Preoperative and postoperative values of neutrophil/platelet/monocyte to lymphocyte ratio with regard to the disease-free period and overall survival in patients with oral cancer

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SUMMARY

This study has shown that preoperative and postoperative neutrophil, lymphocyte, monocyte and platelet counts and NLR, LMR and PLR can be predictive biomarkers for DFS and OS in HNSCC.

Not consuming alcohol significantly lowered the risk of death, the preoperative monocyte count significantly increased the risk of death, the postoperative NLR significantly increased the risk of death and DiffLMR significantly increased the risk of death.

In the multivariate analysis, the preoperative neutrophil count significantly increased DFS and the preoperative monocyte count significantly decreased DFS.

There was a significant decrease of DFS in the preoperative NLR, significant increase in postoperative LMR and a significant increase in DiffNLR.

Preoperative monocyte count significantly increased the risk of death, postoperative NLR, postoperative LMR and DiffNLR significantly decreased the risk of death.

Location of tumor was not associated with preoperative and postoperative NLR or DiffNLR and OS or DFS.

Additionally, to our knowledge this was the first time that the difference in NLR before and after surgery was shown as a significant predictor of OS and DFS. Furthermore, this study showed that neutrophil counts, NLR and DiffNLR are significantly associated with DFS and OS in HNSCC.

Conclusively, the results of this study have shown that preoperative and postoperative NLR and LMR and their components have a significant predictive value for both DFS and OS.

The design of our study should be repeated in other cohorts in order to define cut-off values for HNSCC and include these parameters into clinical practice.

Key words: neutrophils, Neutrophil/Lymphocite ratio, Lymphocite/Monocyte ratio Platelet/Lymphocite HNSCC

PROŠIRENI SAŽETAK

Cilj: Cilj je ovog istraživanja bio odrediti preoperativne i postoperativne vrijednosti odnosa neutrofila/limfocita, limfocita/monocita i trombocita/limfocita u 182 bolesnika s pločastim karcinomima glave i vrata kao i njihovu povezanost s ukupnim preživljenjem i periodom bez recidiva bolesti.

Materijali i Metode: Statistička analiza se napravljena uz pomoć univarijante i multivarijantne analize. Univarijatna i multivarijatna analiza pokazatelja perioda bez recidiva bolesti i ukupnog preživljenja je uključivala podatke o dobi, konzumiranju alkohola i pušenju, lokaciji tumora i provedenoj terapiji.

Rezultati: Univarijatna analiza perioda bez recidiva bolesti znakovito je bila povezana s brojem preoperativnih monocita i sa razlikom odnosa limfocita/monocita, sa marginalnom povezanošću sa konzumiranjem alkohola, postoperativnim odnosom neutrofila/limfocita i preoperativnim odnosom limfocita/monocita. Broj preoperativnih monocita je znakovito povećao opasnost od recidiva bolesti za 3,9 puta, dok je razlika odnosa limfocita/monocita znakovito povećavala opasnost od recidiva bolesti za 14,9% za svaku promjenu jedinice. Univarijatna analiza ukupnog preživljenja pokazala je znakovitu povezanost sa konzumiranjem alkohola, brojem preoperativnih monocita, postoperativnim odnosom neutrofila/limfocita dok je razlika odnosa limfocita/monocita pokazala marginalnu povezanost sa preoperativnim odnosom limfocita/monocita i postoperativnim odnosom trombocita/limfocita. Ne konzumiranje alkohola znakovito umanjuje smrtnost za 42,6%, broj preoperativnih monocita znakovito povećava smrtnosti za 3,4 puta, postoperativni odnos neutrofila/limfocita znakovito povećava smrtnosti za 4,5% za svaku promjenu jedinice, dok razlika odnosa limfocita/monocita znakovito povećava smrtnosti za 17,1% za svaku promjenu jedinice. Kod multivarijatne analize, broj preoperativnih neutrofila znakovito povećava period bez recidiva bolesti za 6,1 puta. Utvrdjeno je znakovito smanjenje perioda bez recidiva bolesti za 25,8% za svaku promjenu jedinice kod preoperativnog odnosa neutrofila/limfocita, znakovito povećanje smrtnosti za 31,2% za svaku promjenu jedinice u postoperativnom odnosu limfocita/monocita kao i znakovito povećanje smrtnosti za 6,0% za svaku promjenu jedinice kod razlike odnosa neutrofila/limfocita. Kod multivarijatne analize, broj preoperativnih neutrofila znakovito umanjuje smrtnosti za 18,5%, preoperativni broj monocita znakovito povećava smrtnosti za 5,8 puta, postoperativni odnos neutrofila/limfocita za 30,2% za svaku promjenu jedinice, postoperativni odnos limfocita/monocita za 34,9% za svaku promjenu jedinice, dok razlika odnosa neutrofila/limfocita znakovito umanjuje smrtnost za 18,6% za svaku promjenu jedinice.

Ne konzumiranje alkohola ima marginalnu znakovitost na umanjenju smrtnosti za 41,6%. Lokacija tumora, njegov stupanj, pušenje, spol i dob nemaju neku znakovitu povezanost ni sa periodom bez recidiva bolesti niti sa ukupnim preživljenjem.

Zaključak: rezultati ovog istraživanja su pokazali da je duže ukupno preživljenje bilo povezano s ne-konzumiranjem alkohola, preoperativnim i postoperativnim brojem neutrofila i limfocita, preoperativnim odnosom neutrofila/limfocita te razlikom između preoperativnog i postoperativnog odnosa neutrofila i limfocita. Dulji period bez recidiva je bio znakovito povezan s ne-konzumiranjem alkohola, preoperativnim broj neutrofila i limfocita, postoperativnim odnosom neutrofila i limfocita i razlikom između preoperativnog i postoperativnog odnosa neutrofila i limfocita.

Ključne riječi: neutrofili, odnos neutrofila/limfocita, odnos limfocita/monocita, odnos karcinomi glave i vrata

LIST OF ABBREVIATIONS

Abbrevation	Term
AFs	Attributable fractions
AJCC	American Joint Committee on Cancer
CLA	Conjugated linoleic acid
CLL	Chronic lymphocytic leukemia
DFS	Disease free survival
EBV	Epstein-Barr virus
FA	Fatty acid
HNSCC	Head and Neck squamous cell carcinoma
HPV	Human papillomavirus
LMR	Lymphocite to monocyte ratio
NLR	Neutrophil to lymphocyte ratio
OS	Overall survival
OSCC	Oral squamous cell carcinoma
PLR	Platelet to lymphocyte ratio
SGT	Salivary gland tumors
T,N,M	Tumour, lymph nodes, metastases
UADT	Upper aerodigestive tract cancer
USA	United States of America

CONTENT

1. INTRODUCTION

Cancer is the second among fatal diseases, next to cardiovascular diseases. Cancer is a broad term used for identifying a large number of diseases. Perhaps the only common feature of these diseases is the ability of uncontrolled cell proliferation that cannot be checked by the normal cell kinetics regulators. A normal cell suddenly turns into a rogue cell and starts dividing continuously without check, leading to the development of solid lumps (tumors) or an abnormal rise in the number of dispersed cells like the blood corpuscles (1). Cancer can occur in any part of the body and in any organ or tissue. Even though most of the cancers are generally associated with old age, no age group is immune to this disease (1).

Cancer originates in our own cells, but several factors, both intrinsic and external to the body, which influence our daily life, can add to the life time cancer risk (2). While cancer, as such, is not infectious, some infections can act as a stimulus to induce and promote cancer development (3). In addition, environmental pollutants such as chemicals, industrial effluents, some therapeutic drugs, and mutagenic agents, including ionizing radiation, can increase the incidence of cancer (1).

About 50% of all cancers are attributed to life style, eg. diet, tobacco habits and alcohol consumption, and exposure to industrial toxins (4).

Carcinogenesis is a multistep process:

1. Initiation which can occur as autonomous growth. This relates to: molecular basis for the stimulation of the rare altered initiated cells, physiological nature of the initiated cells that triggers them to act as the original progenitors for cancer. One of essential part of initiation is cell proliferation

2. Promotion is referred as precancerous lesion such as papilloma's nodules etc. and

3. Progression is a step in carcinogenesis when precancerous lesions evolve to cancer (5).

Overall survival and disease-free survival prognosis may depend on both TNM and patients general information such as gender age etc. Also, cancer development depends on tumor characteristics and inflammation response of the host (6).

Head and neck cancers are the sixth leading causes of death worldwide. The majority of head and neck cancers (around 90%) are squamous cell cancers with very high death rate. The incidence of this type of cancer is higher in males than in females 2:1 even though alcohol

consuming and tobacco consumption are increasing in the last century in females, therefore, the incidence tends to be almost equal for both genders (7, 10).

As aforementioned, the development of oral squamous cell carcinoma (OSCC) for all types of cancers is also a multistep process that requires accumulation of genetic alterations. It is influenced by a patient's genetic predisposition, by environmental influences, as well as lifestyle habits including tobacco, alcohol, chronic inflammation, and viral infection (11).

1.1 EPIDEMIOLOGY OF HEAD AND NECK CANCERS

Epidemiological data regarding HNSCC vary throughout the world. It has been expected that due to the smoking ban policies in some countries, a decrease in HNSCC occurrence will be achieved. However, data are controversial. In some of these countries, an increasing incidence of head and neck cancer (HNSCC) in young adults has been reported. Weatherspoon et al. (12) reported changes in the incidence of oral cancer based on anatomic location and demographic factors over time in the United States. About 75,468 incident oral cancer cases were diagnosed from 2000 to 2010. The tonsil was the most frequently diagnosed anatomic subsite (23.1%). An increasing incidence trend was observed for cancers in the oropharyngeal region, in contrast to decreasing trend seen in the oral cavity region (12). Zhang et al. (13) included 28 studies involving 13.830 patients with head and neck cancer in the past 45 years. The same authors (13) reported that the increased alcohol and tobacco consumption trends increased the risk of head and neck cancer over the past 45 years. Tobacco consumption was found to be a stronger risk factor for head and neck cancer than alcohol consumption (13). Katzel et al. (14) performed a cohort study from 1995-2010 (n = 2.2 million annual members) and identified 1,383 human papillomavirus (HPV)-related and 1,344 HPV-unrelated oral cavity and oropharyngeal cancer cases. The observed increasing HPV-related cancer rates are most evident among non-smokers, whereas the decreasing HPV-unrelated cancer rates are least evident among younger individuals, non-smokers, and those without an alcohol abuse history (14). Winn et al. (15) included 35 studies that have pooled their data from 25,500 patients with head and neck cancer (i.e., cancers of the oral cavity, oropharynx, hypopharynx, and larynx) and 37,100 controls. The same authors (15) have confirmed that tobacco use and alcohol intakes are key risk factors of these diseases. Other risk factors include short height, lean body mass, low education and income, and a family history of head and neck cancer. Zhang et al. (16) analyzed 177 population-based cancer registries distributed in 28 provinces of China with a total of

175,310,169 populations and accounting for 13.01% of the overall national population in 2011. The estimate of new cases diagnosed with oral cancer was 39,450 including 26,160 males and 13,290 females. The overall incidence rate for oral cancer was 2,93/100,000. The estimated number of oral cancer deaths in China in 2011 was 16,933. Oral cancer accounted for 0.80% of all cancer deaths. In addition, the incidence and death rates were increased by the raising of age (16). Hertrampf et al. (17) reported that the annual incidence of oral cancer is about 13,000 in Germany. The same authors (17) reported that men are 2,5-times more likely to be diagnosed with tumor and 3-times more likely to die from this tumor than women. The incidence and death rate in women increased slightly during the last decade, while the incidence and death rate in men remained stable at a high level. While a decline was observed for younger age groups, an increase was seen in the elderly ones. This is probably due to the efforts in non-smoker protection in recent years (15). Toporcov et al. (18) pooled data from 25 case-control studies and conducted separate analyses for adults ≤ 45 years old and >45 years old. The young group of cases had a higher proportion of tongue cancer (16,0% in women; 11.0% in men) and unspecified oral cavity/oropharynx cancer (16,2%; 11,1%) and a lower proportion of larynx cancer (12,1%; 16,6%) than older adult cases. The proportions of never smokers or never drinkers among female cases were higher than among male cases in both age groups. Positive associations with HNSCC and duration or pack-years of smoking and drinking were similar across age groups. However, the attributable fractions (AFs) for smoking and drinking were lower in young individuals when compared with elderly individuals. A family history of early-onset cancer was associated with HNSCC risk in the young (23,2%), but not in the older adults (2.2%). Differences in HNSCC etiology according to age group may exist. The lower AF of cigarette smoking and alcohol drinking in young adults may be due to the reduced length of exposure due to the lower age (19). Guntinas-Lichius et al. (19) analyzed data of 6,291 patients with primary HNSCC from the Thuringian cancer registry. Crude incidences of HNC increased significantly from 13, 77 to 20,39 between 1996 and 2011. The same authors (19) concluded that the incidence of oral cancer is significantly increasing. Bagnardi et al. (20) analyzed a total of 572 studies, including 486, 538 cancer cases. The same authors concluded that alcohol increases risk of cancer of oral cavity and pharynx, esophagus, colorectum, liver, larynx and female breast. Jayasekara et al. (21) observed a dose-dependent association between lifetime alcohol intake and the risk of upper aero-digestive tract cancer (UADT) with an intake of ≥40 g/day of alcohol and for a 10 g/day increment in intake. A positive association with

baseline alcohol intake a 10 g/day increment in intake was found to be a slightly weaker predictor of risk than lifetime intake (21). Sharma et al. (22) reported that upper aero-digestive tract (UADT) cancers in North East India represent 37,6% of all cancers in both sexes, accounting for 53,3% in males and about 27,5% in females of the total cases. There were 5,638 cases registered during the last four years of the study (2008-2011) accounting for 56, 7% (3,198/5,638) of the total in males and 43,3% (2,440/5,638) in females. The male: female ratio was 1.31: 1.00. Esophageal cancer was most common in both sexes, with most appreciable gender variation for the tongue and hypopharynx, presumably reflecting differential exposure to risk factors (22). Krishna et al. (23) reported a significant association between OSCC in middle aged and male subjects. Cases with both habits of tobacco chewing and smoking were at a higher risk for OSCC than just tobacco chewing and duration of risk habits also emerged as a responsible factor for the development of carcinoma. The buccal mucosa was the most common (35,5%) affected oral site (23). Sharp et al. (24) analyzed all HNSCCs that were diagnosed from 1994 to 2009 from the National Cancer Registry Ireland who were classified by smoking status at diagnosis. Follow-up examinations had been performed for 5 years, i.e. until December 31, 2010. In total, 5,652 subjects with head and neck cancers were included. At diagnosis, 24% of them were nonsmokers, 20% of them were ex-smokers, and 56% of them were current smokers. Compared with never smokers, the current smokers had a significantly raised death rate caused by cancer. A significantly increased cancer-related death rate was seen in current smokers with oral cavity, pharyngeal, and laryngeal cancers. The association was stronger in surgically treated patients. Neither radiotherapy nor chemotherapy modified the effect of smoking. Patients with head and neck cancer who smoke have a significantly increased cancer death rate (24).

1.2 ETIOLOGY OF HEAD AND NECK CANCERS

1.2.1 Body height and body mass index

A study performed by Etemadi et al. (25) comprised 218,854 participants aged 50-71 years who were cancer free at baseline (1995 and 1996). Until year 2006, 779 incident HNSCC occurred: 342 in the oral cavity, 120 in the oro- and hypopharynx, 265 in the larynx, 12 in the nasopharynx, and 40 at overlapping sites. There was an inverse correlation between HNSCC and body mass index, which was almost exclusively among current smokers and diminished as initial years of follow-up were excluded. A direct correlation with waist-to-hip ratio,

particularly for cancers of the oral cavity, was seen. Height was also directly associated with total HNSCC, and oro- and hypopharyngeal cancers. The same authors concluded that this finding was probably due to tobacco smoking (25).

1.2.2 Vitamin and food intake

Edefonti et al. (26) reported that greater vitamin E intake from food may reduce the risk of HNSCC, although the authors were not able to explain the heterogeneity observed across studies or rule out certain sources of bias. The study was performed on the sample of 5959 HNSCC cases and 12, 248 controls. The same authors in another study (27) reported that the inverse association of vitamin C intake from food with HNSCC may reflect a protective effect on these cancers; however, the authors cannot rule out other explanations. The objective of the study of Cittadini et al. (28) was to analyze beef consumption, conjugated linoleic acid (CLA) and n-3 fatty acid (FA) serum concentration and their relation to salivary gland tumors (SGT). A questionnaire on non-nutritional risk factors and validated food frequency questionnaire were applied in 20 SGT and 20 control (Co) patients. Serum oleic and linolenic FAs showed a significant negative association with SGT. Fanidi et al. (29) measured plasma levels of vitamins B2, B6, B9 (folate), B12, and methionine and homocysteine in pre-diagnostic plasma samples and analyzed in relation to HNSCC and esophagus cancer risk, as well as post-diagnosis all-cause mortality in 385,747 participants. After controlling risk factors, study participants with higher levels of homocysteine had elevated risk of HNSCC. A slight decrease in HNC risk was also seen among subjects with higher levels of folate. Plasma concentrations of the other investigated biomarkers did not display any clear association with risk or survival (29).

Galeone et al. (30) analyzed 5,127 cases and 13,249 controls and concluded that the highest OPC risk was observed in heavy alcohol drinkers with low folate intake as compared to never/light drinkers with high folate. Maasland et al. (31) reported that consumption of vegetables and fruits (or of specific groups of them) may protect against HNSCC and its subtypes. The study included 120,852 participants and after 20.3 years of follow-up, 415 cases of HNC (131 OCC, 88 OHPC, 3oral cavity/pharynx stayed unspecified or overlapping and 193 LC) were seen. It has been speculated that tea intake might reduce the risk of HNSCC development. Zhang et al. (32) performed a literature search and included 14 studies on this topic. The same authors (32) reported that tea consumption was associated with a decreased risk of oral cancer, while no association was detected with oral/pharyngeal, pharyngeal, or laryngeal cancer.

1.2.3 Other medical conditions

It has been postulated that gastroesophageal reflux plays a role in the etiology of head and neck squamous cell carcinomas (HNSCC). Papagerakis et al. (33) evaluated 596 patients with HNSCC and reported a significant association between the use of histamine receptor-2 antagonists (H2RA) and proton pump inhibitors (PPI), alone or in combination, and various clinical characteristics as well. The findings in this large cohort study pointed to the fact that routine use of antacid medications may have significant therapeutic benefit in patients with HNSCC. Helby et al. (34) measured plasma total IgE in 37,747 individuals from the general population, and the participants were followed prospectively for up to 30 years. During the mean follow-up of 7 years, a first cancer was diagnosed in 3,454 participants. The multivariable adjusted hazard ratio for a 10-fold higher level of IgE was 1,05 [95% confidence interval for any cancer, 0,44 for chronic lymphocytic leukemia (CLL), 0,53 for multiple myeloma, 1,54 for other non-Hodgkin lymphoma, 1,38 for cancer of the oral cavity and pharynx, and 1,12 for lung cancer. High levels of plasma total IgE were associated with low risk of CLL and possibly of multiple myeloma, without convincing evidence for high risk of any cancer type (34).

1.2.4 Occupational hazard

Carton et al. (35) included 296 squamous cell carcinomas of the head and neck in women and 775 controls. An elevated risk was observed for working proprietors working for 10 years or more with a significant trend with duration of employment. An elevated but non-significant risk was observed for street vendors, bakers, welders and flame cutters. The same authors (35) suggested a role of occupational exposures in the development of HNSCC cancer in women. Reijula et al. (36) studied a cohort of 16,134 male and 81,838 female waiters from Denmark, Finland, Iceland, Norway and Sweden. During the follow-up period, from 1961 to 2005, they found that 19,388 incident cancer cases were diagnosed. The highest incidence for cancers in the pharynx, oral cavity and tongue was seen among male waiters. In female waiters, cancers of the larynx, oral cavity and lungs were noticed. The risk of cancer among waiters was higher than that in the general population. The elevated incidence in some cancer sites can be explained by higher alcohol consumption, the prevalence of smoking and occupational exposure to tobacco smoke (36). Petrochemical plant maintenance workers are exposed to various carcinogens such as benzene and metal fumes. Koh et al. (37) analyzed data of 14,698 male workers registered in a regional petrochemical plant maintenance workers union during the years 2002-2007 and concluded that there were potential associations between oral and pharyngeal cancers and temporary maintenance jobs in petrochemical industry.

1.2.5 Viral infections

1.2.5.1 Human papilloma viruses

Within HNSCC, the highest prevalence of HPV-DNA is found in the oropharynx (soft palate, base of the tongue, tonsillar area and posterior pharynx). However, the role of HPV in the development of solely oral cancer is controversial. Patients with HNSCC and HPV infection have better prognosis, treatment response and survival when compared to the HNSCC patients without HPV infection. Furthermore, patients with HNSCC who smoke and drink alcohol but also have HPV infection have better prognosis in comparison to the ones without HPV infection (38). It is thought that HPV starts cancerogenesis but is also considered to be a buffer of other factors which lead to the cancer. It has been recognized that certain sexual practices such as oro-genital and oro-anal sex are highly correlated with HPV positive cancers (39). HPV prevalence depended upon tumor location and significantly higher rate of HPV has been found in oropharynx and larynx. A larger numbers of men with HPV were noticed in males with HNSCC when compared with women.

1.2.5.2 Co-infection with other viruses

Sand and Jalouli (40) reported that apart from HPV, Epstein-Barr virus (EBV) and herpes simplex virus 1 (HSV-1) might play a role in the HNSCC cancerogenesis. Polz-Gruszka et al. (41) identified EBV in 57, 5%, HSV-1 in 7.5% and cytomegalovirus (CMV) in 10% of 80 patients with oral squamous cell cancer having also HPV. A co-infection of two viruses was noticed in 30% of the studied patients, most frequently being EBV and HPV (15%). Beachler et al. (42) concluded that the incidence of oropharyngeal cancer (both HPV positive and negative) was significantly increased in HIV positive patients when compared to the general population, probably as a result of immunosuppression.

1.2.5.3 Coexistence with certain syndromes

Van Monsjou et al. (43) reported that in some patients' genetic syndromes such as Fanconi's anemia and Bloom syndrome may play a role in the development of HNSCC.

1.2.5.4 Hormones and oral cancer

The importance of estrogen and testosterone as well as their receptors as basis for sex differences in the prevalence and etiology of a large number of cancers together with therapy and survival is well known (44).

Estrogen receptors alpha and beta are present in the squamous cell within the oral cancer and it seems that their interaction with receptors of epidermal growth factor leads to the disease progression (45). The results of the study performed on patients with head and neck cancer showed that alcoholics had significantly increased estrogen levels as well as decreased testosterone levels when compared with the patients with head and neck cancers who were not alcoholics (46). Decreased testosterone levels correlate with shorter survival in patients with head and neck cancer (47). The presence of androgen receptors in oral cancer cells correlates with increased proliferation, decreased apoptosis and increased cancer aggressiveness (48). Some recent studies have shown that there were sex differences in the expression of androgen receptors within oral cancer tissues (49). Although the presence of estrogen and androgen receptors in the oral cancer has been analyzed, so far there have been insufficient data regarding the role of estrogen and androgen receptors in the development of tumor and metastases within oral cancer. Furthermore, it is still unknown whether activated stromal cells are present in oral cancer as seen in the stroma of other cancers (50). There is a lack of similar data upon interaction between estrogen and androgen receptors with other stromal markers in oral cancer and their characteristics regarding etiology of oral cancer and survival rate. Estradiol and testosterone are known to be mitogens in certain types of tissues (51), and their effect is tissue specific. The risk of oral cancer is associated with smoking and alcohol consumption. Both of these are mixtures of xenoestrogens from tobacco-nicotine/cotinine as aromatase inhibitor and alcohol as aromatase stimulant (52) the actions of which regarding oral cancer etiology are to be investigated. Etiological role of estrogen and androgen receptors in oral cancer might correlate with histological profile, thus contributing to improved diagnostic performance and therapy of these patients.

1.3 SYMPTOMS OF THE ORAL CANCER

Oral cancer might be asymptomatic at its initial stage; therefore, it is usually diagnosed when symptoms become more obvious and when the disease progresses. Discomfort is the usual presenting symptom seen in 85% of the diseased patients. Some of the patients have sensation of the mass in the oral cavity in the neck area. Burning sensation, dysphagia, odinophagyia, otalgia, restricted mouth movements or bleeding from the oral cavity are less frequently present. Loss of tongue function might affect speech, swallowing and nutrition. Lymphatic spread occurs into upper lymph nodes, submandibular, digastric, upper cervical nodes and other cervical nodes. The most affected lymph nodes are the ones on the same side. If cancer is located on the medial side, more frequent affliction of contralateral and bilateral lymph nodes is seen. Lymph nodes which are connected with cancer are increased and tender on palpation. However, they are not tender when they are connected with inflammatory response. Fixation of the primary tumor with adjacent tissue within bone suggests aperiosteum affection and possible spread through bone.

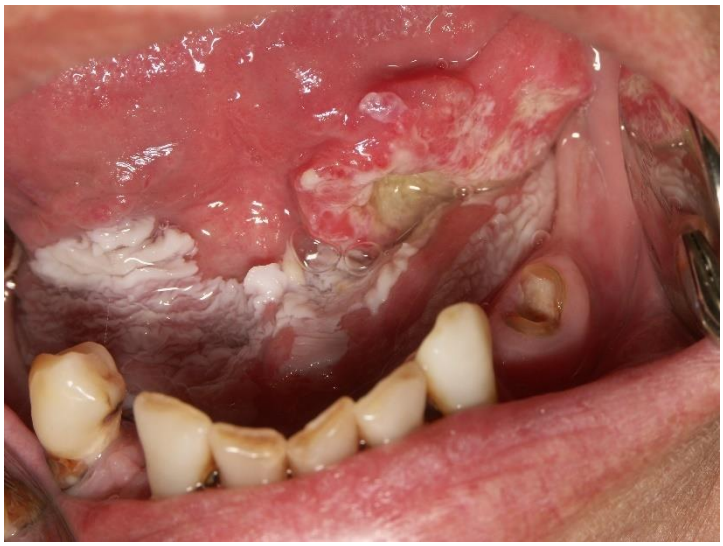

Figure 1: Oral cancer (Department of Oral Medicine, School of Dentistry, University of Zagreb)

1.4 TUMOR, NODE AND LYMPH METASTASES CLASSIFICATION

AJCC has developed the tumor, node, and metastasis (TNM) system of cancer classification (53).

T is the size of the primary tumor, N indicates the presence of regional lymph nodes, and M indicates a distant metastasis.

The staging system for SCC combines the T, N, and M to classify lesions as stages 1 to 4. Classification of the cancer by TNM description is more accurate than use of the four staging groups and may reflect the biology of the tumor. For example, there are differences in biology and response to treatment between a stage 3 tumor that is classified as T1N1M0 and a stage 3 tumor that is classed as T3N0M0.

Various aspects related to TNM staging of cancers of the head and neck are discussed by Patel and Shah (54). It has been suggested that the T4 classification of upper gingival and hard palate carcinomas by defining the boundary between T1–T3 and T4 as extensive invasion of the maxillary sinus, nasal cavity, or external skin (54). Local or regional spread of oral SSC is common and affects the choice of therapy and prognosis. Metastases to cervical lymph nodes are common, but distant metastases below the clavicle are rare.

Oral cancer occurring in the posterior aspect of the oral cavity and oropharynx and inferior in the mouth tends to be associated with a poorer prognosis, which may be explained by diagnosis occurring with advanced disease and a higher incidence of spread to lymph nodes at the time of diagnosis (55). Ipsilateral lymph node metastases are frequent; however, the spread to contralateral nodes also occurs and is more common with midline and posterior lesions.

The most important factor in survival is the stage of disease at diagnosis. Unfortunately, the majority of oral cancers are diagnosed in advanced stages, after becoming symptomatic (56,47).

The incidence of spread is influenced by tumor size. Lesions classed as T1 may show regional spread in 10 to 20% of cases, T2 lesions in 25 to 30% of cases, and T3 to T4 tumors in 50 to 75% (56). A 3-year follow-up with no evidence of disease occurs with approximately 75 to 85% of T1 lesions, 50 to 60% of T2 lesions, and 20 to 30% of T3 to T4 lesions. For patients without lymph node involvement, the overall 3-year no-evidence-of-disease rate is approximately 50 to 60%; however, with lymph node involvement, the rate of cure is approximately 33 to 50% (57).

A classification of oral cancer is made by TNM classification which includes tumor size, presence of regional metastases in the cervical lymph nodes and presence of distant metastases.

Letter T depicts the size of the tumor

T1s-carcinoma in situ

T1-tumor <2 cm

T2-tumor >2 and <4 cm.

T3-tumor >4 cm

T4-tumor > 4cm with infiltration of the surrounding structures (bones, muscles, sinuses and skin)

Letter N depicts the lymph node involvement

N0-without metastases in the cervical lymph nodes

N1-solitary ipsilateral node <3cm

N2a-solitary ipsilateral node >3 cm and <6 cm

N2b-multiple ipsilateral nodes >3cm and <6 cm

N2c-bilateral and contralateral nodes <6 cm.

N3a-ipsilateral node >6 cm

N3b-contralateral node >6 cm

Letter M depicts presence of distant metastases

M1-no distant metastases

M1-presence of distant metastases

Staging of the oral cancer is based upon these criteria:

Stage 1-T1 N0 M0

Stage 2-T2 N0 M0

Stage 3-T1/T2/T3 N1 M0

T3 N0 M0

Stage 4- T4 N0 M0

any T stage N2/N3 M0

any T stage any N M1.

1.5 HYPOPHARYNGEAL, OROPHARYNGEAL AND LARYNGEAL CANCERS

The oropharynx is a middle part of the pharynx which extends from the anterior tonsilar arch to the superior hyiod level and may be divided into tongue base, i.e. posterior part of the tongue, tonsils, tonsillar pad, soft palate, uvula as well as the posterior and lateral phayngeal wall (58).

The oropharnx and hypopharynx have abundant lymphatics and therefore metasteses into cervical lymph nodes are usually the first sign of the disease. Cervical lymph nodes in the region II, III and IV are usually affected. Treatment of the early stages of this cancer consists of the surgery and radiotherapy, while more advanced stages are treated with concomitant chemotherapy. In cases of inoperable cancers, radiotherapy and/or chemotherapy are given to the patients (59).

Overall 5-year survival rate depends on the stage and cancer localization. In the stage 1, 5-year survival rate is 55-85%, in the stage II 50-80%, in the stage III it is 10-60%, while in the stage IV, 5-year survival rate is 10-55%. Relapse time is highest during the first 4 years after the treatment of initial cancer and the incidence is 80-90% (58).

The hypopharynx extends from the top of epiglottis till the lower margin of the crycoid and is divided into pyriform sinuses, posterior pharyngeal wall and postcricoid area. The hypopharynx has the abundant lymph drainage into jugulodigastric, jugulo-omohyoid, upper and medial deep cervical and retrophayngeal lymph nodes. Late diagnosis of advanced cases is a common feature, due to the late appearance of symptoms and abundant lymph drainage. More than 50% of these patients upon admission have metastases in the cervical lymph nodes and the presenting sign is a palpable neck lymph node. Surgery or radiotherapy are the main therapies when treating hypopharyngeal cancers, however in more advanced cases treatment may rely only upon chemotherapy (58).

The upper margin of the larynx is an upper part of the epiglottis, while the lower margin of the larynx is a lower part of the cricoid. It is divided into: supraglottis, glottis and subglottis.

Laryngeal cancer is the second most frequent cancer of the aerodigestive system, followed by the lung cancer. The most frequent type is squamous cell cancer with 5-year survival rate around 60% in the USA. Etiological factors are smoking and alcohol intake, although lately the role of human papilloma viruses has emerged as significant cause. Most frequently, symptoms include hoarsness, feeling of the strange object, while in the advanced cases most frequently dysphagia, hemoptysis, ear pain, difficulties with breathing and body weight loss occur (58).

Laryngeal cancers might show various clinical findings and behavior due to their site of development. Aproximally, 59% of the all laryngeal cancers develop on the glotiss, 40% of them develop on the supraglotiss and 1% of them develop in the subglotiss. Early stages are treated with surgery or radiotherapy, whereas more advanced stages are treated with surgery (if possible) together with concomitant chemo radiotherapy. An overall 5-year survival rate in the stages I and II is around 80-90% (depending on the tumor site i.e. glotis versus supraglotis) while stages III and IV have survival rate of 45-75%.

The supraglottis extends from the top of epiglotis and velicula till the ventricle and lower part of the false vocals and is divided into arytenoid cartilage, ariepiglotic crease, false vocals and epiglotis. Due to the late occurrence of the symptoms, 70% of the patients present with advanced disease with nodal metastases in 55% of the patients. Most frequently, cervical lymph nodes of the region II, III and IV are affected.

The glottis extends from the ventricle till 0.5 cm beneath the vocal margins and is divided in the vocals and the anterior commissure. Symptoms such as hoarseness develop in the early stage of the disease, therefore in the 75% of the patients, fortunately, the disease itself is localized.

Subglottis is a part of the larynx and it extends 5mm from the free margin of the vocals till the lower part of the cricoid, with rare lymph drainage which drains into IV and VI cervical lymph nodes (58).

Table 1: Data from the Croatian National Cancer Registryfor orophayngeal, hypopharyngeal and laryngeal cancers from the year 2011 to 2015.

	Oropharnx			Hypopharnx			Larynx			Total
	Men	Women	Total	Men	Women	Total	Men	Women	Total	
2011	**52**	**5**	**57**	**77**	**8**	**85**	**316**	**26**	**342**	**484**
2012	**51**	**7**	**58**	**80**	**10**	**90**	**302**	**29**	**331**	**479**
2013	**50**	**12**	**62**	**95**	**13**	**108**	**304**	**20**	**324**	**494**
2014	**52**	**6**	**58**	**92**	**11**	**103**	**319**	**26**	**345**	**506**
2015	**47**	**9**	**56**	**76**	**12**	**88**	**272**	**31**	**303**	**447**
TOTAL	**252**	**39**	**291**	**420**	**54**	**474**	**1513**	**132**	**1645**	**2410**

1.6 HISTOPATHOLOGY OF HEAD AND NECK CANCERS

The current method of diagnosing oral cancer involves the microscopic examination. Dysplasia or atypia describes the range of cell abnormalities, cell morphology, an increased rate of mitoses, hyperchromatism and cell maturation. A weak, moderate and severe dysplasia describes epithelial abnormalities which do not affect the entire depth of the epithelium. If the entire epithelium is affected, the diagnosis is *carcinoma in situ*. When basal membrane is destroyed, an invasion of connective tissue develops and the diagnosis of cancer is established. A well-differentiated cancer might have anatomical characteristics of epithelial cells and might produce keratin, while the cancer which is not well differentiated shows a loss of anatomical function and epithelial function. Cancers might correlate with inflammatory infiltrate and those lesions are hard to differentiate from dysplasia (60).

Squamous cell cancers arise either from the multilayered epithelium of the upper respiratory and digestive system or from the cylindrical laryngeal epithelium after metaplasia has occurred. Histopathologically, it does not differ significantly from the squamous cells in the other parts of the body (58).

Table 2: Histopathological grade of squamous cell cancer.

Tumour grade	characteristics
grade I	(well-differentiated tumor) similar to normal squamous epithelium
grade II	(moderately differentiated tumor) obvious nuclear pleomorphy and mitotic activity, including abnormal mitoses, however kertinization is less pronounced
grade III	(poorly differentiated tumors) non-mature cells dominate with numerous typical and atypical mitoses, keratinization is minimal and zones of necrosis could be seen.

Basic histopathological characteristics of squamous cell carcinoma are differentiation of tumor cells and invasive growth accompanied with the basal membrane rupture. Keratin deposits are characteristic for well differentiated cancers; however, they can be found in the weakly differentiated cancers (61).

Based on similarities with normal squamous epithelium, we can distinguish between well differentiated, moderately differentiated and poorly differentiated cancers. Cancers of the oral cavity are well to moderately differentiated, while tongue cancers, hypopharynx and tonsils cancers are characterized by weak differentiation. Clinically, histological grade within oral squamous head and neck cancers have small predictive value with regard to the treatment outcome. Disruption of the basal membrane leads to the spread into surrounding tissues which is usually accompanied with stromal response and intensive inflammation which consists of lymphocytes and plasma cells. It is of utmost importance to describe perineural and perivascular infiltration (62).

1.7 DIAGNOSIS OF HYPOPHARYNGEAL, OROPHARYNGEAL AND LARYNGEAL CANCERS

When diagnosing these cancers, it is of utmost importance to make a clinical examination, which is usually followed by fiber endoscopy. After that, biopsy specimen needs to be taken from the primary tumor which consists of tumorous tissue at the borders together with normal tissue surrounding the diseased one. Subsequently, histopathological analysis is performed. Tumor size and relation to the surrounding tissues has to be determined. Computed tomography (CT) is usually performed in cases of deeper local tumor invasion, nodal metastases or when the surrounding tissues are affected. In cases of soft tissue invasion, magnetic resonance seems to be a superior diagnostic tool. The lymph node status is determined by use of ultrasound and guided punction together with cytological analysis. In certain cases, CT is performed (58).

1.8 THERAPY

The usual treatments of oral cancer are surgery, radiation therapy and, rarely, chemotherapy, which depends on the stage of the disease. Early stages (T1 and T2) are treated surgically, while advanced stages (T3 and T4) are treated by a combination of surgery and radiation therapy. Surgery is recommended in cases in which the side-effects of surgery are less pronounced when compared to radiation therapy, in cancers which are not radiosensitive and in cases of recurrent cancers when the affected area received a maximal dose of radiation therapy. The main causes of failure regarding surgery are cancer recurrence due to the excision which is not wide enough. Surgery results in severe deformities which affect function and esthetics in patient.

Radiation therapy might be a primary treatment option either preoperatively or postoperatively or concomitant therapy to the surgery. Therapeutic dose for the most head and neck cancers is

50-70 Gy. The dose of 50-70 Gy is applied in daily applications. The dose is applied in daily fractions of 2 Gy. Apart from cancer cells, radiation therapy leads to the damage of surrounding structures, which leads to numerous side-effects which are divided into acute and chronic. Acute side-effects arise during the radiation period and last several weeks after the radiation therapy. They include mucositis, loss of taste and xerostomia. Chronic side-effects of the radiation therapy include radiation caries, trismus and osteoradionecrosis (63).

Chemotherapy is usually applied for advanced cases or reoccurrences which have decreased the potential to be successfully treated with surgery and/or radiation therapy. Most frequently used agents are metothrexate, bleomycin, 5-fluorouracil and cysplatin. Chemotherapeutic protocols might cause cancer decrease. However, they do not increase survival, primary tumor control or decrease of metastases prevalence. Protocols which combine radiation and chemotherapy have shown success in controlling local disease. However, they do not affect five year survival of patients (64).

1.9 PROGNOSIS

Local or regional spread of oral cancer is well known, however, distant metastases underneath the clavicle are rare. Oral cancers arising from the posterior parts of the mouth and in the oropharynx have worse prognosis, which is probably due to the fact that cancer is already in advanced stage with higher prevalence of spread into lymph nodes. Ipsilateral metastases into regional lymph nodes are more frequent, however, contralateral metastases into lymph nodes could be found, especially when cancer is located in the central parts of the mouth. The most important factor for survival is the stage of the disease at the time of diagnosis. Most of oral cancers are diagnosed when patients already have symptoms. Cancer spread depends on the tumor size. T1 lesions spread regionally in 10-20% of the cases, T2 lesions in 25-30% and T3, T4 lesions in 50-75%. In T1 lesions, three year disease-free period is seen in 75-85%, in T2 lesions in 50-60%, while in T3 and T4 lesions in 20-30% of cases. In patients with negative lymph nodes, three year disease free period is seen in 50-60%, while in patients with affected lymph nodes, the survival is 33-50%. Patients with localized cancers of the oral cavity and pharynx have survival of 70%. Patients who were only surgically treated have the survival rate of 81%, those treated with surgery and radiation therapy have the survival rate of 70%, whereas those treated with only radiation therapy have the survival rate of 55%. Patients with distant metastases have 33% of overall survival (65).

1.10 ORAL SIDE-EFFECTS OF CHEMOTHERAPY

Chemotherapy disrupts normal synthesis and/or function of deoxyribonucleic or ribonucleic acid, i.e. proteins. Oral side-effects in these patients are frequent and occur due to various causes. Cytostatics strongly affect cells that rapidly divide and, apart from being helpful in targeting cancer cells, they also target normal oral mucosal cells. Damage of the oral mucosa leads to the expression of inflammatory molecules, which leads to the ulceration development, which may lead to various infections and disturbances while the patient is eating food (66).

Furthermore, due to impact on salivary glands, xerostomia develops which in turn results in development of opportunistic and other infections in the oral cavity. Last but not least, the healing process is compromised due to the influence of chemotherapy on the cell reproduction and leukocytes which are important part of the regenerating processes within the body. Oral side effects due to the chemotherapy might be divided into acute and chronic. Acute side-effects last during the entire period of chemotherapy, while chronic side-effects might be seen even years after chemotherapy has stopped (xerostomia, taste disturbances, loss of teeth etc) (67).

Many oral pathological conditions such as cracked teeth, inadequate fillings and periodontal abscesses due to the inadequate hygiene might lead to additional difficulties during and after chemotherapy. Therefore, apart froma detailed clinical examination, x-rays should be taken (panoramic radiography, and if required periapical radiography) (68).

It is important to note that side effects of chemotherapy might decrease with certain actions before commencement of chemotherapy, which have to be performed at least one month before chemotherapy. Therefore, it is wise to extract all the retained teeth, fill all carious lesions and periapical lesions (even asymptomatic ones) and try to restore periodontal tissues (deep scaling and root planning). Additionally, the places which are prone to mechanical irritation should be removed. In addition to that, it is of utmost importance to maintain the proper oral hygiene (68).

Among the most common adverse effects of chemotherapy in the oral cavity are oral mucositis, xerostomia, peripheral neuropathy and the consequences of the bone marrow suppression which manifests itself with infections in the oral cavity i.e. reactivation of dental and periodontal pathological processes as well as loss of taste (68).

Around 50% of people treated with chemotherapy develop oral mucositis, usually 7-10 days after the commencement of chemotherapy. The symptoms do resign 2-4 weeks after the

chemotherapy cycle. The risk of oral mucositis development correlates with certain cytostatics, prolonged and repeated treatment, together with a number of cycles and it depends on the dose per chemotherapy cycle which is given to patients (69).

Oral mucositis has the following four stages:

1. Redness i.e. inflammation of the oral mucosa
2. After 4-5 days, development of painful ulcers
3. 6-12 day-ulcers are seen in the entire mouth
4. 12-16 day-start of the healing phase. (70)

Distribution of oral mucositis according to World Health Organization:

a) stage 0=no pain
b) stage 1= painful ulcers, erythema and weak edema
c) stage 2=painful erythema, oedema or ulcers: the patient can still eat
d) stage 3=painful erythema, edema or confluent ulcers: the patient cannot eat
e) stage 4= need for hospital treatment i.e. parenteral nutrition.

Oral mucositis treatment relies upon improvement of oral hygiene (teeth brushing every 4 hours), use of artificial saliva (Glandosane®, bicarbonate, diluted hydrogen peroxide), usage of agents against oral mucositis (Gelclair®, Caphosol®). In order to diminish pain symptoms, local analgesics could be applied (Dolokain® gel, Xylocain®) and benzydamine hydrochloride (Tantum Verde®) as well as peroral analgesics.

Throughout the world, tretament of oral mucositis relies on use of topical morphine, low-level laser therapy, phototherapy, palifermine (keratinocyte growth factor), mouthwashes with allopurinol, amifostine, antibiotic pastes or lozenges, hydrolytic enzymes, calcium phosphate, magnsium hydroxide (numerous antacides), povidone iodine and zinc sulphate.

Bleeding in the oral cavity might be a relatively frequent finding in patients on chemotherapy as it affects development of thrombocytes in the bone marrow resulting in the prolonged bleeding. Gingiva might spontaneously bleed. However, bleeding might be due to toothbrushing or food intake.

Taste disturbances are well-known side effects of the chemotherapy. Therefore, food is not tasty enough for them. Also, patients might have nutritional defects due to oral lesions

Pain in the oral cavity in patients treated with chemotherapy might arise due to dental and periodontal causes, oral mucositis, bacterial, viral and fungal infections, neuropathic pain and neuralgic symptoms and musculoskeletal syndrome, etc. (71).

It is recommended to dissolve ice cubes in the mouth, use chewing gums or sugar free lozenges (which depends upon dental status), as well as to take fluid by the use of straw. Tobacco and alcohol consumption should be avoided, as well as spicy and harsh food which might additionally damage oral mucosa. Fluoride pastes and soft toothbrushes are recommended.

In conclusion, accompanying dental preventive and therapeutic measures will diminish the risk of oral and systemic complications which will improve both, the treatment and patients' quality of life.

1.11 INFLAMMATION AND CANCER

The development of oral cancer correlates with intact immune response since it is known that immunosuppressed patients are more prone to the development of cancers within body. The total number of T-lymphocytes is decreased in patients with oral cancer. CD8 lymphocytes dominate in the infiltrate and show immunosuppression which develops together with progression of the invasive cancer. Langerhans cells (intraepithelial macrophages) are slightly increased in number in the human neoplastic epithelium.

Defensive immune response by the host against foreign pathogens is defined as inflammation (72). Inflammation is a significant moderator of carcinogenesis, also associated with poorer disease-free survival and overall survival (73).

Tissue injury reaction activate a multiple chain of chemical signals that initiate and keep a host response designed to cure the injured tissue (74).

This involves activation and directed migration of leukocytes (neutrophils, monocytes and eosinophil cationic proteins) from the venous system to sites of damage, and tissue mast cells which also have a significant role (75).

For neutrophils, a four-step mechanism is a supposed coordination of neutrophils to section of affiliated tissue and to the extracellular matrix (ECM) which is transitional site where endothelial cells and fibroblast proliferate and migrate, making a nest for reconstitution of the normal microenvironment (75).

These steps involve: activation of members of the selecting family of adhesion molecules (L-, P-, and E-selectin) that facilitate rolling along the vascular endothelium; triggering of signals that activate and up regulate leukocyte integrin mediated by cytokines and leukocyte-activating molecules; immobilization of neutrophils on the surface of the vascular endothelium by means of tight adhesion through α4β1 and α4β7 integrin binding to endothelial vascular cell-adhesion molecule-1 (VCAM-1) and MadCAM-1, respectively; and transmigration through the endothelium to sites of injury, presumably facilitated by extracellular proteases, such as matrix metalloproteinase (MMPs).

An acute inflammatory response starts with neutrophils (and sometimes eosinophils). At the site of affected tissue, a migration of monocytes is seen, which differentiate into macrophages, guided by chemotactic factors. Macrophages are the essential source of cytokines and growth.

Factors which affect endothelial, epithelial and mesenchymal cells in local microenvironment. The other cells that are important in inflammation are mast cells which are both sensors and effectors in communication among nervous, vascular, and immune systems. They interact with astrocytes, microglia, and blood vessels via their neuroactive stored and newly synthesized chemicals. They are capable of elaborating a vast array of important cytokines and other inflammatory mediators such as histamine, and proteases complexed to highly sulphated proteoglycans (25).

There are three essential mechanisms by which infections can cause cancer:

1. First, an infectious agent may become persistent within the host and thus induce chronic inflammation (76). This is often accompanied by the formation of reactive oxygen and nitrogen species (ROS and RNOS, respectively) by phagocytes at the site of inflammation. ROS and RNOS have the potential to damage DNA, proteins and cell membranes, and modulate enzyme activities and gene expression, thus favoring carcinogenesis. Furthermore, a chronic inflammation often results in repeated cycles of cell damage, and compensating cell proliferation. This process increases the number of cells that are dividing, thus leading to DNA damage. Also, it promotes the growth of malignant cells (77).
2. Infectious agents may directly transform cells, by inserting active oncogenes into the host genome, inhibiting tumor suppressors or stimulating mitosis (3).
3. Thirdly, infectious agents, such as human immunodeficiency virus (HIV), may induce immune-suppression, with consequent reduced immune-surveillance. The cancer course is very aggressive in immune-compromised body, although the risk factors are unchanged (78).

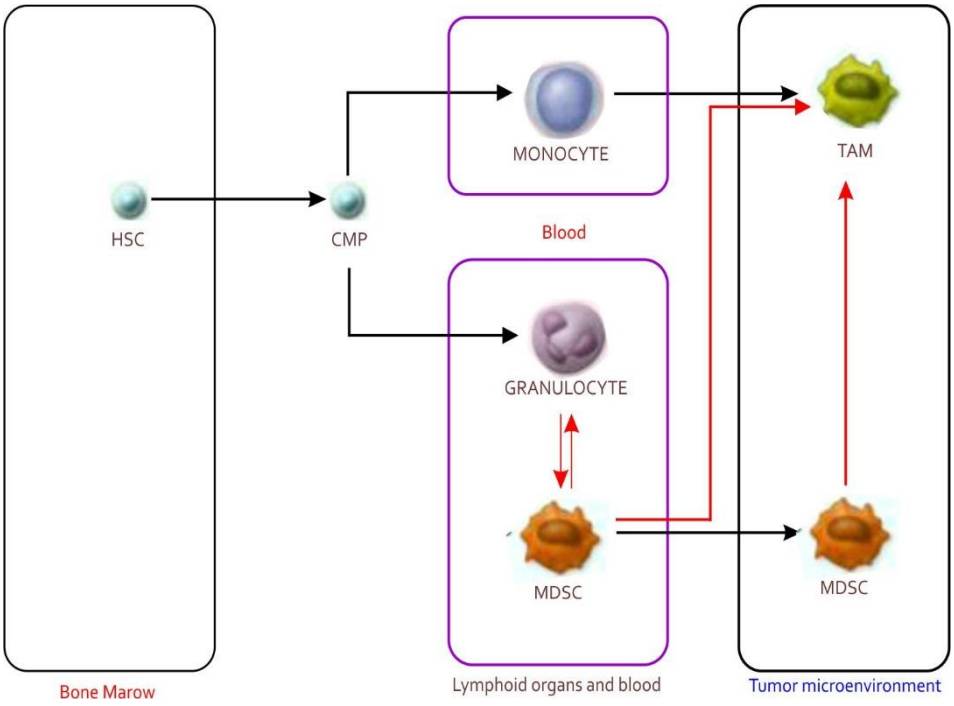

Figure 2: Inflamation and tumor pathway

1.12 NEUTROPHIL/LYMPHOCYTE RATIO (NLR) AND CANCER

The elements of peripheral blood count can predict a disease free survival and overall survival. A complete count of neutrophils derived with complete count of lymphocytes in peripheral blood count is Neutrophil/Lymphocyte ratio that have prognostic value for several types of cancers such as colorectal carcinoma, esophageal carcinoma, lung cancer, etc. (79).

Neutrophils take part in the elimination of pathogens by phagocytosis – generation of reactive oxygen species – via phagocyte NADPH oxidase, the release of antimicrobial and cytotoxic h7 compounds, formation of neutrophil extracellular traps, and secretion of chemokines and cytokines (80)

An increased neutrophil level is characteristic of many cancer types as they promote disease progression by releasing the matrix metalloproteinase-9 (81).

Neutrophils themselves are also a significant source for the hepatocyte growth factor which has been implicated in the regulation of mitogenesis, motogenesis, and morphogenesis of epithelial and endothelial cell (81).

1.13 LYMPHOCYTE/MONOCYTE RATIO (LMR) AND CANCER

Lymphocyte-to-Monocyte ratio (LMR), is also a factor that predicts degree of systemic inflammation, has recently been reported to be a valuable prognostic factor in several types of cancer, such as colon cancer, esophageal carcinoma, lung cancer, diffuse large B cell lymphoma (82).

1.14 THROMBOCYTOSIS AND CANCER

The body produces about 2 x 10^{11} platelets per day. The platelet production is preceded by megakaryocytopoiesis and is regulated by a number of circulating humoral factors, including thrombopoietin. Primitive proliferating progenitor cells are committed to immature megakaryocytes and are finally differentiated to postmitotic megakaryocytes, losing their proliferative capacity in the process. The bone marrow stroma cells produce soluble cytokines allowing the self-renewal and differentiation of thrombopoiesis. To date 19 cytokine growth factors have been identified that regulate thrombopoiesis from early stem cells to postmitotic megakaryocytes (interleukins 1-13, G, M, GM-CSF, leukemia inhibitory factor, SCF and erythropoietin). Thrombopoietin is a specific stimulator of platelet production increasing megakaryocytes and maturation status, whereas cytokines are not specific for stimulating thrombopoiesis. It is this system that regulates megakaryocyte development and the daily output of platelets (83).

Increased levels of thrombopoietin have been found in patients with reactive thrombocytosis and with solid tumors (84). There are indications that a high platelet count is associated with a poor prognosis in carcinomas of the colon and lung and in malignant mesothelioma (83).

2. AIM AND HYPOTHESES

2.1 AIM OF STUDY

The aim of this research was to compare Neutrophil/Lymphocyte ratio, Platelet/Lymphocyte ratio as well as Lymphocyte/Monocyte ratio with the clinical parameters and disease stage according to the TNM classification, disease-free period of patients and overall survival of patients.

The main hypotheses were:

1. The patients with head and neck cancer will have disturbed Neutrophil/Lymphocyte ratio Platelet/Lymphocyte ratio, as well as Lymphocyte/Monocyte ratio
2. The patients with head and neck cancer who will have significantly changed values of these parameters (either increased or decreased values) will have a shorter disease-free period and overall survival.

3. MATERIALS AND METHODS

3.1. STUDY POPULATION

Data were retrieved from patients' charts in the Department of Otorhinolaryngology, at the Clinic for Tumors, Zagreb, Croatia. The study population predominantly consisted of male HNSCC patients (156 male patients; 26 female patients). The results of preoperative and postoperative NLR were associated with demographic characteristics, lifestyle, OS and DFS. For the purpose of analysis, the TNM (tumor-node-metastasis) classification was used for definition of disease stage following criteria of the 8th edition of TNM Classification of malignant tumors.The treatment type was as follows: patients treated only with surgery and patients treated with concomitant radio chemotherapy. The localization of cancer was defined as the oral cavity (anterior 2/3 of the tongue, gingiva and alveolar ridge, hard palate and buccal mucosa) and oropharyngeal (soft palate, pharynx and tonsils). The treatment type was dichotomized as follows: patients' stage I and II were treated only by surgery. Patients' stage III and IV were treated after surgery with adjuvant radiotherapy. Those with involved resection margins and/or extranodular spread of disease additionally (histologically confirmed) received concomitant cisplatin chemotherapy, daily dose of 100 mg/m^2 of body surface, every 3 weeks. Patients with HPV infection were not investigated.

The patients with TNM stage I and II were treated by surgery and stage III and IV received adjuvant therapy after surgery using 3D conformal radiotherapy to target a volume of lymph node regions bilaterally to a prescribed dose of 50 Gy and tumor bed to a prescribed dose of 60 Gy (with or without "boost" dose of 6 Gy) and 6 (or 18) MV photons with linear accelerator (ARTISTE or ONCOR; Siemens Medical Solutions USA, Inc). The patients were irradiated during 6 to 6.5 weeks with daily doses of 2 Gy. Those with involved resection margins and/or extranodular spread of disease additionally (histologically confirmed) received concomitant cisplatin chemotherapy, daily dose of 100 mg/m2 of body surface, every 3 weeks.

3.2. LABORATORY MEASUREMENTS

Blood samples were taken one week before the surgery and postoperatively 7 days after the surgery. Furthermore, data on tumor site, patients' age and gender, alcohol and cigarette intake, type of treatment and TNM classification were collected. The exclusion criteria were: patients with missing data. The Ethics Committee of the Sestre Milosrdnice University Hospital Center approved the study.

Preoperative and postoperative serum neutrophils, platelet, monocytes and lymphocytes were extracted from blood counts of blood samples using fully automated 5-part differential hematology Analyzer (Sysmex XN-1000 (Sysmex, Kobe, Japan). The time of blood sampling (7 days after surgery) ensured that the process of wound healing did not affect the results. The maximal follow-up period was 202.9 months (mean 102.1 months). The NLR was calculated by dividing neutrophil with the lymphocyte count as well as LMR and PLR. Additionally, the difference between preoperative and postoperative NLR (DiffNLR), LMR (DiffLMR), PLR(DiffPLR) was introduced in order to test its predictive capacity for DFS and OS.

3.3. STATISTICAL ANALYSIS

The statistical analysis was conducted using STATISTICA version 12 (StatSoft, Inc., Tulsa, OK, USA) and MedCalc version 18.9 (MedCalc Software, Ostend, Belgium; https://www.medcalc.org; 2016). Categorical variables were recorded as numbers and proportions (%). Quantitative variables were tested for normality of distribution using the Kolmogorov–Smirnov test and recorded as the mean and standard deviation (SD) or median and interquartile range (IQR), depending on the type of data distribution. The Kaplan–Meier survival analysis was used to determine OS and DFS that are presented as the Kaplan–Meier survival curves and median and IQR of OS and DFS time. OS and DFS times were entered either as an exact time or a censored value of time till the last control. The changes in NLR, LMR and PLR before and after surgery were assessed using t-test for dependent samples or Wilcoxon matched pairs test depending on the type of data distribution. Associations of OS and DFS with possible predictors were first analyzed using Cox proportional hazards regression analysis in a univariate models. The variables with the associations having $P < 0.2$ and all the hematological variables measured pre- and post-surgery together with the ratios (NLR, LMR and PLR) and their pre-post differences (DiffNLP, DiffLMR and DiffPLR) were entered into the Cox proportional hazards regression analysis using backward stepwise approach. The results of Cox proportional hazards regression analysis are presented as hazard ratios (HR) and 95% confidence intervals (95% CIs). All of the tests were two-tailed, and $P < 0.05$ was used to indicate significance in all analysis.

4. RESULTS

4.1. BASELINE CHARACTERISTICS

This study included the results of 182 patients treated for HNSCC (85.7% male), whose mean age was 60.0 years (range 23.4–86.3 years). There was a significantly larger number of male patients (85.7%) with a high percentage of patients consuming alcohol (74.7%) and active smokers (65.9%). The baseline characteristics of the study population are presented in (Table 3).

Table 3: Baseline characteristics of the study population (N=182)

Characteristic	
Age (years), mean±SD (range)	60.0±9.7 (23.4 to 86.3)
Sex	
Male	156 (85.7%)
Female	26 (14.3%)
Alcohol consumption	136 (74.7%)
Smoking habit	
Non-smokers	20 (11.0%)
Active smokers	120 (65.9%)
Ex-smokers	25 (13.7%)
Missing data	17 (9.3%)
Tumor location	
Oral	132 (72.5%)
Pharyngeal	48 (27.5%)
TNM stage	
1	21 (11.5%)
2	91 (50.0%)
3	48 (26.3%)
4	22 (12.1%)
Treatment after surgery	
No additional treatment	34 (18.7%)
Radiotherapy	143 (78.6%)
Radio- and chemotherapy	5 (2.7%)
Relapse	124 (68.1%)
Death	122 (67.0%)

There were more patients with oral (72.5%) than with pharyngeal (27.5%) location of oral carcinoma with most of the patients in stage 2, according to the TNM staging (50%). Radiotherapy was the most preferred treatment performed after the initial surgery treatment (Table 3). Relapse of the disease occurred during the follow-up period in 68.1% of patients and death in 67%. DFS had a median of 29.7 months (IQR 11.8–80.9 months) and OS had a median of 31.4 months (IQR 14.8–83.8 months) (Kaplan–Meier survival analysis, Figure 3 and Figure 4).

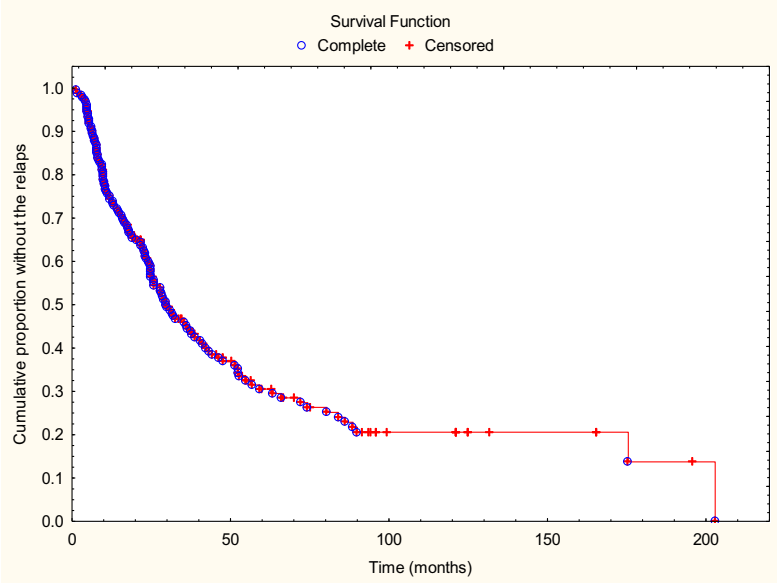

Figure 3: Kaplan–Meier survival curve for disease free survival (DFS)

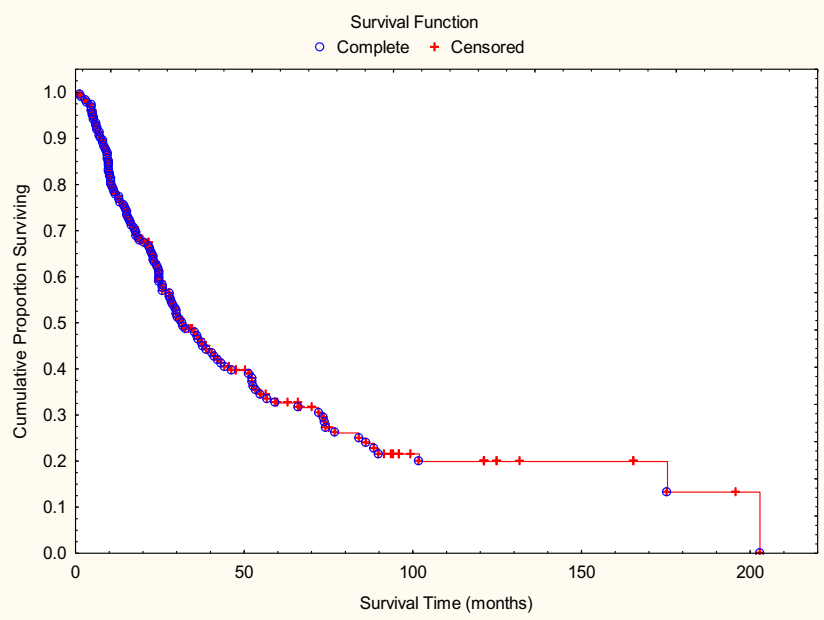

Figure 4: Kaplan–Meier survival curve for overall survival (OS)

4.2. HEMATOLOGICAL MARKERS

The hematological analysis included pre- and postoperative measurements of neutrophil, lymphocyte, monocyte and platelet counts, together with the NLR, LMR, PLR and DiffNLR, DiffLMR and DiffPLR, all presented in (Table 4). It was found that NLR has significantly increased after surgery (Z=7.092, P<0.001), LMR significantly decreased (Z=8.101, P<0.001) and PLR significantly increased (t=6.163, P<0.001).

Table 4: Pre- and post-operative hematological variables and their ratios

Variables	Mean ± SD or median (IQR)
Pre-operative	
Neutrophyl count, $x10^9$/L	5.11 ± 1.88
Lymphocyte count, $x10^9$/L	2.11 ± 0.78
Monocyte count, $x10^9$/L	0.63 ± 0.24
Platelet count, $x10^9$/L	266 ± 86
NLR	2.27 (1.77 to 3.19)
LMR	3.68 ± 1.94
PLR	144 ± 76
Post-operative	
Neutrophyl count, $x10^9$/L	6.34 (4.77 to 8.10)
Lymphocyte count, $x10^9$/L	1.84 ± 0.85
Monocyte count, $x10^9$/L	0.88 ± 0.36
Platelet count, $x10^9$/L	280 ± 104
NLR	3.83 (2.53 to 5.34)
LMR	2.01 (1.45 to 2.82)
PLR	177 ± 93
DiffNLR	1.06 (-0.02 to 2.34)
DiffLMR	-1.24 (-2.26 to -0.27)
DiffPLR	23.93 (-5.63 to 67.18)

Legend: NLR, Neutrophil to Lymphocyte ratio; LMR, Lymphocyte to Monocyte ratio; PLR, Platelet to Lymphocyte ratio, Diff, difference between post- and pre-operative value.

4.3. UNIVARIATE ASSOCIATIONS WITH DISEASE FREE SURVIVAL (DFS) AND OVERALL SURVIVAL (OS)

Univariate associations for possible predictors of DFS and OS were assessed using the Cox proportional hazards regression analysis and the results are presented in (Table 5 and Table 6). Significant univariate associations for DFS were found for preoperative monocyte counts and for DiffLMR (P=0.004 and P=0.043, respectively), with marginal association for alcohol consumption (P=0.054), postoperative NLR (P=0.059) and preoperative LMR (P=0.089) (Table 5). The preoperative monocyte count significantly increased the risk of disease relapse by 3.9 times per each $1x10^9/l$ (HR=3.919, 95% CI 1.565 to 9.812), and the DiffLMR significantly increased the risk of disease relapse by 14.9% per each unit change (HR=1.149, 95% CI 1.004 to 1.314).

Table 5: Univariate associations with disease free survival

Covariate	b	SE	Wald	P	HR	95% CI HR
Age	0.013	0.010	1.831	0.176	1.013	0.994 to 1.032
Sex	-0.307	0.269	1.303	0.254	0.736	0.435 to 1.246
No alcohol	-0.506	0.263	3.699	0.054	0.603	0.360 to 1.010
Smoking	-0.027	0.119	0.052	0.819	0.973	0.770 to 1.229
Grade	0.020	0.187	0.012	0.914	1.020	0.707 to 1.472
Tumor location	0.147	0.200	0.542	0.462	1.158	0.783 to 1.713
Treatment after surgery	0.341	0.243	1.968	0.161	1.406	0.873 to 2.265
Preoperative neutrophil count	0.018	0.046	0.151	0.698	1.018	0.930 to 1.115
Preoperative lymphocyte count	0.025	0.136	0.033	0.856	1.025	0.786 to 1.337
Preoperative monocyte count	1.366	0.468	8.508	**0.004**	3.919	1.565 to 9.812
Preoperative platelet count	0.000	0.001	0.036	0.849	1.000	0.998 to 1.003
Postoperative neutrophil count	0.049	0.032	2.335	0.127	1.050	0.986 to 1.119
Postoperative lymphocyte count	0.057	0.107	0.278	0.598	1.058	0.858 to 1.306
Postoperative monocyte count	0.309	0.269	1.324	0.250	1.362	0.805 to 2.306
Postoperative platelet count	0.001	0.001	1.806	0.179	1.001	0.999 to 1.003
Preoperative NLR	0.067	0.053	1.607	0.205	1.069	0.964 to 1.185
Postoperative NLR	0.044	0.023	3.579	0.059	1.045	0.998 to 1.095
DiffNLR	0.032	0.027	1.383	0.240	1.033	0.979 to 1.090
Preoperative LMR	-0.127	0.075	2.897	0.089	0.880	0.760 to 1.020
Postoperative LMR	0.038	0.082	0.210	0.647	1.039	0.884 to 1.221
DiffLMR	0.139	0.069	4.088	**0.043**	1.149	1.004 to 1.314
Preoperative PLR	0.001	0.001	0.608	0.435	1.001	0.998 to 1.004
Postoperative PLR	0.001	0.001	1.560	0.212	1.001	0.999 to 1.003
DiffPLR	0.001	0.001	0.741	0.390	1.001	0.998 to 1.004

Legend: NLR, Neutrophil to Lymphocyte ratio; LMR, Lymphocyte to Monocyte ratio; PLR, Platelet to Lymphocyte ratio, Diff, Difference between post- and pre-operative value. Bold numbers represent significant associations.

Table 6: Univariate associations with overall survival

Covariate	b	SE	Wald	P	HR	95% CI HR
Age	0.014	0.010	2.109	0.147	1.014	0.995 to 1.034
Sex	-0.241	0.269	0.803	0.370	0.786	0.464 to 1.331
No alcohol	-0.556	0.270	4.227	**0.040**	0.574	0.338 to 0.974
Smoking	-0.041	0.122	0.116	0.734	0.959	0.756 to 1.218
Grade	0.058	0.188	0.095	0.758	1.060	0.733 to 1.532
Tumor location	0.110	0.200	0.300	0.584	1.116	0.754 to 1.653
Treatment after surgery	0.352	0.253	1.936	0.164	1.422	0.866 to 2.336
Preoperative neutrophil count	0.006	0.047	0.018	0.894	1.006	0.917 to 1.104
Preoperative lymphocyte count	-0.032	0.141	0.051	0.821	0.969	0.735 to 1.277
Preoperative monocyte count	1.237	0.486	6.487	**0.011**	3.446	1.330 to 8.927
Preoperative platelet count	0.000	0.001	0.063	0.802	1.000	0.998 to 1.003
Postoperative neutrophil count	0.041	0.033	1.553	0.213	1.041	0.977 to 1.110
Postoperative lymphocyte count	0.026	0.113	0.052	0.819	1.026	0.823 to 1.280
Postoperative monocyte count	0.072	0.281	0.065	0.798	1.075	0.620 to 1.864
Postoperative platelet count	0.001	0.001	2.120	0.145	1.001	0.999 to 1.003
Preoperative NLR	0.082	0.053	2.332	0.127	1.085	0.977 to 1.205
Postoperative NLR	0.044	0.022	3.977	**0.046**	1.045	1.001 to 1.092
DiffNLR	0.031	0.026	1.409	0.235	1.032	0.980 to 1.087
Preoperative LMR	-0.135	0.077	3.056	0.080	0.874	0.752 to 1.016
Postoperative LMR	0.069	0.082	0.718	0.397	1.072	0.913 to 1.259
DiffLMR	0.158	0.071	5.013	**0.025**	1.171	1.020 to 1.345
Preoperative PLR	0.002	0.001	1.441	0.230	1.002	0.999 to 1.004
Postoperative PLR	0.002	0.001	2.929	0.087	1.002	0.999 to 1.004
DiffPLR	0.002	0.001	1.132	0.287	1.002	0.999 to 1.004

Legend: NLR, Neutrophil to Lymphocyte ratio; LMR, Lymphocyte to Monocyte ratio; PLR, Platelet to Lymphocyte ratio, Diff, Difference between post- and pre-operative value. Bold numbers represent significant associations.

Significant univariate associations for OS were found for alcohol consumption (P=0.040), preoperative monocyte counts (P=0.011), postoperative NLR (P=0.046) and for DiffLMR (P=0.025), with marginal association for preoperative LMR (P=0.080) and postoperative PLR (P=0.087) (Table 4). Not consuming alcohol significantly lowered the risk of death by 42.6%

(HR=0.574, 95% CI 0.338 to 0.974), the preoperative monocyte count significantly increased the risk of death by 3.4 times per each $1x10^9$/l (HR=3.446, 95% CI 1.330 to 8.927), the postoperative NLR significantly increased the risk of death by 4.5% per each unit change (HR=1.045, 95% CI 1.001 to 1.092), and DiffLMR significantly increased the risk of death by 17.1% per each unit change (HR=1.171, 95% CI 1.020 to 1.345).

4.4. MULTIVARIATE ASSOCIATIONS WITH DISEASE FREE SURVIVAL AND OVERALL SURVIVAL

Significant independent associations with DFS and OS are presented in (Tables 5 and Table 6) respectively. As seen in (Table 5), not consuming alcohol had a marginal significance in lowering the risk of disease relapse by 40.4% (P=0.083). The treatment step after surgery had a marginal significance in increasing the risk of disease relapse by 66.9% pre each step (no treatment/ radiotherapy/ radio-chemotherapy) (P=0.083). The preoperative neutrophil count significantly lowered the risk of disease relapse by 15.5% per each $1x10^9$/l (P=0.030), and the preoperative monocyte count significantly increased the risk of disease relapse by 6.1 times per each $1x10^9$/l (P=0.001). There was a significant increase in the risk of disease relapse of 25.8% for a unit change in the preoperative NLR (P=0.004), significant increase in this risk by 31.2% for a unit change in postoperative LMR (P=0.007), and a significant increase in this risk by 6.0% for a unit change in DiffNLR (P=0.036).

Table 7: Results of a multivariate (backward stepwise) Cox proportional hazards regression analysis for disease free survival (DFS); chi-squared=22.431, df=7, P=0.002 for the model

Covariate	b	SE	Wald	P	HR	95% CI HR
No alcohol	-0.518	0.299	3.015	0.083	0.596	0.332 to 1.069
Treatment after surgery	0.512	0.295	3.027	0.082	1.669	0.937 to 2.973
Preoperative neutrophil count	-0.168	0.077	4.730	**0.030**	0.845	0.726 to 0.984
Preoperative monocyte count	1.810	0.546	11.003	**0.001**	6.109	2.097 to 17.797
Preoperative NLR	0.230	0.079	8.366	**0.004**	1.258	1.077 to 1.470
Postoperative LMR	0.271	0.101	7.176	**0.007**	1.312	1.076 to 1.599
DiffNLR	0.059	0.028	4.420	**0.036**	1.060	1.004 to 1.120

Legend: NLR, Neutrophil to Lymphocyte ratio; LMR, Lymphocyte to Monocyte ratio; Diff, difference between post- and pre-operative value. Bold numbers represent significant associations.

Table 8: Results of a multivariate (backward stepwise) Cox proportional hazards regression analysis for overall survival (OS); chi-squared=24.008, df=7, P=0.001 for the model

Covariate	b	SE	Wald	P	Exp(b)	95% CI of Exp(b)
No alcohol	-0.538	0.309	3.035	0.082	0.584	0.319 to 1.070
Treatment after surgery	0.577	0.307	3.531	0.060	1.780	0.976 to 3.249
Preoperative neutrophil count	-0.205	0.079	6.709	**0.010**	0.815	0.698 to 0.952
Preoperative monocyte count	1.756	0.564	9.684	**0.002**	5.789	1.916 to 17.497
Postoperative NLR	0.264	0.078	11.327	**0.001**	1.302	1.116 to 1.518
Postoperative LMR	0.300	0.100	8.934	**0.003**	1.349	1.109 to 1.642
DiffNLR	-0.206	0.073	7.901	**0.005**	0.814	0.706 to 0.940

Legend: NLR, neutrophil to lymphocyte ratio; LMR, lymphocyte to monocyte ratio; Diff, difference between post- and pre-operative value. Bold numbers represent significant associations.

As seen in (Table 6), not consuming alcohol had a marginal significance in lowering the risk of death by 41.6% (P=0.082). The treatment step after surgery had a marginal significance in increasing the risk of death by 78.0% pre each step (no treatment/ radiotherapy/ radio-chemotherapy) (P=0.060). The preoperative neutrophil count significantly lowered the risk of death by 18.5% per each $1x10^9$/l (P=0.010), and the preoperative monocyte count significantly increased the risk of death by 5.8 times per each $1x10^9$/l (P=0.002). There was a significant increase in the risk of death of 30.2% for a unit change in the postoperative NLR (P=0.001), significant increase in this risk by 34.9% for a unit change in postoperative LMR (P=0.003), and a significant decrease in this risk by 18.6% for a unit change in DiffNLR (P=0.005).

Tumor location, tumor stage, smoking, sex and age were not significantly associated with either DFS or OS.

5. DISCUSSION

Some studies have found that patients with cancer exhibit abnormal leukocyte fractions such as elevated neutrophil count and diminished lymphocyte count. Also, these studies have found that the Neutrophil-to-Lymphocyte ratio (NLR) provides a surrogate marker for prognosis and response to treatment of patients after radical surgery for several different types of cancer Iimori et al. (120).

5.1. THE CORRELATION BETWEEN COMPLETE BLOOD COUNT AND VARIOUS CANCERS IN THE HUMAN BODY

5.1.1. Complete Blood Count and Gynecological Malignancies

Yildirim et al. (85) analyzed 316 patients with benign and 253 patients with malignant adnexal masses and reported that NLR, PLR neutrophil and platelet values were higher in the group of patients with malignant adnexal masses when compared with patients with benign adnexal masses. Furthermore, the same authors (85) stated that the lymphocyte values were decreased in the group with malignant adnexal masses. Eventually, the same authors (85) concluded that NLR and PLR might serve for detection of ovarian malignancies in their early stages, which naturally, increases the treatment options and improves the survival rates.

Bakacak et al. (86) reviewed 185 benign and 33 malignant cases of adnexal masses and concluded that age, CA-125 levels, NLR, PLR, and lymphocyte count determined preoperatively might differentiate malignant from benign ovarian masses.

Wang et al. (87) reported that NLR was independent prognostic factor for overall survival (OS) and progression-free survival (PFS) in patients with ovarian cancer.

Furthermore, Younes et al. (88) analyzed 56 patients with uterine serous papillary carcinoma and showed that leukocytosis and neutrophilia correlated with aggressive tumor biology might reveal decreased 5 year survival.

Seckin et al. (89) investigated a total of 63 patients, of which 41 patients had borderline, 11 patients had benign, and 11 patients had malignant mucinous ovarian tumors. The same authors stated (89) that together with frozen samples, CA19-9 and NLR had the highest sensitivity to detect mucinous cancers (81 and 78 percent, respectively). Therefore, they suggested an incorporation of all these values into the everyday clinical practice.

Utsumi et al. (90) investigated 77 patients with ovarian cancer and multivariable analysis revealed that an elevated white blood cell count significantly correlated with a shorter overall survival of the patients.

Eo et al. (91) concluded that preoperative LMR is an independent predictor of suboptimal cytoreduction in patients with advanced-stage epithelial ovarian cancer, which revealsan additional piece of prognostic information.

Paik et al. (92) reported data upon 757 patients with epithelial ovarian cancer whose primary treatment consisted of surgical debulking and chemotherapy. The same authors (92) reported that lymphocyte and monocyte count were found to be significant prognostic factors for platinum-sensitivity, platelet count for progression free survival and neutrophil count for overall survival on multivariate analysis.

Huang et al. (93) enrolled 55 ovarian cancer patients and subgroup analysis for primary, platinum-sensitive, and platinum-resistant patients showed that PLR and NLR significantly correlated to the outcome of dose-dense chemotherapy. Lower PLR or lower NLR had better treatment response for dose-dense chemotherapy and are possible markers for representing treatment response in dose-dense chemotherapy. For a clinician, this is useful for timing: when to switch to another chemotherapy regimen.

Miao et al. (94) analyzed 344 patients with epithelial ovarian cancer and the patients with lower value of NLR or PLR had a longer progression-free survival (PFS) and overall survival (OS). In multivariate analysis, NLR and PLR showed a significant association with PFS and OS in predicting platinum resistance in patients with epithelial ovarian cancer.

Chen et al. (95) included 816 Chinese women with epithelial ovarian cancer and concluded that preoperative anemia, leukocytosis or thrombocytosis in these patients was tightly associated with more malignant disease phenotype and poorer prognosis. Moreover, thrombocytosis is an independent indicator of the disease- specific survival in patients with epithelial ovarian cancer.

Ma et al. (96) found out that thrombocytosis and increased platelet aggregation rates were associated with more aggressive tumor biology in patients with epithelial ovarian cancer.

Additionally, the combination of thrombocytosis and increased platelet aggregation rate is an independent negative prognostic factor for the overall survival in these patients.

Furthermore, So et al. (97) analyzed data of 155 women with epithelial ovarian cancer, and the results have shown that 23 (14.8%) had leukocytosis and 132 (85.2%) did not have leukocytosis. Mortality rates were higher as RFS and OS were significantly shorter for women with leukocytosis than for women without leukocytosis. It seems that preoperative leukocytosis might be an independent prognostic factor for RFS and OS.

Njølstad et al. (98) evaluated 557 patients with endometrial carcinoma and reported that patients with anemia, leukocytosis and thrombocytosis had significantly shorter 5-year disease-specific survival when compared to the patients with normal counts as well as advanced disease. Furthermore, lower hemoglobin counts and higher platelet counts were independently associated with poor disease outcome.

5.1.2. Complete blood count and colorectal cancer

It has been shown that NLR and PLR were significantly higher in CRC patients preoperatively compared with healthy participants as reported by Kilincap et al. (99), Emir et al. (100) reported that NLR and PLR might indicate conversion of colonic and rectal neoplastic polyps to invasive tumor. Jia et al. (101) stated that pre-treatment levels of NLR and PLR might provide useful information for the early diagnosis or the therapeutic choices in CRC patients.

He et al. (102) concluded that NLR is an independent factor for OS and PFS in CRC patients. Kim et al. (103) reported that the patients with pre-treatment thrombocytosis had lower 3-year disease-free and overall survival rates when compared with patients who had normal pre-treatment platelet counts.

Kitayama et al. (104) concluded that high lymphocyte counts (>1,800/mm) had an independent association with disease-free survival in patients with CRC. Kitayama et al. (104) concluded that in patients with rectal cancer, peripheral blood lymphocytes have a significant impact on the response to radiotherapy.

However, Shen et al. (105) concluded that an elevated baseline NLR is a valuable and easily available prognostic factor for OS after neoadjuvant therapy.

Besides, Carruthers et al. (106) reported that in addition to postoperative surgical margin (R-status), an elevated NLR is associated with worse OS and DFS.

Kitayama et al. (104) reported that circulating lymphocyte count was most markedly decreased in during the chemotherapy and radiation therapy, which eventually recovered after the surgery, while the numbers of neutrophils and monocytes were comparatively stable.

Ying et al. (105) indicated that preoperative elevated NLR can be considered as an independent prognostic biomarker for recurrent-free survival, the overall survival and cancer specific survival in patients with colorectal cancer.

Wei et al. (107) concluded that colorectal cancer patients receiving adjuvant chemotherapy with baseline platelet count>276×109/L had worse prognosis.

Seong et al. (108) analyzed 265 patients who had undergone curative resection of colorectal cancer and reported that on univariate analysis, CRP, ESR, and NLR were significantly associated with DFS and DSS. On multivariate analysis, CRP and NLR were independently significant prognostic variables for DSS and DFS.

Ikeguchi et al. (109) included 50 patients with unresectable advanced colorectal cancer with regard to the C-reactive protein (CRP), albumin (ALB), neutrophil/lymphocyte ratio (NLR), and carcinoembryonic antigen (CEA). They concluded that aforementioned markers significantly correlated with poor prognosis.

Furthermore, Shibutani et al. (110) included 110 patients with un-resectable metastatic colorectal cancer who underwent palliative chemotherapy. The same authors (80) stated that the overall survival rates were significantly worse in the group with high pre-treatment NLR and CRP.

The 117 stage IV CRC patients who underwent curative resection were analyzed by Ozawa et al. (111) The same authors (111) indicated that that the LMR was an independent prognostic factor for CSS in patients with stage IV CRC who had undergone curative resection, but not for DFS. Therefore, they concluded that the preoperative LMR is a simple and useful prognostic indicator in patients with stage IV CRC who have undergone curative resection.

Preoperative asymptomatic leukocytosis had a prevalence of 5.6% (out of total number of 59,805 patients in colorectal cancer resections and carries and a significant increased risk of mortality and morbidity were reported by Moghadamyeghaneh et al. (112).

Shibutani et al. (110) reported that the overall survival rates were significantly worse in the high preoperative NLR and postoperative NLR group. Furthermore, the postoperative NLR is an independent indicator for disease outcome in these patients.

5.1.3. Complete blood count and breast cancer

Onesti et al. (113) analyzed data of 112 breast cancer patients (79 triple-negative, 33 hormone receptor-negative/HER2-positive) who were treated with standard neoadjuvant chemotherapy. The same authors (113) concluded that relative eosinophil count and eosinophil x lymphocyte product might serve as new prognostic indicators of the response to the neoadjuvant chemotherapy as well as be prognostic tool for longer survival in these patients.

Geng et al. (114) stated that NLR was identified as independent prognostic factor for DFS in 2458 breast cancer patients. Ferroni et al. (115) analyzed pre-treatment NLR in 475 patients with breast cancer and stated that elevated pre-treatment NLR was associated with worse disease-free survival (DFS) and overall survival (OS). NLR might provide important information in stage I BC patients in terms of depicting patients who should be more aggressively treated.

Higher preoperative neutrophil to lymphocyte ratio was found in breast cancer patients, as stated by Fange et al. (116). Liu et al. (117) have shown that eighteen eligible studies demonstrated that elevated NLR was associated with poor DFS, OS and cancer-specific survival of breast cancer patients. Iimori et al. (118) suggested that the NLR may represent a predictive marker for response to endocrine therapy in stage IV breast cancer which they proved on their 34 patients.

Regarding the Platelet/Lymphocyte ratio, Zhang et al. (119) performed meta-analysis and concluded that PLR was associated with poor prognosis of breast cancer and adequately predicted clinicopathological characteristics.

The following study included 110 women with breast cancer and 78 cases of healthy females who were analysed regarding correlation of haematological parameters and clinicopathological characteristics by Sun et al. (120). The results have shown that NLR and PLR were significantly higher in diseased patients when compared to the healthy controls. Warris et al. (121) also confirmed that NLR and PLR are independent indicators of prognosis in 2374 women with breast cancer.

On the other hand, Takeuchi et al. (122) concluded that LMR and NLR could not predict disease free survival in patients with breast cancer, however, they also stated that preoperative CRP and PLR showed a correlation with worse prognosis in these patients.

A higher pretreatment NLR significantly and independently indicated worse prognosis for patients with breast cancer and triple-negative breast cancer, and this finding was superior to lower LMR. However, NLR showed not to be an indicator for other breast cancer subtypes, which was concluded by Jia et al. (123) in a study based on 1579 patients with various types of breast cancer.

Moreover, He et al. (124) concluded that pretreatment LMR might serve as apredictive marker of the overall survival in women with triple negative breast cancer.

Bozkurt et al. (125) analyzed data of 85 patients with triple negative breast cancer and stated that women with early TNBC and elevated pretreatment NLR had a decreased disease free survival as well as the overall survival when compared to the women who did not have an increased NLR.

5.2. THE CORRELATION BETWEEN COMPLETE BLOOD COUNT AND HEAD AND NECK CANCERS

At present, the data for preoperative and postoperative NLR in HNSCC are missing. Therefore, the aim of this retrospective study was to establish preoperative and postoperative NLR levels for HNSCC together with their association with DFS and OS period for the first time. Additionally, multivariate analysis was used in order to check the impact of alcohol and tobacco intake, patient age, tumor location, cancer stage and therapy on NLR’s capability to predict the disease outcome.

This study has shown that preoperative and postoperative neutrophil counts and NLR can be predictive biomarkers for DFS and OS in HNSCC. This study used multivariate analysis, which enabled the testing of neutrophil count, NLR and DiffNLR as predictive biomarkers for DFS and OS by including alcohol consumption, patient age, smoking, and tumor stage and therapy type as modifying parameters. Additionally, this was the first time that the difference in NLR before and after surgery was shown as a significant predictor of OS and DFS. The absence of alcohol consumption was significantly associated with longer DFS and better OS.

Lymphocytes are the most significant components of the adaptive immune system, which when infiltrated into the tumor indicate the generation of an effective antitumor cellular immune response (126). An increased NLR has been associated with an increase in the peri-tumoral infiltration of macrophages and an increase in interleukin 17, interleukin 6, interleukin 8, tumor growth promoting factors, vascular endothelial growth factor, hepatocyte growth factor and matrix metalloproteinases, all of which form tumor microenvironments (127).

Previous studies have shown the association between a low peripheral blood lymphocyte count and shorter survival of patients with different types of cancers (128, 129). However, other cell types also involved in immunological response have been shown to play a significant role in the progression of cancer. Thus, it was reported that neutrophils support metastases by producing leukotrienes which enable colonization of distant tissues with cancer cells (130). Both neutrophils and NLR have been shown to be prognostic factors in nasopharyngeal cancer independent of OS and DFS (131). Tsai et al. (132) reported that neutrophil counts and NLR increased with the advancement of the clinical stage (i.e. T4) and poorer tumor differentiation in patients with oral cancer, which was also accompanied with decreased lymphocyte count.

Location of tumor was not associated with preoperative and postoperative NLR or DiffNLR and OS or DFS which is in line with published data (133).

Our results confirm those from a report by Rachidi et al. (133) stating that NLR is a robust predictor of overall survival in oral, pharyngeal, and laryngeal squamous cell carcinomas. Perisanidis et al. (134) analyzed 97 patients with oral cancer undergoing preoperative chemo-radiotherapy regarding DFS. The same authors (34) reported that a high pretreatment NLR is a significant independent predictor of shorter DFS in patients with oral cancer receiving

preoperative chemo-radiotherapy. Fang et al. (135) analyzed data from 226 patients with oral cancer and reported that elevated levels of NLR were significantly associated with tumor status, nodal metastasis, tumor depth, disease-free survival and overall survival. The level of NLR on DFS and OS has been still shown to exist after adjusting data for tumor status, lymph node metastasis, and tumor cell differentiation. Song et al. (136) showed that a high preoperative NLR was associated with increased wound complications and poorer survival in patients with hypo pharyngeal squamous cell carcinoma after radical resections. Our study is in concordance with these studies which suggested that the preoperative NLR is an independent predictor of head and neck cancer recurrence (137, 138,139) even after other lifestyles and demographic data are included in analysis.

A recent study on healthy population estimated normal NLR values in an adult, non-geriatric, population in good health to be between 0.78 and 3.53, which is a significant added value in application of NLR. However, the obtained control range did not take into account the smoking habit and alcohol intake. It is interesting to note that in our study, where 70-80 % of the subjects were alcoholics and smokers, the mean postoperative NLR value was higher (group value) than the suggested control range values. Alcohol intake was associated with neutropenia, and neutrophils were also shown to be hypo-responsive in cases of exposure to alcohol due to impaired phagocytosis and superoxide generation in humans and in animal models (139, 140). Additionally, the heterogeneous impact of alcohol on OS related with treatment and primary site points to the need for further investigation (141). However, the effect of smoking on measured parameters did not have great significance, as it is known that contrary to alcohol, smoking causes an increase in neutrophil counts (142). Our results confirm the importance of including alcohol consumption and smoking into calculations of NLR.

Our study showed that neutrophil counts, NLR and DiffNLR are significantly associated with DFS and OS in HNSCC. Using neutrophil counts, NLR and DiffNLR therapy and medical surveillance protocols in these patients may be adjusted on the individual level to achieve better DFS and OS. In conclusion, a significant association between alcohol consumption and tumor site with regard to the DFS and OS was noticed. The design of our study should be repeated in other cohorts in order to define cut-off values for HNSCC and include these parameters into clinical practice.

6. CONCLUSIONS

This study has shown that preoperative and postoperative neutrophil, lymphocyte, monocyte and platelet counts and NLR,LMR and PLR can be predictive biomarkers for DFS and OS in HNSCC.

Not consuming alcohol significantly lowered the risk of death, the preoperative monocyte count significantly increased the risk of death, the postoperative NLR significantly increased the risk of death and DiffLMR significantly increased the risk of death.

In the multivariate analysis, the preoperative neutrophil count significantly increased DFS and the preoperative monocyte count significantly decreased DFS.

There was a significant decrease of DFS in the preoperative NLR, significant increase in postoperative LMR and a significant increase in DiffNLR.

Preoperative monocyte count significantly increased the risk of death, postoperative NLR, postoperative LMR and DiffNLR significantly decreased the risk of death.

Location of tumor was not associated with preoperative and postoperative NLR or DiffNLR and OS or DFS.

Additionally, to our knowledge this was the first time that the difference in NLR before and after surgery was shown as a significant predictor of OS and DFS. Furthermore, this study showed that neutrophil counts, NLR and DiffNLR are significantly associated with DFS and OS in HNSCC.

Conclusively, the results of this study have shown that preoperative and postoperative NLR and LMR and their components have a significant predictive value for both DFS and OS.

The design of our study should be repeated in other cohorts in order to define cut-off values for HNSCC and include these parameters into clinical practice.

7. REFERENCES

1. Devi PU. Basics of carcinogenesis. Health Adm. 2004;17(1):16-24.
2. Gelband H, Sloan FA, editors. Cancer control opportunities in low-and middle-income countries.NAP(US); 2007 .
3. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002 ;420(6917):860.
4. Belpomme D, Irigaray P, Hardell L, Clapp R, Montagnier L, Epstein S, Sasco AJ. The multitude and diversity of environmental carcinogens. Environ Res. 2007;105(3):414-29.
5. Farber E. The multistep nature of cancer development. Cancer Res. 1984 ; 44(10):4217-23.
6. Kao HK, Löfstrand J, Loh CY, Lao WW, Yi JS, Chang YL, Chang KP. Nomogram based on albumin and neutrophil-to-lymphocyte ratio for predicting the prognosis of patients with oral cavity squamous cell carcinoma. Sci Rep. 2018; 8(1):1308.
7. Prasad RB, Sharma A, Babu HM. An insight into salivary markers in oral cancer. Dent Res J 2013; 10(3):287
8. Neville BW, Day TA. Oral cancer and precancerous lesions. CA Cancer J Clin 2002 Jul; 52(4):195-215.
9. Müller S, Pan Y, Li R, Chi AC. Changing trends in oral squamous cell carcinoma with particular reference to young patients: 1971–2006. Head Neck Pathol 2008; 2(2):60.
10. Tandon P, Dadhich A, Saluja H, Bawane S, Sachdeva S. The prevalence of squamous cell carcinoma in different sites of oral cavity at our Rural Health Care Centre in Loni, Maharashtra–a retrospective 10-year study. Contemp Oncol . 2017; 21(2):178.
11. Choi S, Myers JN. Molecular pathogenesis of oral squamous cell carcinoma: implications for therapy. J Dent Res. 2008; 87(1):14-32..
12. Weatherspoon DJ, Chattopadhyay A, Boroumand S, Garcia I. Oral cavity and oropharyngeal cancer incidence trends and disparities in the United States: 2000–2010. Cancer Epidemiol. 2015; 39(4):497-504.
13. Zhang Y, Wang R, Miao L, Zhu L, Jiang H, Yuan H. Different levels in alcohol and tobacco consumption in head and neck cancer patients from 1957 to 2013. PloS One. 2015; 10(4):e0124045.
14. Katzel JA, Merchant M, Chaturvedi AK, Silverberg MJ. Contribution of demographic and behavioral factors on the changing incidence rates of oropharyngeal and oral cavity cancers in Northern California. Cancer Epidemiol Biomarkers Prev. 2015 (6):978-84.

15. Winn D, Lee YC, Hashibe M, Boffetta P, Consortium I. The Inhance consortium: toward a better understanding of the causes and mechanisms of head and neck cancer. Oral Dis 2015; 21(6):685-93.

16. Zhang S-K, Zheng R, Chen Q, Zhang S, Sun X, Chen W. Oral cancer incidence and mortality in China, 2011. Chin J Cancer 2015; 27(1):44.

17. Hertrampf K, Eisemann N, Wiltfang J, Pritzkuleit R, Wenz H-J, Waldmann A. Baseline data of oral and pharyngeal cancer before introducing an oral cancer prevention campaign in Germany. J Cranio Maxill Surg . 2015; 43(3):360-6.

18. Toporcov TN, Znaor A, Zhang Z-F, Yu G-P, Winn DM, Wei Q, et al. Risk factors for head and neck cancer in young adults: a pooled analysis in the Inhance consortium. Int J Epidemiol. 2015; 44(1):169-85.

19. Guntinas-Lichius O, Wendt TG, Kornetzky N, Buentzel J, Esser D, Böger D, et al. Trends in epidemiology and treatment and outcome for head and neck cancer: A population-based long-term analysis from 1996 to 2011 of the Thuringian cancer registry. Oral Oncol. 2014; 50(12):1157-64.

20. Bagnardi V, Rota M, Botteri E, Tramacere I, Islami F, Fedirko V, et al. Alcohol consumption and site-specific cancer risk: a comprehensive dose–response meta-analysis. Br J Cancer. 2015; 112(3):580.

21. Jayasekara H, MacInnis RJ, Hodge AM, Hopper JL, Giles GG, Room R, et al. Lifetime alcohol consumption and upper aero-digestive tract cancer risk in the Melbourne Collaborative Cohort Study. Cancer Causes Control. 2015; 26(2):297-301

22. Sharma JD, Kalita M, Barman D, Sharma A, Lahon R, Barbhuiya JA, et al. Patterns of upper aero-digestive tract cancers in Kamrup urban district of Assam: A retrospective study. Asian Pac J Cancer Prev. 2014; 15(17):7267-70

23. Krishna A, Singh R, Singh S, Verma P, Pal U, Tiwari S. Demographic risk factors, affected anatomical sites and clinicopathological profile for oral squamous cell carcinoma in a north Indian population. Asian Pac J Cancer Prev. 2014; 15(16):6755-60.

24. Sharp L, McDevitt J, Carsin A-E, Brown C, Comber H. Smoking at diagnosis is an independent prognostic factor for cancer-specific survival in head and neck cancer: findings from a large, population-based, study. Cancer Epidemiol Biomarkers Prev. 2014:cebp. 0311.2014.

25. Etemadi A, O'Doherty MG, Freedman ND, Hollenbeck AR, Dawsey SM, Abnet CC. A prospective cohort study of body size and risk of head and neck cancers in the NIH–AARP Diet and Health Study. Cancer Epidemiol Biomarkers Prev.2014 ; 23(11):2422-9.

26. Edefonti V, Hashibe M, Parpinel M, Ferraroni M, Turati F, Serraino D, et al. Vitamin E intake from natural sources and head and neck cancer risk: a pooled analysis in the International Head and Neck Cancer Epidemiology consortium. Br J Cancer 2015; 113(1):182-92.

27. Edefonti V, Hashibe M, Parpinel M, Turati F, Serraino D, Matsuo K, et al. N atural vitamin C intake and the risk of head and neck cancer: A pooled analysis in the I nternational Head and Neck Cancer Epidemiology Consortium. Int J Cancer 2015; 137(2):448-62

28. Cittadini MC, Cornaglia PM, Perovic NR, Joekes S, Heinze VM, Bernal C, et al. Beef consumption and fatty acids serum concentration: relationship with salivary gland tumors in Cordoba, Argentina. Anticancer Res. 2014; 34(10):5579-84.

29. Fanidi A, Relton C, Ueland PM, Midttun Ø, Vollset SE, Travis RC, et al. A prospective study of one-carbon metabolism biomarkers and cancer of the head and neck and esophagus. Int J Cancer. 2015; 136(4):915-27.

30. Galeone C, Edefonti V, Parpinel M, Leoncini E, Matsuo K, Talamini R, et al. Folate intake and the risk of oral cavity and pharyngeal cancer: A pooled analysis within the I nternational Head and Neck Cancer Epidemiology Consortium. Int J Cancer 2015; 136(4):904-14.

31. Maasland DH, van den Brandt PA, Kremer B, Goldbohm RA, Schouten LJ. Consumption of vegetables and fruits and risk of subtypes of head-neck cancer in the Netherlands Cohort Study. Int J Cancer. 2015; 136(5):E396-409.

32. Zhang W, Geng T, Han W, Dou H. Tea intake and risk of oral, pharyngeal, and laryngeal carcinoma: a meta-analysis. Med Sci Monit 2014; 20:2142.

33. Papagerakis S, Bellile E, Peterson LA, Pliakas M, Balaskas K, Selman S, et al. Proton pump inhibitors and histamine 2 blockers are associated with improved overall survival in patients with head and neck squamous carcinoma. Cancer Prev Res 2014; 7(12):1258-69.

34. Helby J, Bojesen S, Nielsen S, Nordestgaard B. IgE and risk of cancer in 37 747 individuals from the general population. Ann Oncol 2015; 26(8):1784-90

35 Carton M, Guida F, Paget-Bailly S, Cyr D, Radoi L, Sanchez M, et al. Occupation and head and neck cancer in women Results of the ICARE study. Am J Ind Med 2014; 57(12):1386-97.

36. Reijula J, Kjaerheim K, Lynge E, Martinsen JI, Reijula K, Sparén P, et al. Cancer incidence among waiters: 45 years of follow-up in five Nordic countries. Scand J Public Health 2015; 43(2):204-11.

37. Koh D-H, Chung E-K, Jang J-K, Lee H-E, Ryu H-W, Yoo K-M, et al. Cancer incidence and mortality among temporary maintenance workers in a refinery/petrochemical complex in Korea. Arch Environ Occup Health 2014; 20(2):141-5.

38. Saulle R, Semyonov L, Mannocci A, Careri A, Saburri F, Ottolenghi L, et al. Human papillomavirus and cancerous diseases of the head and neck: a systematic review and meta-analysis. Oral Dis 2015; 21(4):417-31.

39. Pringle GA. The role of human papillomavirus in oral disease. Dent Clin North Am. 2014; 58(2):385-99.

40. Sand L, Jalouli J. Viruses and oral cancer. Is there a link? Microbes Infect. 2014; 16(5):371-8.

41. Polz-Gruszka D, Stec A, Dworzański J, Polz-dacewicz M. EBV, HSV, CMV and HPV in laryngeal and oropharyngeal carcinoma in Polish patients. Anticancer Res. 2015; 35(3):1657-61.

42.Beachler DC, Abraham AG, Silverberg MJ, Jing Y, Fakhry C, Gill MJ, et al. Incidence and risk factors of HPV-related and HPV-unrelated Head and Neck Squamous Cell Carcinoma in HIV-infected individuals. Oral Oncol 2014; 50(12):1169-76.

43. van Monsjou H. Epidemiological characteristics of oral and oropharyngeal squamous cell carcinoma: 9789462598683; 2015.

44. Fucic A, Gamulin M, Ferencic Z, Katic J, Von Krauss MK, Bartonova A, et al. Environmental exposure to xenoestrogens and oestrogen related cancers: reproductive system, breast, lung, kidney, pancreas, and brain. Environ Health. 2012; 11(1):S8.

45. Ann Marie Egloff,1 Mary E. Rothstein,2 Raja Seethala,3 Jill M. Siegfried,2 Jennifer Rubin. Grandis,1,2 and Laura P. Stabile2 Cross-talk Between Estrogen Receptor and Epidermal Growth Factor Receptor in Head and Neck Squamous Cell Carcinoma Clin Cancer Res. 2009; 15(21): 6529–6540.

46. Zárate S, Stevnsner T, Gredilla R. Role of estrogen and other sex hormones in brain aging. Neuroprotection and DNA repair. Front Aging Neurosci 2017; 9:430.

47. Singh HP, Kumar P, Goel R, Kumar A. Sex hormones in head and neck cancer: Current knowledge and perspectives. Clin Cancer Investig J. 2012; 1(1):2.

48. Yadi W, Binhua P. Inflammation A Driving Force Speeds Cancer Metastasis. CJLC 2009; 12(11):1194-201.

49. Li Y, Izumi K, Miyamoto H. The role of the androgen receptor in the development and progression of bladder cancer. Jpn J Clin Oncol. 2012; 42(7):569-77.

50. Zhang J, Liu J. Tumor stroma as targets for cancer therapy. Pharmacol Therapeut. 2013; 137(2):200-15.

51. Stamatiou K, Lardas M, Kostakos E, Koutsonasios V, Michail E. The impact of diabetes type 2 in the pathogenesis of benign prostatic hyperplasia: a review. Adv Urol. 2009:818965.

52. Barbieri RL, Gochberg J, Ryan KJ. Nicotine, cotinine, and anabasine inhibit aromatase in human trophoblast in vitro. J Clin Invest. 1986; 77(6):1727-33.

53. Asare, Elliot MD, Washington, May Kay MD PhD, Gress, Donna M. RHIT, CTR, Gershenwald, Jeffrey E. MD, Greene, Frederick L. MD. C Improving the quality of cancer staging. A: CA Cancer J Clin, 2015; 65(4): 261-263.

54. Patel SG1, Shah JP. TNM staging of cancers of the head and neck: striving for uniformity among diversity. CA Cancer J Clin. 2005; 55(4):242-58.

55. Dechen Wangmo Tshering Vogel, Peter Zbaeren B, Harriet C. Thoeny Cancer of the oral cavity and oropharynx. Cancer Imaging. 2010; 10(1): 62–72..

56. Rivera C. Essentials of oral cancer. Int J Clin Exp Pathol 2015; 8(9):11884.

57. Glynne-Jones R, Saleem W, Harrison M, Mawdsley S, Hall M. Background and current treatment of squamous cell carcinoma of the anus. Oncol Ther. . 2016; 4(2):135-72

58. Shah J, Patel K (2003) Head and Neck Surgery and Oncology 3 edn Mosby Ltd: Edinburgh

59. Milisavljevic D, Stankovic M, Zivic M, Popovic M, Radovanović Z. Factors affecting results of treatment of Hypopharyngeal Carcinoma. Hippokratia. 2009; 13(3):154.

60. Jain S, Dhingra S. Pathology of esophageal cancer and Barrett's esophagus. Ann Thorac Surg 2017; 6(2):99.

61. Yanofsky VR, Mercer SE, Phelps RG. Histopathological variants of cutaneous squamous cell carcinoma: a review. J Skin Cancer. 2011; 2011:210813

62. Baillie R, Tan ST, Itinteang T. Cancer stem cells in oral cavity squamous cell carcinoma: a review. Front Oncol. 2017; 7:112.

63. Hellevik T, Martinez-Zubiaurre I. Radiotherapy and the tumor stroma: the importance of dose and fractionation. Front Oncol. 2014;4:1

64. Arruebo M, Vilaboa N, Sáez-Gutierrez B, Lambea J, Tres A, Valladares M, González-Fernández Á. Assessment of the evolution of cancer treatment therapies. Cancer. 2011 2011; 3(3):3279-330.

65. Vigneswaran N, Williams MD. Epidemiologic trends in head and neck cancer and aids in diagnosis. Oral Maxillofac Surg Clin North Am. 2014; 26(2):123-41.

66. Zur E. Oral mucositis: etiology, and clinical and pharmaceutical management. Int J Pharm Compd 2012; 16(1):22-33.

67. Jones DL, Rankin KV. Management of the oral sequelae of cancer therapy. Tex Dent J. 2012; 129(5):461-8.

68. Müller S, Pan Y, Li R, Chi AC. Changing trends in oral squamous cell carcinoma with particular reference to young patients: 1971–2006. The Emory University experience. Head Neck Pathol 2008; 2(2):60.

69. Ruiz-Esquide G, Nervi B, Vargas A, Maiz A. Treatment and prevention of cancer treatment related oral mucositis. Rev. Méd. Chile 2011; 139(3):373-81.

70. Keefe DM, Schubert MM, Elting LS, Sonis ST, Epstein JB, Raber-Durlacher JE, et al. Updated clinical practice guidelines for the prevention and treatment of mucositis. Cancer: Interdisciplinary CA Cancer J Clin. 2007; 109(5):820-31.

71. Lalla RV, Sonis ST, Peterson DE. Management of oral mucositis in patients who have cancer. Dent Clin North Am 2008; 52(1):61-77.

72. Riera Romo M, Pérez-Martínez D, Castillo Ferrer C. Innate immunity in vertebrates: an overview. Immunology. 2016 Jun; 148(2):125-39.

73. Zhong Z, Sanchez-Lopez E, Karin M. Autophagy, inflammation, and immunity: a troika governing cancer and its treatment. Cell. 2016; 166(2):288-98.

74. Todoric J, Antonucci L, Karin M. Targeting inflammation in cancer prevention and therapy. Cancer prev research. 2016; 9(12):895-905.

75. Newton K, Dixit VM. Signaling in innate immunity and inflammation. Cold Spring Harb Perspect Bio. 2012:a006049.

76. Cassell GH. Infectious causes of chronic inflammatory diseases and cancer. Emerg Infect Dis 1998; (3):475.

77. Di Meo S, Reed TT, Venditti P, Victor VM. Role of ROS and RNS sources in physiological and pathological conditions. Oxid Med Cell Longev. 2016;2016. 1245049.

78. Kuper H, Adami HO, Trichopoulos D. Infections as a major preventable cause of human cancer. J Intern Med.. 2000; 248(3):171-83.

79. Templeton AJ, McNamara MG, Šeruga B, Vera-Badillo FE, Aneja P, Ocaña A, Leibowitz-Amit R, Sonpavde G, Knox JJ, Tran B, Tannock IF. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. JNCI: J Natl Cancer Inst. 2014;106(6).

80. Treffers LW, Hiemstra IH, Kuijpers TW, Van den Berg TK, Matlung HL. Neutrophils in cancer. Immunol Rev. 2016; 273(1):312-28.

81. Grenier A, Chollet-Martin S, Crestani B, Delarche C, El Benna J, Boutten A, Andrieu V, Durand G, Gougerot-Pocidalo MA, Aubier M, Dehoux M. Presence of a mobilizable intracellular pool of hepatocyte growth factor in human polymorphonuclear neutrophils. Blood. 2002; 99(8):2997-3004.

82. Lazos JP, Piemonte ED, Lanfranchi HE, Brunotto MN. Characterization of chronic mechanical irritation in oral cancer. Int J Dent. 2017; 201: 6784526

83. Guo T, Wang X, Qu Y, Yin Y, Jing T, Zhang Q. Megakaryopoiesis and platelet production: insight into hematopoietic stem cell proliferation and differentiation. Stem Cell Investig. 2015; 2:3.

84. Lin RJ, Afshar-Kharghan V, Schafer AI. Paraneoplastic thrombocytosis: the secrets of tumor self-promotion. Blood. 2014; 124(2):184-7.

85. Yildirim MA, Seckin KD, Togrul C, Baser E, Karsli MF, Gungor T, et al. Roles of neutrophil/lymphocyte and platelet/lymphocyte ratios in the early diagnosis of malignant ovarian masses. Asian Pac J Cancer Prev. 2014; 15(16):6881-5.

86. Bakacak M, Serin S, Ercan Ö, Köstü B, Bostancı MS, Bakacak Z, Kıran H, Kıran G. Utility of preoperative neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios to distinguish malignant from benign ovarian masses. J Turk Ger Gynecol Assoc. 2016 ; 17(1):21-5.

87. Wang Y-q, Jin C, Zheng H-m, Zhou K, Shi B-b, Zhang Q, et al. A novel prognostic inflammation score predicts outcomes in patients with ovarian cancer. Clin Chim Acta 2016; 456:163-9.

88. Younes G, Segev Y, Begal J, Auslender R, Goldberg Y, Amit A, Lavie O. The prognostic significance of hematological parameters in women with uterine serous papillary carcinoma (USPC).Eur J Obstet Gynecol Reprod Biol. 2016; 199:16-20.

89. Seckin KD, Karslı MF, Yucel B, Bestel M, Yıldırım D, Canaz E, et al. The utility of tumor markers and neutrophil lymphocyte ratio in patients with an intraoperative diagnosis of mucinous borderline ovarian tumor. Eur J Obstet Gynecol Reprod Biol.. 2016; 196:60-3.

90. Utsumi F, Kajiyama H, Niimi K, Sekiya R, Sakata J, Suzuki S, et al. Clinical significance and predicting indicators of post-cancer-treatment survival in terminally ill patients with ovarian cancer. J Obstet Gynaecol Res 2017; 43(2):365-70.

91. Eo W, Kim H-B, Lee YJ, Suh DS, Kim KH, Kim H. Preoperative Lymphocyte-Monocyte Ratio Is a Predictor of Suboptimal Cytoreduction in Stage III-IV Epithelial Ovarian Cancer. J Cancer. 2016; 7(13):1772.

92. Paik ES, Sohn I, Baek S-Y, Shim M, Choi HJ, Kim T-J, et al. Nomograms Predicting Platinum Sensitivity, Progression-Free Survival, and Overall Survival Using Pretreatment Complete Blood Cell Counts in Epithelial Ovarian Cancer. Cancer Res Treat 2017; 49(3):635.

93. Huang C-Y, Yang Y-C, Wang K-L, Chen T-C, Chen J-R, Weng C-S, et al. Possible surrogate marker for an effective dose-dense chemotherapy in treating ovarian cancer. Taiwan J Obstet Gynecol. 2016; 55(3):405-9.

94. Miao Y, Yan Q, Li S, Li B, Feng Y. Neutrophil to lymphocyte ratio and platelet to lymphocyte ratio are predictive of chemotherapeutic response and prognosis in epithelial ovarian cancer patients treated with platinum-based chemotherapy. Cancer Biomark. 2016; 17(1):33-40.

95. Chen Y, Zhang L, Liu W-X, Liu X-Y. Prognostic significance of preoperative anemia, leukocytosis and thrombocytosis in chinese women with epithelial ovarian cancer. Asian Pac J Cancer Prev. 2015; 16(3):933-9.

96. Ma X, Wang Y, Sheng H, Tian W, Qi Z, Teng F, et al. Prognostic significance of thrombocytosis, platelet parameters and aggregation rates in epithelial ovarian cancer. J Obstet Gynaecol Res2014; 40(1):178-83.

97. So KA, Hong JH, Jin HM, Kim JW, Song JY, Lee JK, et al. The prognostic significance of preoperative leukocytosis in epithelial ovarian carcinoma: a retrospective cohort study. Gynecol Oncol. 2014; 132(3):551-5

98. Njølstad TS, Engerud H, Werner HM, Salvesen HB, Trovik J. Preoperative anemia, and thrombocytosis identify aggressive endometrial carcinomas. Gynecol Oncol. 2013; 131(2):410-5.

99. Kilincalp S, Çoban Ş, Akinci H, Hamamcı M, Karaahmet F, Coşkun Y, et al. lymphocyte ratio, platelet/lymphocyte ratio, and mean platelet volume as potential biomarkers for early detection and monitoring of colorectal adenocarcinoma. Eur J Cancer Prev. 2015; 24(4):328-33.

100. Emir S, Aydin M, Can G, Bali I, Yildirim O, Öznur M, et al. Comparison of colorectal neoplastic polyps and adenocarcinoma with regard to NLR and PLR. Eur Rev Med Pharmacol Sci. 2015;19(19):3613-18.

101. Jia J, Zheng X, Chen Y, Wang L, Lin L, Ye X, et al. Stage-dependent changes of preoperative neutrophil to lymphocyte ratio and platelet to lymphocyte ratio in colorectal cancer. Tumour Biol . 2015; 36(12):9319-25.

102. He W, Yin C, Guo G, Jiang C, Wang F, Qiu H, et al. Initial neutrophil lymphocyte ratio is superior to platelet lymphocyte ratio as an adverse prognostic and predictive factor in metastatic colorectal cancer. J Med Oncol. 2013; 30(1):439.

103. Kim HJ, Choi G-S, Park JS, Park S, Kawai K, Watanabe T. Clinical significance of thrombocytosis before preoperative chemoradiotherapy in rectal cancer: predicting

pathologic tumor response and oncologic outcome. Ann Surg Oncol . 2015; 22(2):513-9.

104. Kitayama J, Yasuda K, Kawai K, Sunami E, Nagawa H. Circulating lymphocyte is an important determinant of the effectiveness of preoperative radiotherapy in advanced rectal cancer. BMC cancer. 2011; 11(1):64.

105. Ying HQ1, Deng QW, He BS, Pan YQ, Wang F, Sun HL, Chen J, Liu X, Wang SK. The prognostic value of preoperative NLR, d-NLR, PLR and LMR for predicting clinical outcome in surgical colorectal cancer patients. Med Oncol. 2014 Dec; 31(12):305.

106. Carruthers R, Tho L, Brown J, Kakumanu S, McCartney E, McDonald A. Systemic inflammatory response is a predictor of outcome in patients undergoing preoperative chemoradiation for locally advanced rectal cancer. Colorectal Dis. 2012; 14(10):e701-e7.

107. Wei EK, Colditz GA, Giovannucci EL, Fuchs CS, Rosner BA. Cumulative risk of colon cancer up to age 70 years by risk factor status using data from the Nurses' Health Study. Am J Epidemiol. 2009; 170(7):863-72.

108. Seong M-K. Prognostic inflammation score in surgical patients with colorectal cancer. J Korean Med Sci . 2015; 30(12):1793-9.

109. Ikeguchi M, Yamamoto M, Arai Y, Maeta Y, Ashida K, Katano K, et al. Fucoidan reduces the toxicities of chemotherapy for patients with unresectable advanced or recurrent colorectal cancer. Oncol Lett. 2011; 2(2):319-22.

110. Shibutani M, Maeda K, Nagahara H, Ohtani H, Iseki Y, Ikeya T, et al. The prognostic significance of a postoperative systemic inflammatory response in patients with colorectal cancer. World J Surg Oncol. 2015; 13(1):194.

111. Ozawa T, Ishihara S, Kawai K, Kazama S, Yamaguchi H, Sunami E, et al. Impact of a lymphocyte to monocyte ratio in stage IV colorectal cancer. J Surg Res. 2015; 199(2):386-92.

112. Ma X, Wang Y, Sheng H, Tian W, Qi Z, Teng F, et al. Prognostic significance of thrombocytosis, platelet parameters and aggregation rates in epithelial ovarian cancer. J Obstet Gynaecol Res . 2014; 40(1):178-83.

113. Onesti CE, Josse C, Poncin A, Frères P, Poulet C, Bours V, et al. Predictive and prognostic role of peripheral blood eosinophil count in triple-negative and hormone receptor-negative/HER2-positive breast cancer patients undergoing neoadjuvant treatment. Oncotarget. 2018; 9(72):33719.

114. Geng S-K, Fu S-M, Fu Y-P, Zhang H-W. Neutrophil to lymphocyte ratio is a prognostic factor for disease free survival in patients with breast cancer underwent curative resection. Medicine. 2018; 97(35):e11898.

115. Ferroni P, Roselli M, Buonomo OC, Spila A, Portarena I, Laudisi A, et al. Prognostic Significance of Neutrophil–to–lymphocyte Ratio in the Framework of the 8th TNM Edition for Breast Cancer. Anticancer Res. 2018; 38(8):4705-12.

116. Fang Q, Tong Y-W, Wang G, Zhang N, Chen W-G, Li Y-F, et al. Neutrophil-to-lymphocyte ratio, obesity, and breast cancer risk in Chinese population. Medicine. 2018; 97(30).

117. Liu X, Qu JK, Zhang J, Yan Y, Zhao XX, Wang JZ, et al. Prognostic role of pretreatment neutrophil to lymphocyte ratio in breast cancer patients: A meta-analysis. Medicine (Baltimore). 2018; 97(30):e1169296(45):e8101.

118. Iimori N, Kashiwagi S, Asano Y, Goto W, Takada K, Takahashi K, et al. Clinical Significance of the Neutrophil–to–Lymphocyte Ratio in Endocrine Therapy for Stage IV Breast Cancer. In Vivo. 2018; 32(3):669-75.

119. Zhang M, Huang X-z, Song Y-x, Gao P, Sun J-x, Wang Z-n. High platelet-to-lymphocyte ratio predicts poor prognosis and clinicopathological characteristics in patients with breast cancer: a meta-analysis. Biomed Res Int.. 2017; 2017.

120. Sun H, Yin C-q, Liu Q, Wang F, Yuan C-h. Clinical significance of routine blood test-associated inflammatory index in breast cancer patients. Med Sci Monit. 2017; 23:5090.

121. Wariss BR, de Souza Abrahão K, de Aguiar SS, Bergmann A, Thuler LCS. Effectiveness of four inflammatory markers in predicting prognosis in 2374 women with breast cancer. Maturitas. 2017; 101:51-6.

122. Takeuchi H, Kawanaka H, Fukuyama S, Kubo N, Hiroshige S, Yano T. Comparison of the prognostic values of preoperative inflammation-based parameters in patients with breast cancer. PloS one. 2017; 12(5):e0177137.

123. Jia W, Wu J, Jia H, Yang Y, Zhang X, Chen K, et al. The peripheral blood neutrophil-to-lymphocyte ratio is superior to the lymphocyte-to-monocyte ratio for predicting the long-term survival of triple-negative breast cancer patients. PloS One. 2015; 10(11):e0143061.

124. He J, Lv P, Yang X, Chen Y, Liu C, Qiu X. Pretreatment lymphocyte to monocyte ratio as a predictor of prognosis in patients with early-stage triple-negative breast cancer. Tumour Biol. 2016; 37(7):9037-43.

125. Bozkurt O, Karaca H, Berk V, Inanc M, Duran AO, Ozaslan E, et al. Predicting the role of the pretreatment neutrophil to lymphocyte ratio in the survival of early triple-negative breast cancer patients. inflammation. J BUON. 2015; 12:14.

126. Reichert TE, Rabinowich H, Johnson JT, Whiteside TL. Human immune cells in the tumor microenvironment: mechanisms responsible for signaling and functional defects. J Immunother 1998;21:295–306.

127. Bobdey S, Ganesh B, Mishra P, Jain A. Role of Monocyte Count and Neutrophil-to Lymphocyte Ratio in Survival of Oral Cancer Patients. Int Arch Otorhinolaryngol 2017; 21: 21-27.

128. Cho H, Hur HW, Kim SW, Kim SH, Kim JH, Kim YT, et al. Pre-treatment neutrophil to lymphocyte ratio is elevated in epithelial ovarian cancer and predicts survival after treatment. Cancer Immunol Immunother 2009; 58:15–23.

129. Yamanaka T, Matsumoto S, Teramukai S, Ishiwata R, Nagai Y, Fukushima M. The baseline ratio of neutrophils to lymphocytes is associated with patient prognosis in advanced gastric cancer. Oncology 2007; 73: 215–220.

130. Wculek SK, Malanchi I. Neutrophils support lung colonization of metastasis-initiating breast cancer cells. Nature 2015; 528: 413-7.

131. Yanming Jiang,1 Song Qu,1 Xinbin Pan,1 Shiting Huang,1 and Xiaodong Zhu1 Prognostic value of neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio in intensity modulated radiation therapy for nasopharyngeal carcinoma. Oncotarget. 2018; 9(11): 9992–10004.

132. Tsai YD, Wang CP, Chen CY, Lin LW, Hwang TZ, Lu LF, Hsu HF, Chung FM, Lee YJ, Houng JY.Pretreatment circulating monocyte count associated with poor prognosis in patients with oral cavity cancer. Head Neck 2014; 36(7):947-53.

133. Rachidi S, Wallace K, Wrangle JM, Day TA, Alberg AJ, Li Z. Neutrophil-to-lymphocyte ratio and overall survival in all sites of head and neck squamous cell carcinoma. Head Neck. 2016; 38 Suppl 1:E1068-74

134. Perisanidis C, Kornek G, Pöschl PW, Holzinger D, Pirklbauer K, Schopper C, Ewers R. High neutrophil-to-lymphocyte ratio is an independent marker of poor disease-specific survival in patients with oral cancer. Med Oncol 2013; 30: 334

135. Fang JF, Huang Y, Chen QX. Preoperative platelet-lymphocyte ratio (PLR) is superior to neutrophil-lymphocyte ratio (NLR) as a predictive factor in patients with esophageal squamous cell carcinoma. World J Surg Oncol 2014; 12: 58.

136. Song Y, Liu H, Gao L, Liu X, Ma L, Lu M, Gao Z. Preoperative neutrophil-to-lymphocyte ratio as prognostic predictor for hypopharyngeal squamous cell carcinoma after radical resections. J Craniofac Surg 2015; 26: e137-40.

137. Rassouli A, Saliba J, Castano R, Hier M, Zeitouni AG. Systemic inflammatory markers as an independent prognosticators of head and neck squamous cell carcinoma. Head Neck 2015; 37: 103-10.

138. Jin Y, Ye X, He C, Zhang B, Zhang Y. Pretreatment neutrophil-to-lymphocyte ratio as predictor of survival for patients with metastatic nasophayngeal carcinoma. Head Neck 2015; 37: 69-75.

139. Huang SH, Waldron JN, Milosevic M, Shen X, Ringash J, Su J, et al. Prognostic value of pretreatment circulating neutrophils, monocytes, and lymphocytes in oropharyngeal cancer stratified by human papillomavirus status. Cancer. 2015; 121(4):545-55.

140. Forget P, Khalifa C, Defour JP, Latinne D, Pel MC, Kock M. What is the normal value of the neutrophil-to-lymphocyte ratio?. BMC Res Notes. 2017; 10(1):12.

141. Szabo G, Mandrekar P. A Recent Perspective on Alcohol, Immunity and Host Defense Alcohol Clin Exp Res 2009; 33: 220–232.

142. Sawabe M, Ito H, Oze I, Hosono S, Kawakita D, Tanaka H, Hasegawa Y, Murakami S, Matsuo K. Heterogeneous impact of drinking according to treatment method on survival in head and neck cancer: a prospective study, Cancer Sci 2017; 108(1):91-100

8. CURRICULUM VITAE

Granita Muhaxheri was born on April 20th 1987 in Peja, Kosova. She was graduated at the Faculty of Medicine, Department of Dentistry, University of Prishtina "Hasan Prishtina" of Kosova in 2012, obtaining degree of Doctor of Dental Medicine. In 2018 she has finished her specialization in Periodontology and Oral Medicine, at the same University, at Faculty of Medicine, Department of Dentistry obtaining the title Specialist of Periodontology and Oral Medicine. Since 2012 she has been working in private practices in Dental Clinics, while last year she worked as a Teaching Assistant in clinical course of Oral Medicine in Faculty of Medicine Department of Dentistry, University of Prishtina "Hasan Prishtina". In October 2013 she has started PhD studies at the School of Dental Medicine University of Zagreb, Croatia as continuation of her commitment to reach highest levels of education. She has actively participated in numerous international scientific conferences local and abroad and is author and co-author of several scientific papers, in relevant international journals.

List of published scientific articles:

1. **Muhaxheri G**,Vucicevic Boras V, Fucic A, Plavec D, Sekerija M, Filipovic M, Grsic K, Stubljar B, Krnić T,Vrdoljak B.Multivariate analysis of preoprative and postoperative neutrophil to lymphocite ratio as an indicator of head and neck squmous cell carcinoma.Int J Oral Maxillofac Surg. 2018; 47(8):965-970.

2. **Muhaxheri G**, Vucicevic Boras V, Gabric D, Terlevic Dabic D, Jurisic Kvesic A. No efficacy of photodynamic therapy with toluidine blue in oral lichen planus.. RJPBCS 2017; 8(1):132-135.

3. **Muhaxheri G**, Gabric D,Vucicevic Boras V, Terlevic D, Vrdoljak B, Filipovic M. The significance of routine blood tests in patients with head and neck cancers. Libri Oncol. 2017; 45(1): 19-22.

4. **Muhaxheri G**, Vucicevic Boras V, Gabric D, Vrdoljak DV, Ćatić A, Verzak Z.The significance of serum blood analysis in patients with oral lichen planus Andamios: Revista de Investigacion Social. 2016; 13(1): 67-74.

5. **Muhaxheri G**, Gabric D, Vucicevic Boras V. Epidemiology and aetioology of head and neck squmous cell carcinoma. Libri Oncol 2015; 43 (1): 75 – 81.

6. **Muhaxheri G**,Vucicevic Boras V, Fucic A, Plavec D, Sekerija M, Filipovic M, Grsic K, Stubljar B, Krnić T,Vrdoljak B. Multivariate analysis of preoprative and postoperative neutrophil to lymphocite ratio as an indicator of head and neck squmous cell carcinoma.Acta Stomatol Croat. 2017; 51(3):249-264. (Poster)