (32,9%) pacijenata, a sekundarni primarni tumori u 40 (13,2%) pacijenata. Mikrocitna anemija bila je značajno povezana s većim rizikom od regionalnih metastaza (71,4% vs. 28,5%, p=0,002), s OR od 5,18 (95% CL 1,52 do 17,2, p=0,009). Konzumacija alkohola bila je nezavisno povezana s povećanjem rizika od sekundarnih primarnih tumora s OR od 2,78 (95% CL 1,01 do 7,63, p=0,04).

Zaključak: Kod pacijenata s OSCC mikrocitna anemija bila je nezavisni prediktor regionalnih metastaza, a konzumacija alkohola je bila nezavisni prediktor za sekundarne primarne tumore. Pacijenti sa sekundarnim primarnim tumorima obično su starije dobi.

Ključne riječi: planocelularni karcinom usne šupljine, hemoglobin, regionalne metastaze, drugi primarni tumor

9. ABSTRACT IN ENGLISH

Prognostic significance of preoperative anemia on occurrence of regional metastases and other primary tumors in patients with early stage oral squamous cell carcinoma

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Year: 2023

The aim of this study was to evaluate the preoperative effects of reduced blood oxygen carrying capacity (anemia) on the risk of regional metastases/second primary tumor occurrence in patients with early stage (T1-T2N0M0) oral squamous cell carcinoma (OSCC) after primary surgical treatment.

Methods: Candidates for inclusion were consecutive patients with OSCC referred to the respective institutions (UH Dubrava and UCCK) between 1 January 2000 and 31 December 2010, meeting the following criteria: 1) adults >18 years of age; 2) verified T1-T2N0M0 stage; 3) available data on clinical and laboratory work-up allowing for assessment of demographics, life-style/habits, anemia, and comorbidity. Patients with concurrent malignancy were not included. The inclusion time-frame allowed for the longest potential censored observation was 15 years and the shortest censored observation was 5 years (patients treated by the end of 2010).

Occurrence of regional metastases and second primary tumors were verified based on clinical, radiological, and histopathological data. Binary logistic regression was used to investigate the effect of “anemia” and “anemia types” after adjusting for other risk factors.

Results: In this study, we included 304 patients with early stage OSCC, mean age was 59.5±11.0, and 79% of the patients were male. Regional metastases were identified in 100 (32.9%) patients, and second primary tumors in 40 (13.2%) patients. Microcytic anemia was significantly associated with higher risk of regional metastases (71.4% vs. 28.5%, p=0.002), with an OR of 5.18 (95% CI 1.52 to 17.2, p=0.009). Alcohol consumption was independently associated with an increased risk of second primary tumors with an OR of 2.78 (95% CI 1.01 to 7.63, p=0.04).

Conclusions: In patients with OSCC, microcytic anemia was an independent predictor of regional metastases, and alcohol consumption was an independent predictor of second primary tumor. Patients with second primary tumors tend to be of older age.

Keywords:
Oral squamous cell carcinoma, anemia, regional metastases, second primary tumors

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11. CURRICULUM VITAE

Enis Gllareva was born on October 30, 1984, in Gllogoc, Kosovo. There he completed primary school and high school in Gllogoc. He studied at the Medical Faculty/Dentistry branch, in University of Prishtina and graduated in the year 2010 with an average grade of 9.81.

In the year 2012/2013 started attending the specialisation in Maxillofacial surgery in University Clinical Center of Kosova. In the year 2018 he took the title 'Maxillofacial surgeon'.

In the year 2013/2014 enrolled in the post-graduated doctoral study in Biomedicine and Health Sciences, PhD program in English in University of Zagreb School of Medicine.

He is employed in University of Prishtina (Medical Faculty/Dentistry brach), as a teaching assistant form the year 2012, where he still work with dedication.

He is a co-author in 2 scientific articles in the field of Maxillofacial surgery, which are published in Q2 and Q3 journals.

He has been member of EACMF (European Association for Cranio Maxillofacial Surgery) and actively participate in professional congresses and trainings.

He was the winner of the University of Prishtina scholarship for the most successful students in the academic year 2005/06, 2006/07 and 2007/2008.

He was the winner of the University of Prishtina award 'Distinguished Student', in the year 2008.

In the year 2017 he took a 'Helen Matras' award from EACMF for best oral presentation in a International Conference 'Contemporary Treamtent of Dentofacial Deformities'.

From 2012 until now, he is CEO and owner of dental and surgical clinic 'ProfessionalDent'.