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HEAD AND NECK CANCER AND ORAL LEUKOPLAKIA: CLINICAL STUDY OF SOME UNRESOLVED ISSUES

PhD Thesis

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List of Abbreviations

b/c	breaks/cell
BCG	bacillus Calmette-Guérin
CIN	chromosomal instability
CT	computer tomography
CUP	cancer of unknown primary
DNA	deoxyribonucleic acid
HE	haematoxylin and eosin
HNSCC	head and neck squamous cell carcinoma
HPV	human papillomavirus
IGF	insulin-like growth factor
MRI	magnetic resonance imaging
OPMD	oral potentially malignant disorders
PET	positron emission tomography
RT	radiotherapy
SPC	second primary cancer
TNM	tumor, node, metastasis
UICC	Union for International Cancer Control
WHO	World Health Organization

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1. Introduction

Head and neck cancer is the seventh most commonly diagnosed cancer types worldwide when considered as a group of malignancies affecting the upper aerodigestive tract. According to GLOBOCAN estimates, head and neck cancer accounts for approximately 800 000 new cases annually. While individual subregions such as the oral cavity, pharynx, or larynx rank lower when considered separately, their combined burden places head and neck cancer among the most prevalent malignancies globally [1–3]. By the time they are detected, these cancers are usually very advanced. Head and neck cancers represent a major public health problem in Hungary. With an annual incidence of 17.2 per 100 000, Hungary has the highest rate of head and neck cancer in Europe. The country also shows one of the highest mortality rates on the continent, underlining the importance of prevention and early diagnosis [2,4]. Environmental, chemical and physical factors, such as smoking and alcoholism, are prominent among the causative factors, and recent studies also suggest a potential association between periodontal disease and the development of oral cancer [5,6]. As the incidence of head and neck cancers is high, therapeutic tools and options are under continuous development. Whereas in the past radical surgery was the solution in almost all cases, nowadays, thanks to radio-chemotherapy, biological agents and the newer generation of pharmacological agents, organ-sparing treatment, brachytherapy and minimally invasive surgery are becoming increasingly popular [7,8]. This is perhaps the most important issue in laryngeal tumors, as the patient's quality of life is greatly impaired by radical laryngectomy. However, the patient's daily life is also affected by tissue defects resulting from tumors in the oral cavity and pharynx, as the upper aerodigestive tract is an area of key functional and aesthetic importance. Prevention is the best way to ensure a good quality of life.

A detailed knowledge of the development, macroscopic and microscopic morphology and all other features of head and neck cancers is essential for prevention and successful treatment. The majority of these tumors (more than 90%) are squamous cell carcinomas in terms of their histological type. The group of head and neck squamous cell carcinomas (HNSCCs) includes several types of tumors according to their anatomical location, the most common being those arising in the oral cavity, oropharynx, hypopharynx and larynx [9]. The anatomical location is also of major importance for

overall survival. Tumors arising in the larynx have a better prognosis than tumors arising in other head and neck regions. Literature suggests that the 5-year survival of laryngeal cancers is about 70%, and this feature has not changed for decades [10]. Overall survival is worse in developing countries than in developed countries. The main reasons for this are late detection and inadequate care, especially lack of complex radio-chemotherapy [11]. The disease is multifactorial. Environmental hazards, lifestyle and genetic predisposition also play a role in the development of cancer. Smoking and excessive alcohol consumption are the two most important etiological factors in the development of head and neck cancer. In Hungary, these addictions are present in the medical history of almost all patients [12]. It is a well-known fact that smoking is a major factor in the development of many malignant lesions. Tobacco smoke contains thousands of harmful compounds, some of which are carcinogenic. Some of the more important carcinogenic compounds are polycyclic aromatic hydrocarbons, benzopyrenes, nitrosamines, aromatic amines, benzene, various metal compounds and even long-lived radioactive elements. As the greatest exposure is to the aerodigestive tract, it is not surprising that this area is at the greatest risk. However, these substances are absorbed into the bloodstream through the alveoli of the lungs and accumulate in the urine in large quantities during excretion, so they can reach virtually any part of the body and have a damaging effect. The role of alcohol is primarily to act as a solvent, enhancing the harmful effects of other carcinogenic substances. Since alcoholics are also usually smokers, the harmful effects are mutually reinforcing. According to some research, when these two addictions are present together, the risk of developing cancer can increase by up to 35 times [13]. In addition, the main metabolite of alcohol, acetaldehyde, is itself highly toxic, forming deoxyribonucleic acid (DNA) adducts, thereby inhibiting DNA synthesis and DNA repair mechanisms. The effect of alcohol on the metabolic processes in our body is also well known, so that not only drugs but also certain toxic substances and carcinogenic compounds can be elevated, especially if there is continuous exposure to alcohol. In addition to the two major addictions, age, diet, physical activity and socioeconomic status are also prognostic factors for head and neck cancer [1,14].

Whereas in the past, HNSCCs were more common in older age groups, with smoking and/or alcohol being the main etiological factors, in recent decades there has been a significant increase in the incidence of human papillomavirus (HPV) associated

oropharyngeal cancers. According to some analyses, the incidence of oral HPV-associated cancers may even overtake the incidence of cervical cancers within a few years. These tumors are mainly found in young patients with better general health and are not associated with alcoholism or smoking. In contrast, HPV-negative tumors tend to affect older people and have a significantly worse prognosis than virus-associated malignancies. Their recent isolation has been particularly justified by the fact that they have a better therapeutic prognosis than other oral cancers. Patients with HPV-associated tumors have a 5-year survival rate of over 80%, compared to around 40% in HPV-negative cases [15]. The reason for the better outcome is mainly due to the different radiobiological characteristics of HPV-positive tumors and the absence of p53 mutation. In these tumors, cancer cells are characterized by a lower degree of differentiation and a lower repopulation intensity of surviving cells after radiation, which also implies that HPV positivity is a favorable prognostic factor in squamous cell carcinoma of the oral cavity. More recently, results have also been encouraging with the use of immunotherapy, which is also an important therapeutic tool for HPV-positive tumors. It is important to note, that the favorable prognosis of HPV-associated tumors is only in the case of oral squamous cell carcinomas. Similar tumors in the larynx and duodenum have a worse prognosis, although they are much less frequent [16,17].

It is well known that only a fraction of patients who smoke and/or drink develop cancer, so intrinsic susceptibility to genotoxic agents may also play a role in carcinogenesis [18]. For determining cancer risk, the mutagen sensitivity assay is a promising technique. It functions as a phenotypic indicator of the combined impacts of an individual's DNA damage response, repair ability, and sensitivity to carcinogen exposure [19]. To ascertain the general population's mutagen sensitivity, Hsu TC conducted a study back in the 1980s. He counted the induced chromatid breaks in cultured lymphocytes treated with bleomycin in late S-G2 phase [20]. In general, a hypersensitive phenotype is defined as an average number of chromatid breaks greater than one per cell. Excessive sensitivity increases the risk of developing squamous cell head and neck and lung cancer [21], but also breast cancer [19].

In patients with squamous cell carcinoma of the oral cavity, pharynx and larynx, not only local recurrence but also a second primary cancer (SPC) often develops. The development of SPCs is the leading cause of morbidity and mortality in these patients

[22]. Most recurrences occur within the first two years of follow-up, although second initial tumors frequently appear five to ten years later. Common SPCs are found in the digestive and respiratory systems, mostly in the esophagus and lung. The development of SPCs in patients with malignancies of the oral cavity, pharynx, and larynx may be influenced by factors such as smoking status, alcohol use, initial tumor site, and disease stage, according to previous studies [23–25]. However, these factors do not fully account for all cases of SPCs, indicating that genetic susceptibility may also play a role in their development [26,27]. Mutagen sensitivity has been emphasized in a number of research articles as a possible biomarker for susceptibility to certain primary cancers [28–30]. In Hungary, we were the first to investigate the relationship between mutagen sensitivity and the development of SPCs, and we also obtained long-term results, due to the long follow-up time.

In the head and neck area, cancer of unknown primary (CUP) can also occur. CUP is a metastatic disease defined by the absence of a clinically identified primary malignancy at the time of diagnosis, despite appropriate diagnostic work-up. CUP is a relatively frequent cancer type causing incomparable difficulties in pathological diagnosis as compared to other tumor types [31,32]. In some cases, the autopsy may not even identify the primary malignancy because of its microscopic size or previous regression. Depending on the definition, demographic, and time of diagnosis, CUP may represent between 2-5% of all malignancies, even several developing countries have CUP rates that are higher than 10%. [32,33]. Not knowing the primary site of origin of the cancer is a challenge for accurate diagnosis, therapy and prognosis. Most of the neck node metastases from CUP are squamous cell carcinomas. The rate of head and neck cancers with unknown primary can be reduced after appropriate investigation [pan-endoscopy, computer tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET)/CT examination] [34]. The majority of neck lymph node squamous cell carcinoma metastases in CUP originate from the head and neck site. The oropharynx contains a significant percentage of primary tumors seen in patients with squamous cell CUP that are associated to the HPV, with HPV16 being the most common high-risk subtype. As mentioned earlier besides tobacco and alcohol consumption, HPV is an accepted risk and prognostic factor for oropharyngeal squamous cell carcinoma. Because of the epidemic rise of HPV-mediated oropharyngeal squamous cell carcinoma, the

incidence of oropharyngeal squamous cell carcinoma is rising. Non-keratinizing squamous cell carcinoma is the most common pathology in HPV-related squamous cell CUP [35]. When CUP metastasizes to the neck nodes, a non-keratinizing morphology suggests tonsillar or base-of-the-tongue location [36]. The following morphologic traits may be associated with HPV-related metastatic carcinoma: large size, cystic nature, and limited extracapsular expansion [35]. Better outcomes are predicted by cystic neck node metastases [37]. The relevance of mutagen sensitivity in the development of squamous cell neck node metastases from CUP, with or without multiple distant site primary malignancy - remains an open question.

In some cases, a precancerous lesion of the oral cavity precedes oral cancers. According to more recent classifications, we prefer to use the term oral potentially malignant disorders (OPMD) for these, however both terms are used in the literature [38]. Leukoplakia (white spot) is a common OPMD of the oral mucosa. The prevalence in some countries ranges from 0.1-11.7% of the total population, whereas in Hungary it is between 1.3-5.6% [39,40]. The term was first used by the Hungarian dermatologist *Ernő Schwimmer* in 1878 to describe white-colored lesions of the oral mucosa [41]. It is worth highlighting that another Hungarian expert, Professor *Jolán Bánóczy*, has conducted numerous studies on oral leukoplakia [42–45]. The definition has undergone several modifications and the current definition of oral leukoplakia is a predominantly white plaque that cannot be characterized clinically or pathologically in any other disease group [38]. Oral leukoplakia has been classified in several ways over the decades. The most widely used classification divides leukoplakia into homogeneous and non-homogeneous types based on their clinical appearance. The non-homogeneous leukoplakia is further subdivided, according to most classifications, into three main clinical types: speckled, nodular, and verrucous. It is important to note that the term erythroleukoplakia is often used interchangeably with speckled leukoplakia in more recent literature, particularly to emphasize the presence of red areas within the lesion, which is associated with a higher risk of malignant transformation. Erythroplakia is often associated with not only dysplasia but also in situ or invasive carcinoma. Additionally, proliferative verrucous leukoplakia represents a distinct clinical entity characterized by multiple, simultaneous leukoplakic lesions that are often multifocal and extensive, with a particularly high risk of progression to oral squamous cell carcinoma, and is therefore often discussed separately from other

leukoplakias [38,46,47]. It is important to clarify that leukoplakia is a purely clinical concept and that differential diagnosis has a key role. Histopathological diagnosis can be obtained after histopathological sampling. Microscopic examination of leukoplakia usually reveals the presence of hyperkeratosis. It commonly develops as a result of chronic irritation, often caused by smoking, although excessive alcohol consumption may also contribute, and is particularly common in India due to betel nut chewing, but idiopathic cases are also seen. Leukoplakia does not often transform into oral cancer, but significantly increases the risk of developing cancer, which is the most serious consequence. The range of malignant transformation from oral leukoplakia to squamous cell carcinoma amounts from 0.13% to 34%. For laryngeal leukoplakia, the range is from 0% to 64.7% [48–50]. This considerable variation in transformation rates can partly be attributed to differences in diagnostic criteria for leukoplakia, geographical factors, variations in study populations, potential etiological influences, and the duration of follow-up [50]. Traditionally, the prediction of malignant potential is based on the histological grading of dysplasia. An increase in the dysplasia grade elevates the risk of malignant transformation to squamous cell carcinoma [49–51]. Dysplasia cases are grouped into mild, moderate and severe dysplasia (grade I, II, III). Moderate to severe dysplasia carries a significantly higher risk of cancer progression compared to mild dysplasia [52,53]. A clinical evaluation alone does not reliably predict the malignancy risk. After biopsy, there is no dysplastic lesion in more than half of the cases. Molecular testing and biomarkers provide a more precise diagnosis and risk assessment on malignant transformation. The therapy of oral leukoplakia varies, depending on the clinical picture, the severity of the lesion and the extent of dysplasia, among other factors. Therapy may include observation (“watchful waiting”), conservative topical treatment, laser treatment or complete surgical removal [54].

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2. Objectives

Study 1

To determine the predictive value of mutagen sensitivity for the development of SPC in HNSCC patients, to estimate the rate of SPC and the outcome with SPC.

Study 2

To study the clinical and histological characteristics in patients with head and neck node metastasis with CUP.

Study 3

To examine the malignant transformation rate of oral or laryngeal leukoplakia: a comparative study.

To study the malignant transformation of oral leukoplakia, and the risk factors of malignant transformation.

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3. Methods

At the National Institute of Oncology (Budapest, Hungary), 432 patients with HNSCC underwent mutagen sensitivity testing between 1996 and 2006 before receiving treatment. The aim of that test was to clarify the usefulness of the bleomycin sensitivity assay as a biomarker of HNSCC. In addition, to explain the association between HNSCC susceptibility and exposure to carcinogens. The results were published elsewhere [55,56]. Of the total number of patients, four younger (≤50 years) patients were found with squamous cell carcinoma of neck lymph nodes from CUP, and 124 patients had primary HNSCC with the following criteria: smoking and chronic alcoholic, ≤50 years of age at the time of bleomycin test, head and neck cancer not caused by HPV, treatment and follow-up at our institute, squamous cell cancer in oral cavity, pharynx (except nasopharynx) or larynx. We examined the pre-treatment mutagen sensitivity of patients with and without SPC by reviewing patient data. SPC was partly defined according to the criteria of second primary tumor prevention trial of the M.D. Anderson Cancer Center [27]: the SPC must be diagnosed as malignant by the following criteria: it has to be at least 2 cm away from the site of the index tumor, and has to occur ≥4 months after the diagnoses of the index tumor. Following tissue sampling, the histopathological diagnosis was established on the haematoxylin and eosin (HE) stained slides at the Department of Surgical and Molecular Pathology at the National Institute of Oncology. The assessment of HPV positivity was also performed at this department, using p16 immunohistochemistry and HPV DNA detection. Mutagen sensitivity was tested *in vitro* in lymphocytes by counting chromatid breaks induced by bleomycin. The method has been described previously [55,56]. Briefly, lymphocytes were cultured from peripheral blood for 72 hours. At 5 hours before the end of this period, cell cultures were treated with bleomycin at a final concentration of 30 μg/ml, followed by colcemid blocking and the usual cell disruption and staining. Cells were harvested, and chromatid breaks (Figure 1.) were scored in 100 metaphases per sample, and recorded as the mean number

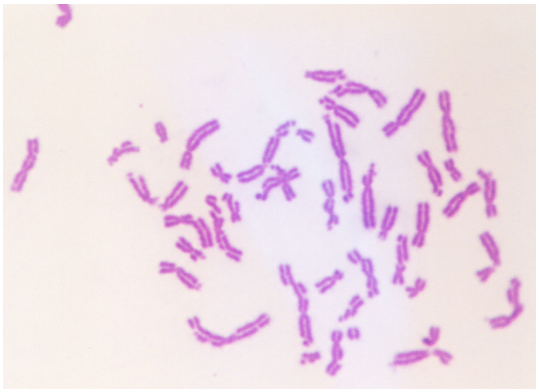

Figure 1. *Chromatid breaks induced by bleomycin*

of breaks per cell (b/c). Patients were divided into hypersensitive and non-hypersensitive groups. The patient was classified hypersensitive if the mean number of b/c was >1. The following survival endpoints were used: any death for overall survival, death from head and neck cancer for cancer-specific survival, death from SPC for survival with SPC, the appearance of SPC for SPC-free survival (time to SPC). Intervals to endpoints were examined with Kaplan–Meier method [57]. The log-rank test was used to compare the curves. The effect of the possible prognostic factors on the probability of the incidence of a SPC were examined in the Cox regression model [18]. Statistical differences in proportions and means were assessed both by the 2-sample t-test and by Fisher exact test. GraphPad Prism (version 5.01 for Windows, Graph-Pad Software, San Diego, CA, USA) and Statistica (version 13.5.0.17, TIBCO Software Inc., Palo Alto, CA, USA) program packages were used for data analysis. A p value ≤ 0.05 were regarded as statistically significant.

We reviewed the medical records of 253 patients treated for laryngeal or oral leukoplakia at the National Institute of Oncology (Budapest, Hungary) over a 26-year period (January 1996 to January 2022). In the computerized institutional database, the number of patients with oral or laryngeal leukoplakia was 221 and 32, respectively. The histopathology of leukoplakia from the biopsy material was classified as no dysplasia, dysplasia and carcinoma. Three levels of dysplasia were identified: mild, moderate, and severe (grade I, II, III). The Department of Surgical and Molecular Pathology at the National Institute of Oncology classifies oral and laryngeal dysplasia according to the World Health Organization (WHO) guidelines [58,59]. The initial procedure for treatment included observation alone, CO2 laser vaporization biopsy or surgical excision. If symptoms (such voice quality, swallowing, or pain) or the lesion's appearance (endoscopically for laryngeal lesions and to the naked eye for oropharyngeal lesions) worsened, a re-biopsy was conducted. The histological findings were classified as follows: no dysplasia, dysplasia (grade I, II, III) or cancer. The treatment consisted of surgery with the removal of the lesion, CO2 laser treatment or observation. Initially, and before any treatment, at least one biopsy was routinely performed prior to laser vaporization. In addition, surgical excisions were performed for dysplastic leukoplakia with a histological examination of the entire lesion. Regular follow-up (two visits/year) was suggested for patients [54]. To assess the associations between the different study

variables and the risk of developing cancer, a survival analysis was performed using the Kaplan–Meier method [57]. The number of patients progressing towards malignancy was evaluated in each of the groups. The follow-up period was defined as the interval from the time of a leukoplakia clinical diagnosis to death or the last follow-up. The following survival endpoints were used: death from leukoplakia-associated cancer for cancer-specific survival, and the time to an appearance of cancer for malignant transformation-free survival. The survival curves were compared with a log-rank test. The effect of the possible prognostic factors on the probability of the incidence of leukoplakia-related cancer was examined in a Cox regression model [60]. Statistical differences in proportions and means were assessed with a Fisher-exact test and chi-squared test. All tests were two-sided, and p values of ≤ 0.05 were accepted for statistical significance. GraphPad Prism (GraphPad Prism version 5.01 for Windows, GraphPad Software, San Diego, CA, USA) and IBM SPSS Statistics for Windows (version 25.0, Armonk, NY, USA: IBM Corp) program packages were used for a data analysis.

Between April 2021 and April 2024, 75 patients diagnosed with oral leukoplakia had histological samples taken at the Department of Dentoalveolar Surgery, Department of Oral Diagnostics, Semmelweis University. Histopathological examination was performed at the Department of Pathology and Experimental Cancer Research, Semmelweis University. Histopathological samples were analyzed on the basis of HE staining, supplemented in some cases by immunohistochemistry (p53, p16, ki67). Dysplasias were classified into grades of mild, moderate and severe dysplasia (grade I, II, III) according to national and international protocols [61]. For statistical analysis, Fisher-exact test was used, and results with $p \leq 0.05$ were considered significant.

4. Results

In our research, out of the 432 HNSCC patients (who underwent mutagen sensitivity testing) 124 patients met the criteria mentioned above. Cytogenetic and clinical characteristics of the patients are given in Table 1 [62]. Oral cavity (34%) represented the largest group of the four anatomical sites. Most patients had a stage IV disease (43%). Few patients (n=12) were treated with chemotherapy alone. Most patients have undergone surgery and adjuvant radiotherapy (RT). Surgery was performed on 86 patients. The number of R0 or R1 resection was 61 and 25, respectively. Of the 86 cases, 84 underwent lymphadenectomy: 64 were node positive and 20 were node negative. Extracapsular tumor extension was seen in 14 node-positive patients. Response rate (complete or partial response) for radio-chemotherapy alone, definitive RT or chemotherapy alone, was 9/10, 6/7 and 7/12, respectively. Mean follow-up time for all patients and alive patients was 68 months (range: 5–288 months) and 222 months (range: 184–249 months), respectively. Nine patients are still alive, and 115 have died. The crude overall survival rate is 7%. Ten patients died of internal disease. The crude rate of cancer-specific survival is 15%. The estimated rate of 15-year overall or cancer-specific survival is 14.5 and 19%, respectively. Out of 124 patients, 20 (16.1%) developed SPC. The characteristics of the 20 patients with SPC are given in Table 2 [62]. The majority (n=13, 65%) of SPC were HNSCC, the rest of them (n=7,

Table 1. *Clinical and cytogenetic characteristics of 124 patients with HNSCC*

Characteristics	***Patients, n (%)***
Mean age (years)	
45.8 years (range: 23-50 years)	124 (100)
Mutagen sensitivity	
Hypersensitive (>1 b/c)	65 (52)
No hypersensitive (≤1 b/c)	59 (48)
Anatomical subsite	
Oral cavity	48 (38)
Oropharynx	27 (22)
Hypopharynx	32 (26)
Larynx	17 (14)
Gender	
Male	107 (86)
Female	17 (14)
UICC stage	
I	3 (2)
II	20 (16)
III	48 (39)
IV	53 (43)
Treatment	
Surgery alone	9 (7)
Radiotherapy alone	7 (6)
Surgery + adjuvant radiotherapy	71 (57)
Surgery + radio-chemotherapy	6 (5)
Radio-chemotherapy	10 (8)
Chemotherapy alone	12 (10)
Palliative therapy	9 (7)

b/c chromatid breaks/cell; *UICC* Union for International Cancer Control, TNM Classification of Malignant Tumors 7th edition

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Table 2. *Characteristics of the 20 second primary cancer patients*

	Gender	*Index cancer*	*b/c*	*UICC stage*	*Site of SPC*	*UICC stage*	*Time to SPC (months)*	*Histology*	*Survival with SPC (months)*
1	male	oral cavity	0.83	II	oropharynx	II	109	SCC	31
2	male	oral cavity	0.97	I	oral cavity	III	161	SCC	15
3	male	oral cavity	1.58	II	esophagus	III	165	SCC	15
4	male	oral cavity	1.27	II	oral cavity	III	99	SCC	82
5	male	hypopharynx	1.02	III	oral cavity	III	24	SCC	30
6	male	hypopharynx	0.59	III	lung	III	36	SCC	14
7	male	hypopharynx	1.51	III	esophagus	III	24	SCC	10
8	male	larynx	0.55	II	oropharynx	IV	208	SCC	10
9	male	larynx	0.64	II	oropharynx	III	84	SCC	12
10	male	larynx	0.52	III	oropharynx	IV	170	SCC	13
11	male	larynx	0.87	III	oral cavity	IV	236	SCC	8
12	male	oral cavity	0.88	II	lung	III	100	SCC	11
13	male	oral cavity	0.72	III	esophagus	III	73	SCC	8
14	male	larynx	0.78	III	lung	III	15	SCLC	12
15	male	oral cavity	1.52	II	oral cavity	III	152	SCC	48
16	male	oropharynx	1.14	III	oral cavity	III	4	SCC	81
17	female	larynx	1.34	III	oropharynx	III	156	SCC	16
18	female	oral cavity	1.60	II	larynx	III	74	SCC	12
19	female	hypopharynx	1.05	III	oropharynx	II	235	SCC	10
20	male	oropharynx	1.45	II	prostate	III	272	AC	16*
Mean	-	-	1.02	-	-	-	118	-	22

b/c chromatid breaks/cell, *UICC* Union for International Cancer Control, TNM Classification of Malignant Tumors 7th edition, *SPC* second primary cancer, *SCC* squamous cell cancer, *SCLC* small cell lung cancer, *AC* adenocarcinoma, *19 patients died of cancer and 1 died of coronary disease

35%) developed outside of head and neck region (esophagus, lung, prostate). The mean time to SPC was 118 months (range: 4–272 months). The 10-, 15-, or 20-year estimated rate of SPC was 24, 41 and 65%, respectively. In 3 patients, the index cancer and the SPC occurred in the same subregion (oral cavity, contralateral edge of the tongue). In these 3 cases the time to SPC was 161, 99 and 152 months (more than 5 years), and in every case the distance between the index cancer and SPC was greater than 2cm. None of the patients with SPC had persistent disease or *per continuitatem* invasion associated with SPC. The therapy of index cancer for patients with SPC was as follows: surgery alone (n=2, R0 resection), surgery and adjuvant RT (n=17, R0 or R1 resection 16 and 1), surgery and adjuvant radio-chemotherapy (n=1, with R1 resection and extracapsular tumor

extension). The number of hypersensitive (>1 b/c) patients of the 124 HNSCC patients were 65 (mean b/c: 1.43±0.39). Ten of them (15%) developed SPC. In the not hypersensitive group (n=59, mean b/c: 0.74±0.18), 10 patients (17%) also developed SPC (p=0.4272). The mean value of b/c for patients with SPC and without SPC was 1.02±0.37 (range: 0.52–1.6) and 1.12±0.48 (range: 0.35–2.8; p=0.4062). Eighty-four patients underwent lymphadenectomy: 20 were node negative and 64 were node positive. The rate of hypersensitive patients was 0.45 (9/20) and 0.52 (33/64), respectively (p=0.7983). The rate of SPC in the two groups was 0.35 (7/20) and 0.19 (12/64), respectively (p=0.1394). The mean value of b/c was separately evaluated for patients with ≥36 months to SPC development. The mean b/c value of patient with "late" (≥36 months) SPC (n=16) or patients without SPC (n=104) was 1.02 (range: 0.52–1.60) and 1.12 (range: 0.35–2.80), respectively (p=0.5724). The 15-year estimated rate of overall survival for hypersensitive or not hypersensitive patients was 16.9 and 11.0%, respectively (p=0.4164). The second cancer-free survival curves by mutagen sensitivity are shown in Figure 2 [62]. The 15-year estimated rate of SPC for not hypersensitive and hypersensitive patients was 48 and 36%, respectively (p=0.3743). The median and mean survival time with SPC was 23 months (range: 8–82 months) and 15 months. The 2- and 3-year cancer-specific survival with SPC was 38 and 23%, respectively (Figure 3 [62]). The 45% of SPC was developed after 10 years (between 152 and 272 months). The crude

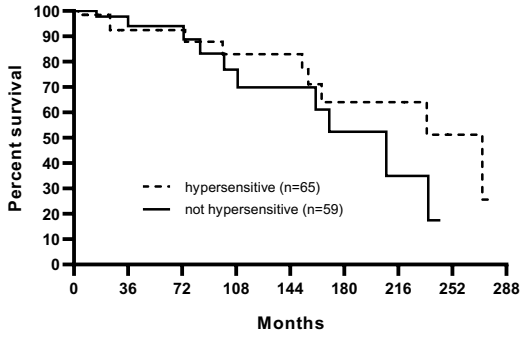

Figure 2. *Second cancer-free survival by mutagen sensitivity.* The 15-year estimated rate of second primary cancer of not hypersensitive or hypersensitive patients was 48 and 36%, respectively (p=0.3743).

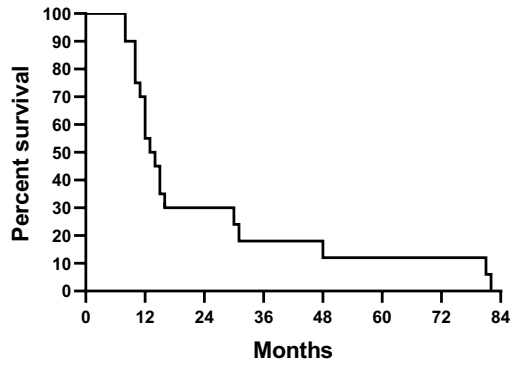

Figure 3. *Cancer-specific survival with second primary cancer (n=20).* The estimated rate of 2- or 3-year survival was 38 and 23%, respectively.

rate of SPC for men and women was 16% (17/107) and 18% (3/17), respectively (p=0.9999), and for irradiated and non-irradiated patients 18% (18/94) and 7% (2/30), respectively (p=0.1540). It should be noted that 80% (n=24) of the non-irradiated patients had stage IV disease. The rate of SPC by anatomical site of index cancer and Union for International Cancer Control (UICC) stage is given in Tables 3 and 4 [62]. The crude rate of SPC was significantly higher among patients with limited disease. However, the majority (62%) of our patients had stage III–IV disease and disease stage had a significant impact on the cancer-specific survival. The 15-year cancer-specific survival with stage I, II, III or IV disease was 67, 52, 22 and 0%, respectively (multigroup $p<0.0001$). All of the patients with stage IV disease died within 77 months. The short survival time might be one of the reasons that none of these patients developed SPC. The effect of the individual patient characteristics (gender, index cancer site, UICC stage, mutagen sensitivity, RT) on the risk of SPC was also examined in the Cox proportional hazards model. Results are presented in Table 5 [62]. None of the studied variables proved to be a significant predictor of the risk of SPC [62]. Among the long-term surviving patients

Table 3. *Second primary cancer rate by index cancer site*

Index cancer	*Patients, n (%)*	*b/c, mean ± SD*	*Mean FUP time* months (range)*	*Crude rate, %(n)*
Oral cavity	48 (38)	1.18±0.51	70 (5–240)	13 (6)
Oropharynx	27 (22)	1.10±0.42	75 (6–288)	15 (4)
Hypopharynx	32 (26)	1.10±0.50	49 (6–244)	13 (4)
Larynx	17 (14)	0.90±0.28	84 (7–244)	35 (6)
All	124 (100)	1.10±0.47	68 (5–288)	16 (20)

b/c chromatid breaks/cell, *SD* standard deviation, **FUP* follow-up time, oral cavity + oropharynx *vs.* hypopharynx + larynx p=0.6253

Table 4. *Second primary cancer rate by UICC stage*

Stage	*Patient, n (%)*	*b/c, mean ± SD*	*Mean FUP time* months (range)*	*Crude rate, % (n)*
I	3 (2)	1.09±0.42	216(176–240)	33 (1)
II	20 (16)	1.20±0.40	145 (21–288)	45 (9)
III	48 (39)	1.01±0.48	82 (11–249)	21 (10)
IV	53 (43)	1.15±0.47	17 (5–77)	0 (0)
All	124 (100)	1.10±0.47	68 (5–288)	16 (20)

b/c chromatid-breaks/cell, *SD* standard deviation, *Stage* TNM Classification of Malignant Tumors 7th edition, **FUP* follow-up time, stage I-II *vs.* stage III-IV p=0.0009

Table 5. *The 15-year estimated rate of second primary cancer by variables (univariate analysis)*

Variables	*%*	*p-value*	*RR (CI 95%)*
Gender	-	0.5071	-
male	46	-	1.00
female	24	-	0.6698 (0.2277-1.970)
Site of index cancer	-	0.1049	-
oral cavity + oropharynx	39.5	-	1.00
hypopharynx + larynx	44.1	-	2.009 (0.7924-5.084)
UICC stage	-	0.9615	-
early (I-II)	46.4	-	1.00
locally advanced (III-IV)	31.9	-	0.9797 (0.4078-2.354)
Mutagen sensitivity	-	0.3072	-
not hypersensitive	47.6	-	1.00
hypersensitive	36.1	-	0.6463 (0.2634-1.586)
Radiotherapy	-	0.8767	-
no	28	-	1.00
yes	43	-	1.120 (0.2427-5.172)

RR relative risk, *CI* confidence interval, *UICC* Union for International Cancer Control, TNM Classification of Malignant Tumors 7th edition

Table 6. *Change in mutagen sensitivity during follow-up time*

Index cancer	*b/c value (before treatment)*	*b/c value (after treatment)*	*Follow-up time (months)*
Oropharynx	0.65	0.85	195
Oropharynx	1.60	2.38	176
Oral cavity	1.52	2.45	194
Hypopharynx	1.05	1.09	216
Oropharynx	1.45	1.20	216
Oral cavity	0.87	0.92	181
Oral cavity	0.63	0.77	172
Mean	**1.11**	**1.38**	**193**

b/c chromatid breaks/cell

under follow-up, seven consented to a repeated assessment of mutagen sensitivity. The results are presented in Table 6. No substantial changes were observed in the values.

Out of our patient database of 432 HNSCC patients four younger (≤50 years) patients were found with squamous cell carcinoma of neck lymph nodes from CUP. The first patient, a 44-year-old female smoker, presented with cystic mass measuring 5 cm on the right side of neck in 1999. The patient had a history of treatment for TisN0M0 urothelial cancer of urinary bladder. In 1997, she underwent transurethral excision and BCG (bacillus Calmette-Guérin) installation at another hospital, but the details are unknown. She was subjected to ipsilateral upper node dissection. The largest size of squamous cell carcinoma metastasis was 25 mm in the metastatic node. The HE staining described well differentiated squamous cell cancer without extracapsular tumor extension. Ten nodes were negative (T0pN1M0). MRI and panendoscopy examination

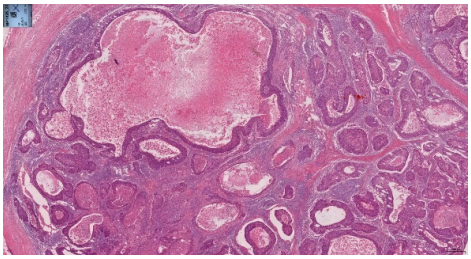

Figure 4. *Extensive cystic changes can be seen in tumor cell nests.* Stained with haematoxylin and eosin.

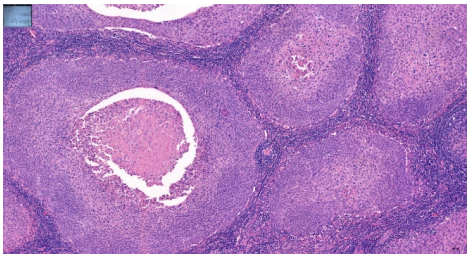

Figure 5. *Lymph node metastasis of squamous cell carcinoma.* Tumor cells show mainly basaloid morphology. Tumor cell nests show extensive central necrosis. Stained with haematoxylin and eosin.

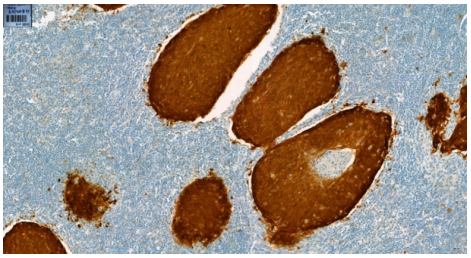

Figure 6. *Immunohistochemistry for p16.* Diffuse intensive p16 expression suggests HPV association.

did not find a primary tumor. Pretreatment value of mutagen sensitivity assay by bleomycin test was 1.17 b/c. Observation was recommended. Five years later (2004), the neck node metastasis recurred. Four lymph nodes were dissected, and two of them had squamous cell carcinoma metastasis. The size of the largest deposit was 20 mm with capsular invasion, but no extracapsular tumor extension was observed. The histopathologic examination showed squamous cell carcinoma. Biopsies from the tonsils were negative. The patient underwent unilateral epi- and oropharyngeal and regional (upper neck nodes) RT (to 50 Gy, 25 fractions). In 2011, she underwent sigmoid polypectomy (pTisN0M0 adenocarcinoma). In 2021, the molecular pathology examination of neck node metastasis (from a regional relapse in 2004) showed HPV16-genotype and p16 over expression by immunohistochemistry. She had distant site metachronous in situ cancers: urothelial cancer and sigmoid colon adenocarcinoma. In situ cancer does not give metastasis and her neck node metastasis was squamous cell carcinoma both in 1999 and 2004. The patient is alive without relapse. The overall survival is 274 months. The second patient, a 43-year-old female smoker presented with enlarged (55 mm) right submandibular lymph node of the neck in 2000. An ipsilateral upper node dissection was performed and 1 out of 19 lymph nodes showed poorly differentiated non-keratinizing-cystic squamous cell carcinoma (Figure 4 [32]) metastasis without extracapsular extension (T0pN1M0), which may indicate tonsillar cancer, but histologic findings of tonsils biopsies were negative. The postoperative PET/CT and panendoscopy did not show primary cancer. Postoperative RT was given

(epi- and oropharynx and ipsilateral upper neck nodes to 50 Gy, 2 Gy fraction). The pretreatment value of the bleomycin test for mutagen sensitivity was 1.04 b/c. She was subjected to right-side colon cancer surgery at another hospital in 2014: adenocarcinoma, pT3pN0M0. In 2014, she underwent left-side nephrectomy: clear cell cancer, pT1apN0M0. She developed abdominal lymph node metastasis from colon cancer in 2015 and was treated with chemotherapy. In 2019, hepatic and pulmonary metastases were diagnosed. In 2022, the retrospective molecular pathology of neck node metastasis showed no HPV16 DNA infection or p16 over expression. She is living with a progressive disease. The histology of her distant site metachronous invasive cancers was adenocarcinoma and clear cell kidney cancer but the neck node metastasis was squamous cell carcinoma. The overall survival is 243 months. The third patient, a 34-year-old nonsmoking female patient presented with cervical lymph node enlargement in 2006. Both MRI and PET/CT showed bilateral suspect neck nodes and increased glucose metabolism was found in the base of the tongue by PET/CT. She underwent the pretreatment mutagen sensitivity examination. The bleomycin test value was 1.60 b/c. She underwent bilateral cervical lymph node dissection and excisional biopsy from the base of the tongue. Fifty-two cervical lymph nodes were dissected and the histopathological examination revealed poorly differentiated squamous cell carcinoma metastasis in five nodes (three on the right side and two on the left side) (Figure 5 [32]). The largest node size was 55 mm, and the largest metastasis was 30 mm. No extracapsular tumor extension was detected (T0pN2 M0). The biopsy specimen from the base of the tongue was free of cancer. The panendoscopic examination was also negative. No primary tumor was found. After surgery, radio-chemotherapy was given (Cisplatin 6 × 152 mg; 1.8 Gy/fraction, neck lymph nodes (bilateral) and hypopharynx to 55.8 Gy; epi- and oropharynx to 66.6 Gy). Molecular pathology was performed in 2020. Tumor cells were p16 positive and the presence of HPV16 was confirmed from tumor DNA (Figure 6 [32]). The patient was followed up as an outpatient and after 198 months, there was no evidence of recurrence. The fourth patient, a 50-year-old male smoker and alcoholic presented with bilateral fixed metastatic neck nodes (T0N3M0) in 2001. Biopsy of a fixed node showed non-keratinized squamous cell carcinoma. The panendoscopic examination did not find primary cancer. The pretreatment value of mutagen sensitivity was 2.06 b/c. Radio-chemotherapy resulted in a partial response. He died of a progressive disease in 2003.

Overall survival was 30 months. Molecular pathology was performed in 2022: no HPV DNA was detected and no p16 stain was seen in the squamous epithelium [32].

On the group of oral or laryngeal leukoplakia (n=253) the mean or median follow-up time was 148.8 months and 144 months (range: 14–328 months), respectively. Seventeen patients presented with in situ or invasive cancer, and half of the invasive cancers had III–IV stages. In 47 patients, the cancer was developed during the follow-up time, between 6 and 204 months (mean time: 53.6 months). Six patients developed cancer after 120 months. The early-stage cancer (stage 0–I–II) rate was 48.4% (31 of 64). In total, 11 of the 64 cancer patients (in situ cancers are also included) are currently alive, 40 died of leukoplakia-associated head and neck cancer, 3 died of SPC and 10 died of internal diseases. The average survival time with cancer was 64.3 months (10–221 months). The 5-year estimated survival with leukoplakia-associated cancer for patients with oral or laryngeal leukoplakia was 40.9% and 61.1% ($p>0.337$), respectively. The number of biopsy/patients (prior to malignant transformation) was 1/148, 2/17, 3/4 and 4/1. Dysplasia progressed in 20 of 22 patients with multiple biopsies (oral, 13; laryngeal, 7). The average time to the malignant transformation of laryngeal leukoplakia or oral leukoplakia patients was 55.6 months (range: 6–204 months) and 52.7 months (range: 6–204 months), respectively ($p=0.913$). The grade of dysplasia had a significant effect on the time to malignant transformation with oral leukoplakia. The mean metastasis transformation-free survival with a low grade or with a high grade was 88.0 and 11.3 months, respectively ($p<0.0001$). In the laryngeal group, the difference between the two grades was not significant ($p=0.982$). The crude rate of malignant events and the 10-year estimated malignant transformation rate using characteristics are shown in Table 7 [54]. The 10-year estimated malignant transformation rate of leukoplakia for all (253) patients was 18.5%. The laryngeal leukoplakia patients have a significantly increased risk of malignant transformation compared with oral patients (univariate Cox Hazard Ratio (HR): 3.13). The 10-year estimated malignant transformation rate was 42.0% and 15.1%, respectively (Figure 7 [54]). The results of the multivariate Cox regression model, run for all patients, are shown in Table 8 [54]. The non-homogenous lesion and higher grade of dysplasia remained independent negative predictors of malignant transformation-free survival. A separate analysis of patients with oral leukoplakia is shown in Table 9 [54].

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Table 7. *Characteristics of the 253 patients with oral or laryngeal leukoplakia*

Charac-teristic	*Malignant events n (%)*	*p*	*10-year MTFS % (±SD)*	*p*	*Univariate Cox HR (CI 95%)*	*p*
All patients	64/253 (25.3%)		81.5±2.6			
Gender						
female	29/138 (21%)	0.11	86.0±3.2	0.061	1	0.065
male	35/115 (30.4%)		76.3±4.3		1.72 (0.97-3.06)	
Age (years)						
≤60	38/152 (25%)	>0.999	79.8±4.7	0.546	1	0.548
>60	26/101 (25.7%)		81.9±3.3		1.20 (0.66-2.16)	
Smoking						
never	5/34 (14.7%)	0.0004	87.8±5.7	0.004	1	0.009
past and present	47/96 (49%)		62.6±5.6		4.01 (1.42-11.30)	
unknown	12/123 (9.8%)					
Oral vs laryngeal						
oral	48/221 (21.7%)	0.002	84.9±2.6	<0.0001	1	<0.0001
laryngeal	16/32 (50%)		58.0±9.4		3.13 (1.71-5.72)	
Lesion type						
homogenous	53/236 (22.5%)	0.0004	83.2 ± 2.6	0.003	1	0.005
non-homogenous	11/17 (64.7%)		53.8 ± 13.8		3.46 (1.46-8.18)	
Biopsy						
no	4/83 (4.8%)	<0.0001	95.9±2.4	<0.0001	1	<0.0001
yes	60/170 (35.3%)		74.0±3.7		6.48 (2.33-18.05)	
Histology						
no dysplasia	5/88 (5.7%)	<0.0001	95.3±2.3	<0.0001	1	<0.0001
grade I dysplasia	7/29 (24.1%)		79.3±7.5		4.76 (1.51-15.03)	
grade II dysplasia	16/20 (80%)		18.3±10.3		32.62 (11.51-92.44)	
grade III dysplasia	15/16 (93.8%)		18.8±9.8		29.79 (10.73-82.73)	
in situ cc.	5					
invasive cc.	12					
Dysplasia						
no	5/88 (5.7%)	<0.0001	95.3±2.3	<0.0001	1	<0.0001
yes	38/65 (58.5)		45.9±6.4		14.58 (5.71-37.24)	
Dysplasia						
low grade (I)	7/29 (24.1%)	<0.0001	79.3±7.5	<0.0001	1	<0.0001
high grade (II,III)	31/36 (86.1%)		18.3±7.0		6.78 (2.94-15.63)	

MTFS malignant transformation-free survival, *SD* standard deviation, *HR* hazard ratio, *CI* confidence interval

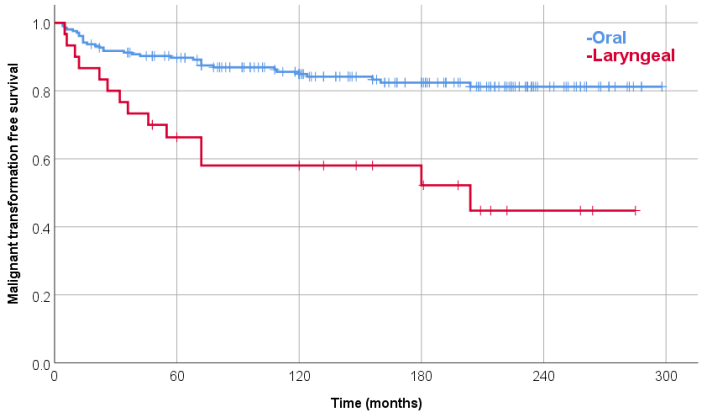

Figure 7. *Malignant transformation-free survival with oral or laryngeal leukoplakia.* The 10-year estimated malignant transformation rate was 15.1% and 42.0%, respectively (p<0.0001).

Table 8. *Multivariate model for all patients (oral or laryngeal leukoplakia)*

Characteristic	*Multivariate Cox HR (CI 95%)*	*p*
Smoking		
never	1	0.142
past and present	2.49 (0.74-8.44)	
Lesion type		
homogenous	1	0.014
non-homogenous	3.74 (1.31-10.09)	
Dysplasia		
no	1	
low grade (I)	4.23 (1.11-16.16)	<0.0001
high grade (II,III)	12.25 (4.10-36.66)	

HR hazard ratio, *CI* confidence interval

The mean follow-up time was 149.9 months (14–328 months). Smoking, the lesion site, the lesion type, dysplasia and the grade of dysplasia had significant effects on malignant transformation-free survival. In the multivariate Cox regression model, only dysplasia and the grade of dysplasia remained independent predictors of malignant transformation-free survival (Table 10 [54]). Four patients with oropharyngeal leukoplakia developed cancer. None of them had an HPV-positive histology with a p16 immunohistochemistry examination. The mean follow-up time for laryngeal patients was 140.9 months (36–285 months). To separately evaluate the patients with laryngeal leukoplakia, some of the subgroups have underpowered materials regarding the number of patients and malignant events (Table 11 [54]). The rate of malignant events was significantly higher only for patients with dysplastic leukoplakia: no or yes, 11.1% and 80%, respectively (p=0.002). In total, 73% (11 of 15) of patients with dysplastic leukoplakia were smokers. The 10-year estimated malignant-free survival was 88.9% and 30.5%, respectively (p=0.002). The presence of dysplasia significantly increased the risk of malignant transformation (HR: 12.43; 95% Confidence Interval: 1.59–97.38; p=0.016). Furthermore, seven dysplastic lesions progressed to a higher grade over the follow-up time.

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Table 9. *Results of patients with oral leukoplakia using characteristics*

Charac-teristic	*Malignant events n (%)*	*p*	*10-year MTFS % (±SD)*	*p*	*Univariate Cox HR (CI 95%)*	*p*
All patients	48/221 (21.7%)		84.9±2.6			
Gender						
female	26/128 (20.3%)	0.621	85.8±3.3	0.756	1	0.756
male	22/93 (23.7%)		83.9±4.1		1.12 (0.56-2.23)	
Age (years)						
≤60	25/126 (19.8%)	0.51	86.2±3.2	0.235	1	0.239
>60	23/95 (24.2%)		81.9±4.8		1.52 (0.76-3.02)	
Smoking						
never	4/33 (12.1%)	0.0004	90.6±5.2	0.004	1	0.009
past and present	35/73 (48%)		63.4±6.5		4.93 (1.48-16.38)	
unknown	9/115 (7.8%)					
*Lesion site**						
tongue	14/60 (23.3%)	0.003	82.6±5.4	0.007	2.89 (0.90-9.20)	0.003
sublingual	6/24 (25%)		76.7±9.2		3.67 (0.98-13.66)	
palate	3/8 (37.5%)		71.4±17.1		5.00 (0.92-27.35)	
buccal	6/60 (10%)		96.5±2.4		1	
gingiva	6/13 (46.1%)		52.4±15.7		7.17 (1.93-26.74)	
lips	0/27 (0%)		100		0	
oropharyngeal	4/10 (40%)		75.0±15.3		4.24 (0.78-23.13)	
multifocal	9/19 (47.4%)		73.3±11.4		5.47 (1.47-20.39)	
Lesion type						
homogenous	37/203 (18.2%)	0.0002	87.0±2.6	<0.0001	1	0.001
non-homogenous	11/18 (61.1%)		53.8±13.8		4.57 (1.88-11.13)	
Biopsy						
no	3/77 (3.9%)	<0.0001	96.9±2.2	<0.0001	1	<0.0001
yes	45/144 (31.3%)		77.9±3.8		6.60 (2.01-21.63)	
Histology						
no dysplasia	4/79 (5.1%)	<0.0001	96.1±2.2	<0.0001	1	<0.0001
grade I dysplasia	4/25 (16%)		88.0±6.5		3.43 (0.86-13.75)	
grade II dysplasia	12/15 (80%)		22.2±12.2		40.71 (11.91-139.11)	
grade III dysplasia	10/10 (100%)		0		54.33 (15.15-194.84)	
in situ cancer	4					
invasive cancer	11					
Dysplasia						
no	4/79 (5.1%)	<0.0001	96.1±2.2	<0.0001	1	<0.0001
yes	26/50 (52%)		50.0±7.4		13.79 (4.79-39.72)	
Dysplasia						
low grade (I)	4/25 (16%)	<0.0001	88.0±6.5	<0.0001	1	<0.0001
high grade (II,III)	22/25 (88%)		11.3±7.1		14.63 (4.28-50.03)	

MTFS malignant transformation free survival, *SD* standard deviation, *HR* hazard ratio, *CI* confidence interval, * p16 immunohistochemistry was negative for oropharyngeal patients

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Table 10. *Multivariate Cox model for patients with oral leukoplakia*

Characteristic	*Multivariate Cox HR (CI 95%)*	*p*
Dysplasia		
no	1	
low grade (I)	2.47 (0.41-15.03)	<0.0001
high grade (II,III)	18.19 (4.71-70.25)	
Lesion type		
homogenous	1	0.063
non-homogenous	2.79(0.95-8.24)	
Smoking		
never	1	0.098
past and present	3.51 (0.79-15.51)	

Table 11. *Malignant events of patients with laryngeal leukoplakia using characteristics*

Characteristic	*Malignant events n (%)*	*p*
All patients	16/32 (50%)	
Gender		
female	3/10 (30%)	0.252
male	13/22 (59.1%)	
Age (years)		
≤60	13/26 (50%)	>0.999
>60	3/6 (50%)	
Smoking		
never	1/1 (100%)	>0.999
past and present	12/23 (52.2%)	
unknown	3/8 (37.5%)	
Site		
unilateral	9/20 (45%)	0.717
bilateral	7/12 (58.3%)	
Lesion type		
homogenous	16/32 (50%)	>0.999
non-homogenous	0/0	
Biopsy		
no	1/6 (16.7%)	0.172
yes	15/26 (57.7%)	
Histology		
in situ cancer	1	
invasive cancer	1	
Dysplasia		
no	1/9 (11.1%)	0.002
yes	12/15 (80%)	
Dysplasia		
low grade (I)	3/4 (75%)	>0.999
high grade (II,III)	9/11 (81.8%)	

The characteristics of the 75 patients diagnosed with oral leukoplakia between 2021 and 2024 are shown in Table 12 [61]. The incidence of oral leukoplakia was slightly higher in women (women: 53.5% vs. men: 46.7%). There was no significant difference between sexes in the presence of dysplasia (p=0.8052). The mean age was 59.5 years (range: 23-88). Leukoplakia was more common in older patients (>50 years) (57/75, 76%). Age had no significant effect on the prevalence of dysplasia (p=0.5648). Of the 75 patients, 32 are smokers and 43 are non-smokers. Smoking in our patient did not significantly increase the incidence of dysplasia (p=0.6208) or the severity of dysplasia (p=0.3256) in leukoplakias. The most frequent site of leukoplakia was gingiva / edentulous ridge (26/75, 34.7%), followed by bucca (16/75, 21.3%), floor of the mouth (12/75, 16%), tongue (11/75, 14.7%), palate (6/75, 8%) and lip (2/75, 2.7%). Multifocal leukoplakia (present in several anatomical regions at the same time) occurred in 2 cases (2/75, 2.7%). Sixty-one homogeneous (81.3%) and 14 non-homogeneous (18.7%) cases were found. Dysplasia was

Table 12. *Characteristics of the 75 patients with oral leukoplakia*

Characteristics	n (%)
All patients	75 (100)
Gender	
male	35 (46.7%)
female	40 (53.3%)
Age (years)	
≤50	18 (24%)
>50	57 (76%)
Smoking	
past and present	32 (42.7%)
never	43 (57.3%)
Lesion type	
homogenous	61 (81.3%)
non-homogenous	14 (18.7%)
Lesion site	
gingival / edentulous ridge	26 (34.7%)
buccal	16 (21.3%)
floor of the mouth	12 (16%)
tongue	11 (14.7%)
palate	6 (8%)
lips	2 (2.7%)
multifocal	2 (2.7 %)
Histopathology	
hyperkeratosis	51 (68%)
mild dysplasia	19 (25.3%)
moderate dysplasia	5 (6.7 %)
severe dysplasia	0 (0%)

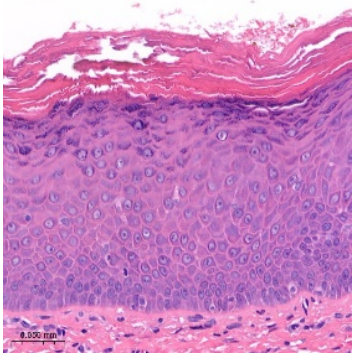

Figure 8. *Histological image without dysplasia, hyperkeratosis.* Stained with haematoxylin and eosin.

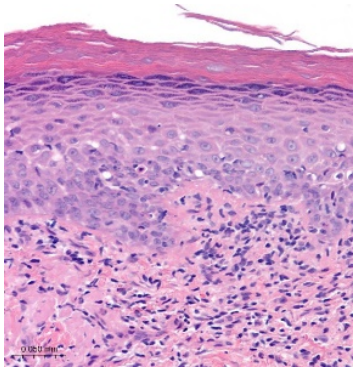

Figure 9. *Histological image showing mild dysplasia.* Stained with haematoxylin and eosin.

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significantly more frequent in lesions with clinically non-homogeneous leukoplakia (p=0.0088). Histopathological findings showed 51 cases of hyperkeratosis (without dysplasia) (Figure 8), 19 cases of mild dysplasia (Figure 9) and 5 cases of moderate dysplasia (Figure 10) [61]. Severe dysplasia was not encountered. Average follow-up time: 17.4 months (range: 1-38 months). Out of the 75 patients, p53 immunohistochemistry was performed in 37 cases (20 with dysplasia and 17 without). Among the dysplastic cases, 55% (11/20) were p53 positive, compared to 11.8% (2/17) of the non-dysplastic cases (p=0.014).

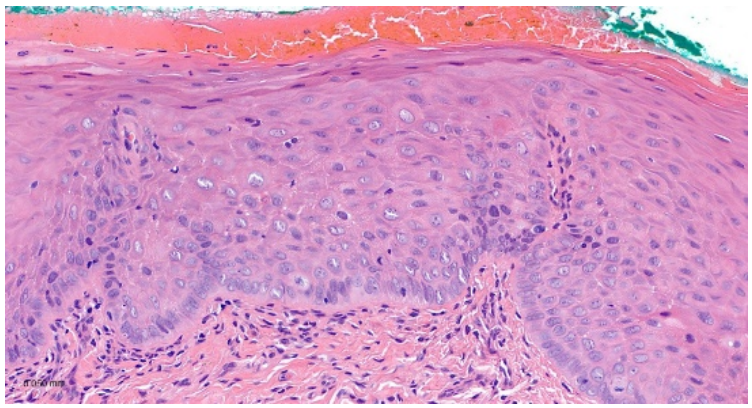

Figure 10. *Histological image showing moderate dysplasia.* Stained with haematoxylin and eosin.

5. Discussion

5.1 Second primary cancer

Head and neck cancer survivors are known to have a higher risk of morbidity and mortality. These include concurrent liver, heart, and lung conditions, therapeutic side effects, and the development of SPCs as a result of alcohol and tobacco use [63]. In these patients, it is important to examine markers that may predict the risk of recurrence or a SPC. Several studies reported that bleomycin-induced mutagen sensitivity, which reflects latent genetic instability, serves as a significant predictor of SPC. Of note: it is well known that the bleomycin-induced b/c value does not change during the life span [64]. In our patients we had the same observation. HNSCC patients with SPC exhibit higher b/c scores compared to those without SPC [28–30,65–67]. *Schantz et al.* [28] from the M. D. Anderson Cancer Center (Houston) were among the first to estimate the bleomycin test to assess the risk of SPC in HNSCC patients. The rate of SPC was significantly higher in the hypersensitive (b/c>1) than in the not hypersensitive group (33% vs. 8%), and hypersensitivity increased the risk by 4.4 times. A limitation of their study was the relatively short follow-up time: median <2 years (range, 4–31 months). Later, the study was expanded to include 278 patients, and the updated results published in 1994 confirmed that mutagen hypersensitivity (b/c>1) also increased the risk of developing SPC. The mean b/c value for patients with SPC was 1.17 (±0.54) compared with 0.98 (±0.44) for SPC-free patients (p=0.044). However, the follow-up time remained short, with a mean time of only 10.5 months from diagnosis to SPC development [29]. In our research for patients, the mean time to SPC was 118 months, which was more than ten times longer. *Cloos et al.* [65] in their study, also examined whether mutagen sensitivity may be utilized as a biomarker to identify patients who are at risk of developing SPC in the upper digestive or respiratory tract. Of all patients (n=218) 19 developed SPC (8.7%), and the follow-up time was relatively short (median: 4.5 years). In this prospective study, no significant difference was found between those who developed SPC and those who did not, with respect to mutagen sensitivity. Additionally, they discovered that the mean b/c value was significantly higher for patients who had SPC ≥3 years after the primary malignancy than for those who developed SPC earlier. In our study, the mean b/c value of patient with “late” (≥36 months) SPC (n=16) or without SPC (n=104) was not

significantly different [62]. *Minard et al.*, from the M. D. Anderson Cancer Center investigated the risk of SPC in early stage HNSCC patients, who were enrolled in a placebo-controlled chemoprevention trial of low-dose 13-*cis*-retinoic acid to reduce the occurrence of SPC. It was possible to analyze a sample of 303 Caucasian patients out of the 1080 participants. In all, SPC developed in 50 out of 303 patients (16.5%). There was no information on average follow-up time. The incidence of SPCs was not significantly decreased by the low-dose 13-cis-retinoic acid treatment. There was no increase in risk linked to the chromatid breaks induced by bleomycin. One reason for the lack of correlation between SPC and mutagen sensitivity was that the patients who were recruited had early UICC stage I–II. [66]. They reported their experience with 991 patients six months later, in which study the mean follow-up time for living patients was 7 years. As in previous studies, prediagnostic chromatid breaks induced by bleomycin were examined as a sign of latent genetic instability. Additionally, they examine how latent genetic instability and increased proliferation potential work together to influence the likelihood of SPC. To examine the proliferative potential, serum insulin-like growth factor (IGF) levels were measured. For mutagen sensitivity, the b/c cut-off value was 0.50. Among patients who developed SPC, the hypersensitive ($b/c \geq 0.5$) and not hypersensitive ($b/c < 0.5$) rate was 82% (n=243) and 18% (n=55), respectively, p=0.036 [67]. According to their findings, early-stage head and neck malignancies are more likely to develop second malignancies due to latent genetic instability and increased proliferation potential. In our research, the threshold for hypersensitivity was $b/c > 1$, as in most available publications. Our patients with HNSCC before treatment underwent bleomycin sensitivity assay and the method was not suitable for the assessment of individual index cancer risk due to overlapping of b/c values with those of controls [55,56]. We examined the association between SPC and mutagen sensitivity in younger adults. The mean time for all patients and alive patients was 5.8 and 18.5 years, respectively. This is one of the longest published follow-up time for patients with HNSCC patients. Even ten years after starting treatment, patients are at a high risk of developing SPC. Therefore, it is important to highlight the long follow-up time. In our series, 45% of SPC developed after 10 years. *Coca-Pelaz et al.* published a systematic review that analyzed articles on SPCs in the head and neck region between 1979 and 2019 [63]. They mentioned that most publications did not report accurate data on follow-up time. The median follow-up time

was reported as 4.6 years, with an even shorter follow-up time of 2.6 years for prospective studies. Differences in follow-up times may affect the proportion of SPCs reported. In our research, there was no significant correlation seen between mutagen hypersensitivity and an elevated risk of SPC. The 15-year estimated rate of SPC for hypersensitive (b/c>1.0) and not hypersensitive (b/c≤1.0) patients was 36 and 48%, respectively [62]. *Cloos et al.* [30] found significantly higher b/c values in patients with multiple cancer (mean, 1.2), than in patients with a single cancer (mean, 0.96; p=0.025). Our comparable values were 1.02 and 1.10 (p=0.4062), respectively. The investigators from the M. D. Anderson Cancer Center changed the experimental parameters several times. First, the cut-off value for b/c was 1.0, and later it was decreased to 0.5. Additionally, in their large-scale study, not only SPCs but also local relapses were included [67]. In our series, the number of patients with b/c≤0.5 was only nine and local relapse was excluded from the study. Local relapse or metastasis were not regarded as SPC.

In a study published in 2023 [68], *Ruan et al.* drew attention to the fact that the incidence of SPCs has been rising quickly over the past few decades due to longer life expectancies and longer cancer survival. For oral and pharyngeal cancers, they found that, these patients have a significantly increased risk to develop second primary non-Hodgkin lymphoma. In our study [62] most of the SPC was HNSCC (65%). Outside of head and neck region, SPC was found in the esophagus, lung and prostate (35%), but non-Hodgkin lymphoma has not developed. Otolaryngologists from Oslo studied 2063 patients with head and neck cancer over a 15-year period [69]. Based on their results the frequency of SPCs was the following: head and neck region (32%), lung (21%), and distant organs (46%). The development of any SPC has a negative impact on survival. Several studies investigated in head and neck region the risk of SPC and its impact on survival independently of mutagen sensitivity, and published long-term (more then 10-year) results [69–71]. The 20-year cumulative rate of SPC was 36% in a large multicentric study with 99 257 participants, where the largest percentage of SPCs were associated with lung cancer, which had a 13% cumulative risk over 20 years [70]. The poor prognosis for patients with head and neck cancer who have an SPC may be attributed to a high frequency of second primary lung cancer. They also mentioned that according to prior research, during a 10-year follow-up period following the first primary head and neck cancer, head and neck was the most common site of SPC (35%–73%). The risk of SPC

was elevated by alcohol consumption and smoking. The idea of field cancerization, which was put forth by *Slaughter et al.* in 1953 [72], describes how alcohol and tobacco's carcinogenic effects might affect different areas of the aerodigestive tract mucosa at the same time, leading to the formation of several primary cancers that are unrelated to one another. After the first primary was diagnosed, the increased risk of SPCs continued for ten years. Our patients also had a lifelong risk of developing SPC and were all smokers and drinkers without giving up these harmful habits. *Rennemo et al.* studied 2 063 patients with head and neck tumor [69], and out of them, 351 had SPC, so the crude rate of SPC was 17%. Our results of the crude rate of SPC were 16%, close to their rate. In their study, the mean time to SPC was more than 4 years. The median survival time with SPC was 12 months, 5-year survival was 16%. The majority of their patients (90%) died of SPC as a result. In terms of anatomical localization, the oral cavity was among the most common subsite (39%). We obtained almost the same figure of 38%. According to our analysis, the anatomical subsite had no significant impact on the risk of SPC [62]. In their study SPC was most common in patients with limited disease (stage I or II). In our series, none of the patients with stage IV disease developed SPC. The explanation might be that patients with a poor prognosis did not live long enough to develop SPC. The survival with SPC was also very poor. The median and mean survival time was 23 and 15 months, respectively. In the study of *Tiwana et al.* from British Columbia, Canada [71], the follow-up time was 25 years (median time for alive patients: 23.2 years), with a 26% of crude incidence of SPC. Oral cavity and oropharyngeal index cancer were more likely to develop SPC. The estimated 5-year overall survival with SPC was 15%. In a German study [73], 118 patients with HNSCC were selected, which number is close to our sample. The crude rate of SPC was 18%. Interestingly, 52% of SPC were diagnosed within 2 years. At our patients, only two SPCs were diagnosed within 2 years (4 and 15 months). *Bugter et al.* [74] from the Erasmus Cancer Institute, Rotterdam studied the risk factor of SPC in a Cox model. The crude rate of SPC was 15.6%. Smoking and alcohol consumption, comorbidity, and the oral cavity subsite were risk factors for SPCs. All of our patients were drinkers and smokers; therefore, we did not investigate these potential risk factors. In a current, population-based study from the United States, the smoking-related cancers were studied. Among 10 smoking-related cancer sites head and neck cancer patients had the highest risk of developing a SPC [75]. We studied the following

risk factors of SPC in a Cox model: gender, UICC stage, site of index cancer, RT, and mutagen sensitivity (all of our patients were smokers and drinkers). None of them proved to be a significant predictor of SPC development [62]. *Arie et al.* [76] evaluated in their retrospective cohort study the incidence of SPCs also by treatment modality in HNSCC patients. Out of the 184 patients, SPC developed in 31 cases (17%). With 13 cases (42%) of all SPC, the lungs were the most frequently affected site. Regarding the frequency of SPC the most frequent location of the primary tumors was the oral cavity and oro-hypopharynx (20%). The rate of SPCs was higher for early-stage tumors, but this finding was not statistically significant. Compared to patients with early-stage cancer, those with advanced-stage cancer had a shorter time to develop SPC. Due to the persistent risk of SPC, it is imperative to emphasize the lifelong follow-up. Chemotherapy and RT were not associated to the development of SPC. In our study, none of these variables (index cancer site, UICC stage, RT) proved to be a significant marker of risk of SPC. *Boakye et al.* [77] examined the incidence and sites of SPCs stratified by the HPV-associated primary head and neck cancer compared with HPV-negative cancer. Incidence of SPCs was higher among those with HPV-negative HNSCC than from potentially HPV-associated HNSCC. Among 109 512 patients with HNSCC, 13 517 (12.3%) developed SPC (more often in patients diagnosed with HPV-negative HNSCC). Due to most patients with HPV-positive cancer have better prognoses and are less likely to consume alcohol and tobacco, people with a possible HPV-associated HNSCC may have a lower relative risk of developing SPC. In our study, all the patients had HPV-negative malignancy, HPV positivity was an exclusion criterion

For cancers, it is important to consider chemoprevention. Chemoprevention of SPC in HNSCC patients are discussed in several articles [28,65,67,78,79]. The conclusion of a large multicentric trial in this topic was that further prevention trials are required to find an appropriate compound [80]. In normal and precancerous epithelial cells, retinoid is a possible compound for regulating cell differentiation and preventing carcinogenesis. According to their findings, the low-dose 13-isoretinoic acid did not decrease the incidence of SPC. The study did not use mutagen sensitivity as a biomarker to identify patients at high risk of developing SPC. In the MD Anderson chemoprevention trial, they also used the 13-isoretinoic acid [66,67]. *Bhatia et al.* declared: “Our findings did not confirm findings from the pivotal MD Anderson trial that used high-dose, short-term

isotretinoin in patients with stage I–IV HNSCC. There were no statistically significant benefits in either overall survival or second primary tumors" [80]. Based on the achievable publications, there is now no suitable biomarker to identify the risk of SPC development, nor is there a substance to prevent it from developing in patients with HNSCC. Both former and current publications [81,82] suggest long-term follow-up and early detection to improve outcome of patients with SPC. Patients have a lifelong risk to develop SPC, especially smokers and drinkers.

5.2 Cancer of unknown primary

Among the cancer patients at the time of clinical diagnosis, distant metastases are present in nearly one out of every three patients [83]. The primary tumor and its metastases are typically detected nearly simultaneously, however in certain patients, the primary tumor cannot be found. Making the diagnosis, selecting the best course of treatment, and estimating the prognosis are all challenging with CUP. For the diagnosis, in addition to the medical history of the patient, biopsy, HE-stained sample and immunohistochemistry prove to be the most important methods. The histological type of CUP in head and neck region is usually squamous cell carcinoma, but undifferentiated carcinoma may also occur. External risk factors such as smoking and alcohol consumption have been extensively studied in head and neck cancers, and both are major risk factors for HNSCC. A study by *Hermans et al.* examined smoking and alcohol consumption as potential risk factors for CUP in general [84]. However, there is limited data specifically regarding the role of smoking as a risk factor in head and neck CUP. In a study *Filippini et al.*, it is emphasized that, similar to the known primary HNSCC tumors, smoking may be a risk factor for head and neck CUP [85]. Among the four CUP patients in our cohort, smoking was noted in the medical history of three patients. The appropriate treatment for HNSCC patients with CUP has not been determined. Various methods are used to identify the primary cancer in neck node squamous cell carcinoma metastasis of CUP. The objective of *Aro et al.* [86] was to evaluate the use of different diagnostic methods, to decrease the incidence of CUP in head and neck region. Based on their results *Aro et al.* emphasize the use of pan-endoscopy including tonsillectomy. *Wichmann et al.* [87] examined in patients with squamous cell carcinoma metastasis from CUP the following: PET/CT

imaging, bilateral tonsillectomy, neck node dissection, risk-factor-adapted therapy. They suggest standardized diagnostic workup followed by neck node dissection and risk factor adapted treatment increase the survival in these patients. As the therapy of head and neck tumors is usually post-operative neck node dissection and RT or radio-chemotherapy, these principles should be followed in squamous cell carcinoma metastasis from CUP. Treatment generally consisted of neck node dissection and RT or radio-chemotherapy. In operable cases, the 5-year overall survival is around 70% with neck nodes dissection followed by RT with or without chemotherapy [87,88].

The majority (80%) of head and neck CUP is HPV-positive cancer [88,89]. HPV positivity may confirm that oropharyngeal cancer is often the underlying cause. In our two HPV-positive cases, the samples taken from the oropharyngeal region were cancer-free. The patients with HPV-positive tumors are younger and the result of the histopathology is more likely squamous cell carcinoma. In our case, both HPV-positive patients were young and diagnosed with squamous cell carcinoma. Compared to HPV-negative head and neck cancers, HPV-positive cases have a better prognosis, and this is also observed in CUP [90,91]. *Jensen et al.* [91] analyzed 60 cases, out of them, 13 were HPV-associated. Of this group, 10 cases had cystic morphology. It has been previously recognized that squamous cell carcinoma of the Waldeyer's ring is more likely to have neck metastases with cystic morphology [92]. In the Danish study [91] HPV positivity was an independent predictor of improved overall and disease-free survival. The fact that the HPV-positive tumors reacted better to treatment was linked to the improved outcome. It was suggested in the study that the presence or absence of HPV should be determined early in patients with CUP, because treatment decisions may be influenced by their status. It is noteworthy that in their research only one patient was female but our three patients with HPV-positive or cystic histology were women. In our research two patients had HPV-positive squamous cell carcinoma and both of them are still alive. However, the diagnosis of HPV infection was made almost 15 years after the original manifestation.

Rassy et al. [35] from the Institute Gustave Roussy emphasized that when cervical metastasis is identified as HPV-positive, the primary tumor is most likely located in the oropharynx, making additional diagnostic procedures, such as tonsillectomy, essential. The best approach for managing extranodal extension or advanced lymph node involvement remains a subject of discussion, with differing opinions on whether neck

node dissection followed by adjuvant RT, a combination of RT and chemotherapy, or initial radio-chemotherapy followed by neck node dissection (if fluorodeoxyglucose F 18-PET/CT yields positive results) is preferable. The study highlights that p16-negative cases are indeed HPV-negative, whereas p16-positive samples require additional testing to confirm HPV infection. They concluded that HPV-associated squamous cell CUP requires treatment approach similar to the standard treatment for non-HPV cases. The most important factors are staging and general condition. Due to the risks associated with potential overdiagnosis, both excessive and insufficient testing should be avoided. There is also disagreement over the clinical utility of site-specific therapy for CUP patients based on gen-expression profiling [93]. In a review about CUPs by *Tomuleasa et al.* [83] they suggest treatment for HNSCC from CUP the same therapy as in the case of any other advanced HNSCC. This mean surgery with radical neck dissection, followed by radio-chemotherapy. The significance of immunohistochemistry, molecular testing, molecular diagnostics, and translational research is substantial. Based on our experience [32], we believe that neck node HPV-positive or cystic metastases from CUP can be effectively treated with standard multimodality treatment: neck node dissection + RT + platinum-based chemotherapy. The PET/CT scan showed a suspect area at the base of the tongue in our patient with bilateral neck node metastasis, but the biopsy revealed no malignancy. After receiving radio-chemotherapy and bilateral radical neck node dissection, one of our patients survived without relapse. To date, her primary cancer location has not been identified.

Further research on mutagen sensitivity would yield more specific data on the cancer risks associated with HPV. Recent research has examined the activation of DNA damage repair factor in HPV-positive oropharyngeal cancers. While DNA damage repair pathways in the cervix play a crucial role in viral replication, their function in oropharyngeal squamous cell carcinoma remains largely unknown. The HPV proteins E6 and E7 constitutively activate both the ataxia telangiectasia mutated-dependent and ataxia telangiectasia mutated-dependent DNA-related pathways in HPV-positive cervical malignancies. Compared to HPV-negative lesions or normal epithelia in marginal locations, HPV-related oropharyngeal squamous cell carcinoma exhibits higher activation of the ataxia telangiectasia mutated-dependent DNA-related pathway. The

findings suggest that members involved in these pathways might have a significant role in oropharyngeal diseases caused by HPV [94].

The relationship between oropharyngeal HNSCC and cystic neck node metastasis has been the subject of several studies [95,96]. Most cases of cystic squamous cell carcinoma metastasis in the upper neck nodes are associated with HPV-positive oropharyngeal primary cancer [92]. In the majority of CUP patients with cystic squamous cell carcinoma lymph node metastasis of the head and neck region, occult primary cancers are localized in the oropharynx [96]. A biopsy of the tonsils or base of the tongue in our female patients revealed no evidence of malignancy. Twenty cases of cystic neck node metastases were examined at the Pennsylvania State Medical Center. Out of the 20 patients, 17 had primary tumors arising in the palatine or lingual tonsil and three were CUPs [92]. In the past, lateral neck cystic tumors were commonly diagnosed as branchial cleft cysts. It was hypothesized that remnants of the branchial clefts were the source of malignant lesions on the lateral side of the neck. These lesions were classified as branchiogenic carcinomas at the time, with no attempt to identify the primary tumor site. It is now understood that, in adults, lateral solitary cystic masses often originate from the epithelium within the Waldeyer's ring and represent occult primary tumors. Weak histological differentiation and the absence of transitions from benign epithelium to malignant carcinoma in lymph node metastases serve as indicators that squamous cell carcinoma metastases originate from Waldeyer's ring rather than from a primary branchiogenic carcinoma. The survival is good with the cystic lymph node metastasis [37,95]. Our patient with cystic morphology has been alive for more than 15 years after the initial presentation but has progressive distant metastases from a second primary (colon) cancer. The other two female patients with HPV-related cancer are still cancer-free [32].

Squamous cell carcinoma of the upper aerodigestive tract has high propensity to develop multiple primary malignancies. An explanation for this phenomenon was proposed by Slaughter, who gave the concept of a condemned mucosa developing after chronic carcinogenic exposure [72]. He considered "field cancerization" to be an important factor in the recurrence or persistence of oral cancer. However, field cancerization cannot account for metachronous three primary cancers with different histology and distant site origin, as in our two cases [32]. There may be a possible

association with genetic and/or immunologic alterations. *Vikesa et al.* [97] from the University of Copenhagen investigated the relationship between CUP and chromosomal instability (CIN), identifying DNA double-strand breaks as a characteristic feature of CUP. Through the early accumulation of distinct genetic and epigenetic changes in both the primary tumor and metastases, CIN is probably going to promote parallel progression. Due to their independent selection, tumor cells are expected to disseminate and settle at unusual locations before the original cancer manifests clinical signs. The uncommon clinical presentation, chemoresistance, and poor prognosis in CUP patients may be attributed to CIN, highlighting the need for targeted diagnostic and therapeutic strategies. An external risk factor for head and neck cancer as well as cancers of the bladder or colon is tobacco use. Individual susceptibility varies, therefore only a small percentage of those exposed to environmental carcinogens will get cancer. HNSCC has a complex etiology that includes endogenous risk factors. Individual susceptibility to malignancy may be influenced by factors such as DNA repair capacity and mutagen sensitivity [98,99]. Mutagen sensitivity also plays a role in developing urothelial and colorectal cancers [100,101]. In our patients with known primary cancer, the majority (19 of 20, 95%) of the metachronous SPC was located in the upper aerodigestive tract [62]. Two of our patients with CUP developed distant site primary cancers: bladder and colon or kidney and colon cancer [32]. Our patient with in situ bladder and in situ colon cancer is living without relapse. The other patient is living with a progressive disease. Although distant site new primary cancer is an uncommon occurrence, patients with HNSCC are at elevated risk of acquiring multiple malignancies in the upper aerodigestive region [102,103]. In our study dealing with SPC [62], the number of hypersensitive or not hypersensitive patients was 65 (52.4%) and 59 (47.6%), respectively. All CUP patients in our research had elevated mutagen sensitivity (b/c>1.0). However, in our 124 patients with bleomycin test no significant difference was observed between the lymph node-positive and lymph node-negative cases nor in mutagen sensitivity or the rate of SPC. The association between mutagen sensitivity and CUP has not been previously studied.

The available literature suggests that compared to other cancers, the difficulties in diagnosing and treating CUP continue to be challenging. In the last decade, new methods such as immunohistochemistry, molecular markers, and translational research have enhanced the efficiency of diagnostic assays. More studies are needed to extend survival,

and there are many unanswered questions yet [83], but based on our experience of neck node HPV-positive or cystic metastases from CUP signify a relatively good prognosis and can be effectively treated with the standard multimodality treatment.

5.3 Leukoplakia

OPMD are locally formed, morphologically altered tissues in which the likelihood of developing a tumor is higher than in normal tissues. Of all the OPMD, oral leukoplakia is the most common. There are several open questions in the available literature regarding the prognosis and prevention of oral leukoplakia malignant transformation [104]. Numerous studies have documented widely disparate rates of laryngeal leukoplakia malignant transformation and recurrence [105]. In our study, we evaluated the malignant transformation rate of patients with oral (oropharynx is included) or laryngeal leukoplakia. Up until now, no study compared the malignant transformation risk of leukoplakia of the two anatomical sites. The 10-year estimated rates were 15.1% and 42.0%, respectively (p<0.0001) [54]. The mean time to malignant transformation was longer with 3 months for laryngeal leukoplakia compared to an oral lesion, but the difference was not significant (laryngeal or oral: 55.6 months and 52.7 months, respectively, p=0.913). The relative risk of the malignant transformation of leukoplakia was more than three times higher for patients with laryngeal cancer. The dysplasia of leukoplakia significantly increased the malignant transformation rate in both groups. The grade of dysplasia also had a significant effect on the malignant transformation (p<0.0001). The survival with leukoplakia-associated cancer was similar in the two groups.

We made a detailed analysis for patients with oral leukoplakia because of the appropriate number of patients (n=221) with a long follow-up time (mean: 149.9 months). The 10-year estimated rate of the malignant transformation of oral leukoplakia was 15.1%. Smoking habit (never vs. ever), the lesion type (homogenous vs. non-homogenous), dysplasia (yes vs. no) and the grade of dysplasia had a significant effect on malignant transformation-free survival. The presence of dysplasia and high-grade (grade II, III) dysplasia remained independent negative predictors of malignant transformation-free survival in a multivariate Cox model.

In an earlier study from China, a retrospective evaluation was conducted on 218 patients who were diagnosed with oral leukoplakia both clinically and histopathologically [106]. The mean follow-up time was 5.3 years. Out of the 218 patients 39 developed cancer, the malignant transformation rate was 17.9%. High-grade epithelial dysplasia proved to be an independent predictor of malignant transformation-free survival, but a smoking habit did not. The importance of the early diagnosis and follow-up period was emphasized. Tongue leukoplakia had higher malignant incidence than those found at other sites, but the anatomical site was not an independent risk factor for malignant transformation. In their study, leukoplakia was most common in the tongue and buccal site, which is in line with our study with a similar number of cases. Two years later, also from Shanghai [107], 320 cases of oral leukoplakia were studied. The multivariate analysis revealed that the following four factors were significant independent predictors for the malignant transformation of oral leukoplakia: patient age of >60 years, lateral/ventral tongue localization, non-homogenous type and high-grade dysplasia. For all the 320 patients, the 3-year and 5-year cancer free survival was 86.6% and 82.0%, respectively. In our study the 10-year estimated malignant transformation rate with oral leukoplakia was 15.1%. In high-risk patients, they recommend control biopsy for early detection of a malignant event, which is also recommended based on our own experience. In a study by de *Vincente et al.* [108], histopathological grading (low-grade vs. high-grade dysplasia) was also significantly associated with oral cancer risk and proved to be a significant independent predictor in the multivariate Cox model. In a systematic review study with 11 423 patients [50], they highlighted the importance of the ability to predict who may develop cancer or not. In that study the malignant transformation rate for oral leukoplakia had a wide range between 0.13% and 34.0%. An advanced age, female sex, leukoplakia larger than 200 mm^2, non-homogeneous type, and higher grades of dysplasia were significant predictors of the malignant transformation of oral leukoplakia. They concluded that more research is necessary to fully understand the determinants revealed in the review. In another systematic review and meta-analysis of the last 5 years, 16 604 patients with oral leukoplakia were involved [104]. The percentage of malignant transformation ranged from 1.1% to 40.8%. The female gender, an advanced age (>50 years), non-homogeneous type and presence of dysplasia were significantly related to malignant transformation. The size of the oral leukoplakia and the smoking habit do not

show a significant effect on malignant transformation. In a Swedish study [109] of the 234 included patients 27 developed cancer. The median follow-up time was 9 years. In the multivariate Cox regression model, the rates of malignant transformation were significantly higher for non-homogeneous oral leukoplakia, leukoplakia with dysplasia, and tongue-localized leukoplakia. In our patients, oral leukoplakia with dysplasia or non-homogenous lesions were also independent predictors of malignant transformation, but the most common site of malignant transformation was the gingiva [54]. The mean follow-up time of our patients was longer than 9 years (148.8 months). Malignant transformations (n=6) were seen even over 10 years after the diagnosis of leukoplakia. The longer duration of our follow-up period may be a factor in the higher likelihood of malignant transformation. To the best of our knowledge, the longest time to the malignant transformation of oral leukoplakia was observed in a Spanish study [110]: 15 years and 2 months. In our patients, the longest time to the malignant transformation of leukoplakia was 204 months, which is longer with 22 months. The severe dysplasia also significantly increased the rate of malignant transformation in their patients. Furthermore, the rate of patients with early-stage cancer was much higher than in our patients, 19.2% and 48.4%, respectively. Most patients of *Jäwert et al.* [109] had follow-up visits with a specialist every 3 to 6 months, and the malignancies linked to OPMD were identified early which greatly increased cancer survival. A large retrospective cohort study from Northern California [111] examined 4 886 oral leukoplakia patients with 4.62 mean years of follow-up. The grade of dysplasia significantly raised the chance of developing oral cancer. One of their main findings was that leukoplakias, which were first identified as non-dysplastic lesions, were the cause of a large percentage of oral cancer cases. Due to the moderate accuracy of the leukoplakia biopsy decision, they recommended a biopsy for all clinically confirmed leukoplakias. In our cohort study, 77 patients with oral leukoplakia were not subjected to biopsy and only 3.9% of them developed oral cancer. The oral cancer rate of patients with a non-dysplastic histology was also low (5.1%). They need close (two times/year) monitoring for signs of early cancer, followed by biopsy if necessary.

Some of the subgroups have underpowered information regarding the number of patients and malignant occurrences in order to assess the patients with laryngeal leukoplakia independently. The presence of dysplasia increased the risk of malignant

transformation in our patients. In a Chinese study [112], 263 patients were analyzed. The rate of non-dysplastic leukoplakia was very high (54.4%). In our study, this rate was only 11.1%. The diagnostic classification of moderate to severe dysplasia was the independent risk factor for the malignant transformation and recurrence of laryngeal leukoplakia, according to the multivariate analysis ($p<0.05$). In a retrospective study from Israel [113], with 52 laryngeal leukoplakia patients, severe dysplasia and smoking were the risk factors of malignant transformation. In our patients, the malignant transformation rate was high even with grade I, II dysplasia [54]. However, initial dysplasia progressed over the follow-up period, and 73% of our patients with dysplasia were smokers. A study by *Zhang et al.* [114] included 32 patients with laryngeal leukoplakia. The malignant transformation rates for mild, moderate and severe dysplasia were 33%, 75% and 75%, respectively. Our patients with dysplasia also had high malignant transformation rates. *Leduchowska et al.* [115] used endoscopic and stroboscopic examinations to estimate the degree of dysplasia in laryngeal leukoplakia. The rate of low-grade or high-grade dysplasia was 61.8% and 38.2%, respectively. The decision between an immediate biopsy and watchful waiting can be guided by the study's findings. Our patients had high (75%) malignant transformation even with mild dysplasia [54].

Even though a number of previous epidemiological studies on oral leukoplakia have been published, the exact prevalence is still controversial and does not take geography or demographic stratification into account [116]. Most of the studies on prevalence was derived from single center analyses, and it varied greatly. Epidemiological data currently do not support the exact prevalence of oral leukoplakia. *Zhang et al.* published a comprehensive systematic review and meta-analysis of the global prevalence of oral leukoplakia [116]. From 1996 to 2022, a total of 69 studies were conducted, involving 1 263 028 patients. Among them, 17,524 were diagnosed with oral leukoplakia, resulting in an overall prevalence rate of 1.39%. According to their findings, population-based research found that the pooled prevalence across various continents varied from 0.33 to 11.74%.

In our clinic from 2021 to 2024 leukoplakia was diagnosed in 75 cases [61]. Results show that oral leukoplakia is slightly more common in women (women: 53.5% vs. men: 46.7%), which is further corroborated by evidence from other studies, including our previous research [54,117]. Less than half of our patients were smokers. Smoking did

not significantly increase either the frequency of dysplasia (p=0.6208) or the severity of dysplasia (p=0.3256) in leukoplakia lesions. Although smoking is considered a significant risk factor in both the national and international literature, further studies are needed to clarify its exact role [43,118,119]. The most common site of leukoplakia was the gingival / edentulous ridge (26/75, 34.7%). In a large case-control study by *Rubert et al*., leukoplakia was also most common in the gingiva (168/412, 40.8%) [117]. Anatomical localization may also be a factor influencing malignant transformation. Another study on our patient population showed that malignant transformation was most frequent in the gingival area (46.1%). It should be noted that malignant transformation was most frequent in the head and neck region in the laryngeal area (50%), but this area was not included in the oral leukoplakia [54]. According to the clinical presentation of leukoplakias, a distinction is made between homogeneous and non-homogeneous forms, with homogeneous cases being more common. In our study, we found 61 homogeneous (81.3%) and 14 non-homogeneous (18.7%) cases. *Rubert et al.* reported quite similar results in their study with a large number of cases: 81.6% of homogeneous cases and 18.4% of non-homogeneous cases. Based on our previous study, we stated that non-homogeneous appearance increases the risk of malignant transformation [54]. Considering homogeneous and non-homogeneous groups, dysplasia was significantly more frequent in the non-homogeneous group (p=0.0088) [61].

Since the definition of oral leukoplakia has undergone numerous revisions and its diagnosis relies on exclusion, achieving an accurate diagnosis and predicting its malignant transformation continue to pose significant challenges in clinical practice [38,54,61,116]. The available literature encompasses numerous studies on biomarkers that can predict the risk of malignant transformation or the severity of dysplasia. Biomarkers and molecular tests allow more accurate risk assessment and diagnosis. Histopathological examination of oral leukoplakias has highlighted the importance of p53 and Ki-67 markers [120–124]. Mutations are common in the tumor suppressor gene p53, which is considered an early event in carcinogenesis. P53 is located on the short arm of the chromosome 17 and is involved in cell cycle regulation, apoptosis and maintenance of genomic stability, among various other essential cellular processes. The mutated form of the p53 protein can be detected by immunohistochemistry. Another important marker is the Ki-67 antigen, which is involved in cell proliferation. The presence of p53 and Ki-

67 markers is associated with oral squamous cell transformation, the extent of dysplasia and malignant transformation. The literature suggests that simultaneous testing of these two markers gives the most useful results, but the studies recommend further investigation [125]. We have started the immunohistochemical analysis of p53 and Ki-67 on our patient material. Based on our results, p53 over-expression is significantly more frequent in dysplastic leukoplakias compared to non-dysplastic cases (p=0.014). This correlates with results published in the international literature [121,122,125]. We cannot yet draw conclusions about the severity of dysplasia and the presence of p53 in our patients due to the small number of cases, but the analysis of these markers may be important for the prognosis and therapy of the lesions. In addition to p53 and ki-67, other markers such as podoplanin, which is a transmembrane glycoprotein, may also be predictive markers. *Monteiro et al.* [126] reported a systematic review and meta-analysis about podoplanin expression and malignant transformation of oral leukoplakia. Building on the findings of the included studies, their results demonstrate evidence that podoplanin expression, as identified through immunohistochemistry, is strongly associated with the risk of malignant transformation in patients with oral leukoplakia. This underscores the potential utility of podoplanin as a predictive biomarker for assessing the likelihood of malignant transformation in such cases. They also emphasize the need for further studies.

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6. Conclusions

Study 1

HNSCC survivors had an increased lifelong risk of developing SPC. The risk of developing SPC was higher in patients with less advanced cancer. Its incidence rate is high even after 10-year follow-up. Therefore, lifelong follow-up is suggested for patients with head and neck cancer. Survival is poor in patients with SPC. Our results show that mutagen hypersensitivity does not increase the risk of SPC development. Therefore, mutagen sensitivity cannot be used as a biomarker to predict which patients will develop SPC. The rate of SPC and survival with SPC after long follow-up time was analyzed first in Hungary by us and in international respect our publication is among the few ones with the very long follow-up time.

Results were published in Strahlentherapie und Onkologie journal (2022).

Study 2

We conclude that neck node squamous cell carcinoma from CUP is characterized by elevated mutagen sensitivity which indicates decreased DNA repair capacity, but clinical significance of mutagen sensitivity in CUP requires further examination. HPV positivity or cystic morphology of neck node metastasis from CUP signifies good outcome and can be treated effectively with conventional site-specific therapy. HPV examination should be performed before treatment of CUP.

Results were published in Case Reports in Oncology journal (2023).

Study 3

Patients with oral or laryngeal dysplastic leukoplakia have an increased risk of malignant transformation, but the risk is about three times higher for patients with laryngeal leukoplakia. There is no significant difference between the groups regarding survival with leukoplakia-associated cancer. We made the first comparative study of the two anatomical sites. Patients with non-dysplastic lesions have a low risk of malignant transformation especially in the oral group. Grade of dysplasia of oral leukoplakia have a significant effect on the risk of malignant transformation. The late transformation (over 10 years) is common. An immediate surgical complete excision and strict and long-term follow up are

suggested for high-risk (grade II, III) patients to diagnose cancer in an early stage and to control late (over 10 years) malignant events. In other condition in the case of progression repeated excision is suggested. In international respect our publication is among the few ones with the very long follow-up time.

The presence and degree of dysplasia is associated with an increased risk of malignant transformation. Based on our results, p53 over-expression is significantly more frequent in dysplastic leukoplakias compared to non-dysplastic cases.

Results were published in the Journal of Clinical Medicine (2023).

Novel findings in national or international contexts:

Study 1

Mutagen sensitivity cannot be used as a biomarker to predict which patients will develop SPC. HNSCC survivors had an increased lifelong risk of developing SPC and its incidence rate is high even after 10-year follow-up. Survival is poor in patients with SPC. The rate of SPC and survival with SPC after long follow-up time was analyzed first in Hungary by us and in international respect our publication is among the few ones with the very long follow-up time.

Study 2

Neck node squamous cell carcinoma from CUP is characterized by elevated mutagen sensitivity which indicates decreased DNA repair capacity, but clinical significance of mutagen sensitivity in CUP requires further examination.

Study 3

Patients with oral or laryngeal dysplastic leukoplakia have an increased risk of malignant transformation, but the risk is about three times higher for patients with laryngeal leukoplakia. Grade of dysplasia of oral leukoplakia have a significant effect on the risk of malignant transformation. The late transformation (over 10 years) is common.

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7. Summary

HNSCC constitutes a major global health burden, with tobacco and alcohol consumption representing the most significant etiological factors. Despite advances in multimodal treatment strategies, the prognosis remains poor due to advanced disease and frequent occurrence of SPCs. In parallel, OPMD, particularly oral leukoplakia, are recognized as important precursor lesions to malignancy, highlighting the need for early detection and effective risk assessment. Mutagen sensitivity, assessed by bleomycin-induced chromosomal breakage in lymphocytes, has been investigated as a potential biomarker of genetic instability and cancer risk. Elevated mutagen sensitivity is observed in patients with HNSCC compared to the general population. However, its ability to predict the development of SPCs is controversial. The lifelong risk of SPC development, particularly among smokers and alcohol consumers, underscores the necessity for continuous long-term follow-up and comprehensive patient management strategies. In the field of OPMD, particularly oral and laryngeal leukoplakias, histopathological dysplasia remains the most reliable predictor of malignant transformation. Higher grades of epithelial dysplasia significantly correlate with an increased risk of progression to squamous cell carcinoma. Furthermore, non-homogeneous clinical presentation is closely associated with a greater likelihood of dysplastic alterations and malignant transformation, emphasizing the importance of thorough clinical and pathological evaluation. These observations reinforce the critical need for regular and frequent surveillance for early diagnosis and intervention. Identification of further biomarkers are necessary to enhance personalized patient care.

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Mutagénérzékenység és második primer daganat kialakulása fiatal fej-nyaki laphámrákos betegeknél

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Fej-nyaki rákos betegeknél nagy a kockázata második primer rák (MPR) kialakulásának. A megemelkedett kockázat kapcsolatos lehet a mutagénérzékenységgel. A kapcsolat kiderítéséhez olyan 124 laphámsejtes fej-nyaki rákban szenvedő fiatal (≤50 év) beteg klinikai adatait elemeztük, akiknél a daganatos betegségük kezelése előtt mutagénérzékenységi vizsgálat történt 1996 és 2006 között. A mutagénérzékenység mértékének megállapítása a perifériás vér limfociták *in vitro* bleomicinkezelése következtében kialakuló kromatidtörések egy sejtre eső átlaga alapján (kromatidtörés/sejt, t/s) történt. A betegeket két csoportba soroltuk: hiperszenzitív (>1 t/s) vagy nem hiperszenzitív (≤1 t/s). Az átlagos követési idő 64 hónap (tartomány: 5–244 hónap) volt. Tizennyolc betegnél (15%) alakult ki MPR. Kialakulásának 10 éves valószínű gyakorisága a hiperszenzitív (n=65) vagy a nem hiperszenzitív (n=59) betegeknél 17% és 30% volt, azonos sorrendben (p=0,4272). Az MPR-ek 39%-a 10 éves követés után alakult ki. Az 5 éves daganatspecifikus túlélés MPR kialakulása után 17% volt. Eredményeink szerint a mutagén-hiperszenzitivitás nem növeli az MPR kialakulásának kockázatát. Magy Onkol 65:39-45, 2021

Kulcsszavak: mutagénérzékenység, fej-nyaki rák, második primer rák

Head and neck cancer patients are at high risk for secondary primary cancer (SPC) development. Mutagen hypersensitivity may be associated with elevated risk of SPC. A survey was made of SPC among 124 young (≤50 years) patients with squamous cell carcinoma of the head and neck who were enrolled in a pretreatment mutagen sensitivity investigation during 1996–2006. Mutagen sensitivity was assessed by exposing lymphocytes to bleomycin in vitro *and quantitating the bleomycin-induced chromatid breaks per cell (b/c). Patients were classified as hypersensitive (>1 b/c) or not hypersensitive (≤1 b/c). The mean follow-up time was 64 months (range: 5–244 months). Eighteen patients (15%) developed a SPC. The 10-year estimated rate of SPC for hypersensitive (n=65) or not hypersensitive (n=59) patients were 17% and 30%, respectively (p=0.4272). Thirty-nine percent of SPC was developed after 10-year follow-up. The 5-year cancer-specific survival was 17% following the development of SPC. According to our findings, mutagen hypersensitivity does not increase the risk of developing SPC.*

Bukovszky B, Fodor J, Székely G, Kocsis S. Z, Oberna F, Major T, Takácsi-Nagy Z, Polgár C, Jurányi Z. Mutagen sensitivity and risk of second cancer in young patients with head and neck squamous cell cancer. Magy Onkol 65:39-45, 2021

Keywords: *mutagen sensitivity, head and neck cancer, second primary cancer*

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BEVEZETÉS

A dohányzás és a túlzott alkoholfogyasztás meghatározó szerepet játszanak a laphámsejtes fej-nyaki rákok kialakulásában. Hazánkban ezek a szenvedélybetegségek szinte minden beteg kórtörténetében szerepelnek (1). Az utóbbi évtizedekben jelentősen növekedett a HPV- (humán papillómavírus) asszociált szájgarati daganatok előfordulása is. Keletkezésükben nincs szerepe az alkoholizmusnak vagy dohányzásnak (2). Jól ismert, hogy a dohányzó és/vagy alkoholista betegeknek csak egy részénél fejlődik ki rák, tehát a genotoxikus anyagokkal szembeni belső érzékenység szintén szerepet játszhat a karcinogenezisben (3). Hsu még az 1980-as években kifejlesztett egy vizsgálatot a lakosság mutagénérzékenységének a meghatározására. Az indukált kromatidtöréseket számolta tenyésztett limfocitáknál, melyeket bleomicinnel kezelt a késői S-G2 fázisban (4). Általában hiperérzékeny fenotípusról beszélünk, ha egynél nagyobb a sejtenkénti kromatidtörések átlagos száma. A túlzott érzékenység növeli a laphámsejtes fej-nyaki és tüdőrák kialakulásának kockázatát (5), de az emlőrák kialakulásának kockázatát is (6).

Laphámsejtes szájüregi, garat- és gégerákos betegeknél gyakran fejlődik ki MPR a légúti és emésztő- (főleg nyelőcső) szervekben. Kialakulásukban szerepe van a daganatstádiumnak, a betegek nemének, a dohányzásnak és az alkoholizmusnak (7–9), de a mutagénérzékenységnek is (10–12).

Az Országos Onkológiai Intézetben 1996 és 2006 között 432 fej-nyaki rákos betegnél történt mutagénérzékenységi vizsgálat, közvetlenül a daganatos betegségük kezelése előtt. Kiderült, hogy a fej-nyaki rákos betegek 58,3%-a és az egészséges kontrollesetek 43,3%-a fokozottan mutagénérzékeny. Daganatos betegeknél a bleomicin által indukált kromatidtörések átlagos száma szignifikánsan emelkedett volt a kontrollcsoporthoz viszonyítva: 1,11 *versus* 0,97 törés/sejt. Továbbá, a magyar lakosság körében kétszer gyakoribb a túlérzékenység a nyugat-európai országok lakosaihoz viszonyítva (13–15). Tanulmányunkban az Országos Onkológiai Intézetben a mutagénérzékenységi vizsgálatokba bevont fiatal (≤50 év) betegeknél vizsgáltuk az MPR kialakulásának jellegzetességeit és gyakoriságát, valamint a mutagénérzékenység klinikai jelentőségét, mint kockázati tényezőt az MPR keletkezésében.

ANYAG ÉS MÓDSZER

1996 és 2006 között az Országos Onkológiai Intézetben 432 fej-nyaki laphámrákos betegnél a kezelések elkezdése előtt mutagénérzékenységi vizsgálat történt. A mutagénérzékenységi vizsgálat módszerét korábban már ismertették (13). Röviden: a hagyományos módszerrel megegyezően 72 óráig tenyésztették a limfocitákat. A tenyésztés vége előtt 5 órával bleomicinkezelést kaptak, amit kolcemides blokkolás követett. Festés után 100 metafázist számoltak betegenként. A genetikai fogékonyság mértékét az egy sejtre jutó kromatidtörések átlagos számával (kromatidtörés/sejt, t/s) jellemezték. A 432 beteg közül 124 felelt meg az alábbi feltételeknek: dohányzik és idült alkoholista, a daganatellenes kezelések elkezdésekor 50 éves vagy fiatalabb volt, fej-nyaki daganatát nem HPV okozta, a kezelések és a kezelések utáni követés is intézetünkben történt, laphámsejtes szájüregi, garat- (kivéve felgarat) vagy gégerákban szenvedtek. A betegeket, ha 100 metafázist számolva átlagosan több mint 1 volt a törések száma, hiperérzékenynek (túlérzékeny) tekintettük. A daganatos események számát és idejét a kórlapokból kigyűjtöttük. A túlélést Kaplan–Meier-módszerrel számítottuk (16). A görbék szerkesztéséhez az alábbi végpontokat használtuk: elhalálozás bármilyen okból (teljes túlélés), elhalálozás rákban (daganatspecifikus túlélés), MPR megjelenése (MPR-től mentes túlélés).

1. TÁBLÁZAT. A betegek citogenetikai és klinikai jellemzői

Jellemzők	Betegek száma (%)
Mutagénérzékenység	
Fokozott (>1 t/s)	65 (52)
Nem fokozott (≤1 t/s)	59 (48)
Daganat helye	
Szájüreg	48 (38)
Szájgarat	27 (22)
Algarat	32 (26)
Gége	17 (14)
Nem	
Férfi	107 (86)
Nő	17 (14)
TNM-stádium	
I.	3 (2)
II.	20 (16)
III.	48 (39)
IV.	53 (43)
Műtét	
Igen	86 (69)
Nem	38 (31)
Kemoterápia	
Igen	12 (10)
Nem	112 (90)
Sugárterápia	
Igen	94 (76)
Nem	30 (24)

t/s: kromatidtörés/sejt

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2. TÁBLÁZAT. A 18 második primer rák jellemzői

	Nem	Első primer rák helye	Átlagos t/s	TNM-stádium	Második primer rák helye	*Idő
1.	férfi	szájüreg	0,83	II.	szájgarat	109
2.	férfi	szájgarat	0,97	I.	szájgarat	161
3.	férfi	szájüreg	1,58	II.	nyelőcső	165
4.	férfi	szájüreg	1,27	II.	szájüreg	99
5.	férfi	algarat	1,02	III.	szájüreg	24
6.	férfi	algarat	0,59	III.	tüdő	36
7.	férfi	algarat	1,51	III.	nyelőcső	24
8.	férfi	gége	0,55	II.	szájgarat	208
9.	férfi	gége	0,64	II.	szájgarat	84
10.	férfi	gége	0,52	III.	szájgarat	170
11.	férfi	gége	0,87	III.	szájüreg	236
12.	férfi	szájüreg	0,88	II.	tüdő	100
13.	férfi	szájgarat	0,72	III.	nyelőcső	73
14.	férfi	gége	0,78	III.	tüdő	15
15.	férfi	szájüreg	1,52	II.	szájüreg	152
16.	férfi	szájgarat	1,14	III.	szájüreg	3
17.	nő	gége	1,34	III.	szájgarat	156
18.	nő	szájüreg	1,60	II.	gége	74

*t/s: kromatidtörés/sejt; *az első és második daganat kialakulása között eltelt idő hónapokban*

A túlélési görbéket log-rank teszttel hasonlítottuk össze. Az átlagértékeket kétszélű t-próbával és Fisher-egzakt teszttel vizsgáltuk. Az adatok elemzéséhez GraphPad Prism (GraphPadPrism version 5.01 for Windows, GraphPad Software, San Diego, CA) programcsomagot használtunk, $p<0,05$ értéket tekintettük szignifikánsnak.

EREDMÉNYEK

A betegek citogenetikai és klinikai jellemzőit az *1. táblázat* ismerteti. Az összes beteg vagy a még élő betegek átlagos követési ideje 64 hónap (tartomány: 5–244 hónap) és 192 hónap (tartomány: 144–226 hónap) volt, azonos sorrendben. Tizennégy beteg még él, 110 már meghalt (nyers túlélési arány 11%). Hét betegnél az elhalálozás oka belgyógyászati betegség volt. A 10 éves teljes túlélés vagy daganatspecifikus túlélés becsült aránya 21% és 24% volt, azonos sorrendben. Százhuszonnégy beteg közül 18-nál (14,5%) alakult ki MPR. Az MPR kialakulásáig eltelt átlagos idő 111 hónap volt (tartomány: 3–236 hónap). Az MPR 10 éves becsült aránya 23%. A 18 beteg jellemzőit a *2. táblázat* ismerteti. Nyolc beteg volt hiperszenzitív és 10 nem az. Közülük egy él és 17 meghalt. A daganatspecifikus elhalálozás aránya MPR-rel 94%. Az MPR-ek 39%-a 10 év után alakult ki, 152 és 236 hónap között. MPR kialakulása után az átlagos túlélés csak 23 (tartomány: 8–71) hónap volt, és a Kaplan–Meier-módszerrel becsült 5 éves daganatspecifikus túlélés aránya 17%.

Az összes beteg t/s átlaga 1,10±0,47 (tartomány: 0,35–2,8), a 18 MPR-es betegé 1,02±0,37 (tartomány: 0,52–1,6) és az MPR-től menteseké (n=106) 1,12±0,48 (tartomány: 0,35–2,8) volt. A két csoport (MPR igen vagy nem) t/s átlaga nem különbözött szignifikánsan egymástól (p=0,4062). A túlzottan mutagénérzékeny (>1 t/s) betegek száma 65 volt (átlagos t/s: 1,43±0,39). Ezek közül 8-nál (13%) alakult ki MPR. A nem hiperérzékenyek (n=59, átlagos t/s: 0,74±0,18) közül pedig 10-nél (17%). Az MPR-től mentes túlélés Kaplan–Meier-görbéit az *1. ábra* szemlélteti. Az MPR 10 éves valószínűségi gyakorisága 17% volt a túlérzékeny csoportban és 30% az összes többinél. Tehát, a várttal ellentétben, nagyobb volt az MPR gyakorisága a kevésbé érzékeny csoportban, de a különbség nem volt szignifikáns (p=0,4272).

A mutagénérzékenység szintje a daganatspecifikus túlélésre sem volt szignifikáns hatással (p=0,4732). A 10 éves túlélés becsült aránya a hiperérzékenyeknél vagy az összes

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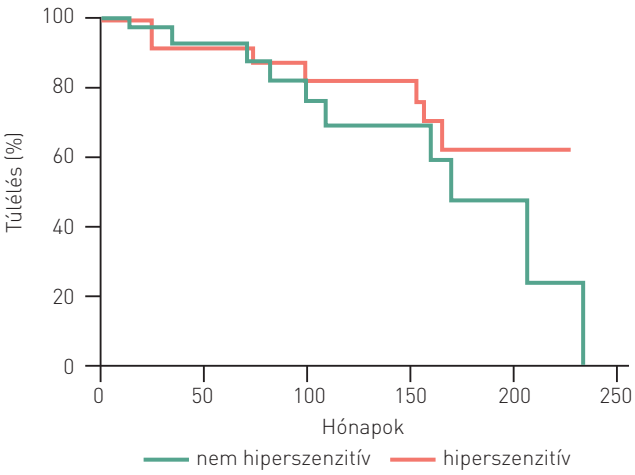


1. ÁBRA. Második primer ráktól mentes túlélés a mutagénérzékenység szintje szerint. A második primer rák 10 éves gyakorisága: hiperszenzitivitás igen (n=64) vagy nem (n=59) 17% és 30%, p=0,4272

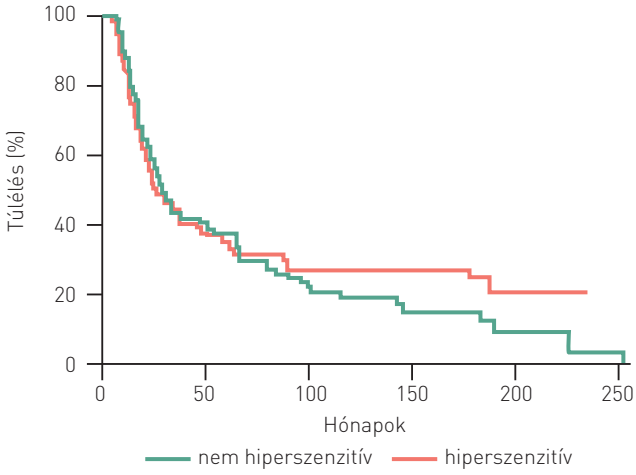
többinél 28% és 20% volt, azonos sorrendben *(2. ábra)*. A túlzottan mutagénérzékeny betegek közül már meghalt rákban 50 (77%), a másik csoportból pedig 53 (90%).

Beteganyagunkra jellemző volt az előrehaladott (III. és IV.) stádium magas aránya, 82%. A stádiumnak szignifikáns hatása volt a daganatspecifikus túlélésre. A 10 éves daganatspecifikus túlélés becsült aránya I., II., III. vagy IV. stádiummal 100%, 62%, 29% és 0% volt *(3. ábra)*, azonos sorrendben (p<0,0001). A IV. stádiumban szenvedő betegek valamennyien meghaltak 77 hónapon belül. A rövid túlélés lehet az egyik oka, hogy a IV. stádiumba tartozó betegeknél egyetlen MPR sem alakult ki. Az MPR gyakoriságát stádium szerint a *3. táblázat* szemlélteti. A 10 éves becsült arány I., II., III. vagy IV. stádiummal 0%, 30%, 17%, és 0% volt, azonos sorrendben (p=0,6114). Kétcsoportos elemzésnél, a II. vagy a III. stádiumot hasonlítva a IV. stádiumhoz a hatás szignifikáns, p<0,0001 és p=0,0008. Viszont a II. és III. stádiumban szenvedő betegeknél az MPR nyers aránya nem különbözött lényegesen, p=0,1218. A t/s arány TNM-stádium szerint nem különbözött szignifikánsan egymástól.

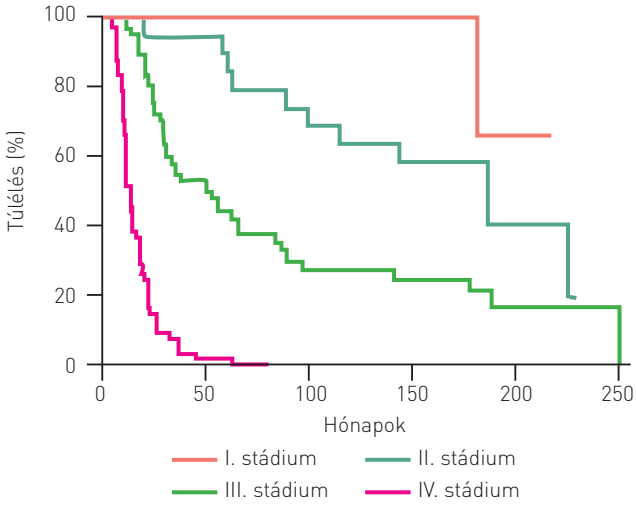
Az MPR gyakoriságát az anatómiai lokalizáció szerint a *4. táblázat* ismerteti. Az anatómiai lokalizációnak nem volt szignifikáns hatása az MPR 10 éves becsült gyakoriságára (p=0,4212). Az MPR nyers aránya gégerákban volt legmagasabb, 35%, és a hat MPR közül négy 150 hónapos követés után alakult ki. A t/s arány legalacsonyabb (0,90) volt a gégerákos betegeknél és legmagasabb szájüregi daganattal (1,18). A két csoport között a különbség szignifikáns (p=0,0382). Ezenkívül egy nem szignifikáns (p=0,0646) tendencia is megfigyelhető volt, hogy az MPR nyers aránya magasabb gégerákos betegeknél, mint szájüregi rákkal, 35% és 13%, azonos sorrendben *(4. táblázat)*.

MEGBESZÉLÉS

Laphámsejtes fej-nyaki rákban nagy a kockázata az MPR kialakulásának. Az MPR megjelenése tovább rontja a beteg túlélési esélyét, ezért fontos a gondos követés és korai felismerés (17, 18). Az M. D. Anderson Cancer Center munkacsoportja (10) feltételezte, hogy a Hsu és munkatársai (19) által kifejlesztett citogenetikai vizsgálat (bleomicinnel szembeni mutagénérzékenység) alkalmas módszer azon betegek azonosítására, akiknél a laphámsejtes fej-nyaki rákot köve-



2. ÁBRA. Daganatspecifikus túlélés mutagénérzékenység szintje szerint. 10 éves túlélés: hiperszenzitivitás igen (n=65) vagy nem (n=59) 28% és 20%, p=0,4732



3. ÁBRA. Daganatspecifikus túlélés TNM-stádium szerint. 10 éves túlélés: I. stádium (n=3) 100%, II. stádium (n=20) 62%, III. stádium (n=48) 29%, IV. stádium (n=53) 0%; többcsoportos összehasonlításnál p<0,0001

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3. TÁBLÁZAT. Második primer rák aránya stádium szerint

Stádium	Betegek száma (%)	t/s átlag±SD	Átlagos követési idő hónapokban (tartomány)	Nyers arány % (n)	10 éves becsült arány %	p-érték
I	3 (2)	1,09±0,42	194 (176–210)	33 (1)	0	0,6114*
II	20 (16)	1,20±0,40	139 (21–222)	40 (8)	30	
III	48 (39)	1,01±0,48	77 (11–244)	19 (9)	17	
IV	53 (43)	1,15±0,47	17 (5–77)	0 (0)	0	
Összes	124 (100)	1,10±0,47	64 (5–244)	14,5 (18)	23	_

*t/s: kromatidtörés/sejt; SD: standard deviáció; *többcsoportos összehasonlítás*

4. TÁBLÁZAT. Második primer rák gyakorisága az index rák lokalizációja szerint

Index rák	Betegek száma (%)	t/s átlag±SD	Átlagos követési idő hónapokban (tartomány)	Nyers arány % (n)	10 éves becsült arány %	p-érték
Szájüreg	48 (38)	1,18±0,51	66 (5–222)	13 (6)	25	0,4212*
Szájgarat	27 (22)	1,10±0,42	69 (6–226)	11 (3)	15	
Algarat	32 (26)	1,10±0,50	47 (6–216)	9 (3)	23	
Gége	17 (14)	0,90±0,28	83 (7–244)	35 (6)	20	
Összes	124 (100)	1,10±0,47	64 (5–244)	15 (18)	23	_

*t/s: kromatidtörés/sejt; SD: standard deviáció; *többcsoportos összehasonlítás*

tően nagyobb valószínűséggel alakul ki MPR. Nyolcvannégy fej-nyaki rákos betegnél mutagénérzékenységi vizsgálatot végeztek és követték őket. Tizenhárom betegnél alakult ki MPR (15%). A tanulmány egyik gyenge pontja a rövid követési idő volt: medián kevesebb, mint 2 év, tartomány 4–31 hónap. Az MPR kialakulása sokkal gyakoribb volt a hiperérzékeny (átlagos t/s: >1) csoportban, mint a nem hiperérzékenyben, 33% és 8%, azonos sorrendben. A túlérzékenység az MPR kifejlődésének kockázatát 4,4-szeresére növelte. A túlérzékenység a DNS-javítás elégtelenségére utal, ami többszörös daganatok keletkezéséhez vezethet. A szerzők előzetes tanulmánynak szánták ezt a kevés esetszámot tartalmazó vizsgálatot. 1994-ben már egy nagyobb számú beteganyag (n=278) elemzésével kapott eredményeiket közölték (11). A betegek 40%-a volt hiperérzékeny (átlagos t/s: >1). A hiperérzékenyek 13,1%-ánál alakult ki MPR szemben a nem hiperérzékenyek 7,7%-ával. A túlérzékenység 2,67-szorosára növelte az MPR kialakulásának kockázatát. Az M. D. Anderson Cancer Center kutatói egy másik beteganyagon is validálták, hogy a fokozott mutagénérzékenység növeli az MPR kialakulásának kockázatát fej-nyaki laphámrákos betegeknél (20). A Retinoid Head and Neck Second Primary Trial-ben azt vizsgálták, hogy fej-nyaki rákos betegeknél korai stádiumban (I.-II.) a 13-cisz-retinsav adása csökkenti-e az MPR vagy a recidíva kialakulásának a kockázatát. Ebben a tanulmányban a mutagénérzékenységi profilban a kritikus szintet jelentősen csökkentették. Hiperérzékenységet állapítottak meg, ha az átlagos t/s ≥0,5 volt. A fokozott mutagénérzékenység szignifikáns prediktora volt az MPR/recidíva kialakulásának. Tehát együtt számolták az MPR-t és a recidívát.

Európai beteganyagon is megpróbálták validálni az M. D. Anderson Cancer Center eredményeit. Cloos és munkatársai holland betegeknél vizsgálták a mutagénérzékenység klinikai jelentőségét fej-nyaki rákos betegeknél (21). Kettőszáztizennyolc beteg közül 19-nél (8,7%) alakult ki MPR 6 éves átlagos követésnél. A két csoport (MPR igen vagy nem) mutagénérzékenysége nem különbözött egymástól szignifikánsan. A dohányzás szignifikánsan növelte az MPR kialakulásának kockázatát. Az MPR kialakulásáig eltelt idő hossza szerint is csoportosították a betegeket: MPR ≥3 év vagy MPR <3 év. Az első csoportban (n=10) a kromatidtörések átlagos gyakorisága 0,97 és a második csoportban (n=9) 0,69 volt (p=0,019). Tehát a két csoport mutagénérzékenységi profilja szignifikánsan különbözött.

Betegeinknél nem tudtuk megerősíteni az M. D. Anderson Cancer Center eredményeit. Az MPR-ben szenvedő betegeink kromoszómaérzékenységi profilja nem különbözött szignifikánsan a többiekétől (p=0,4062). A fokozottan vagy a nem fokozottan érzékeny csoportban az MPR aránya sem volt szignifikánsan különböző (p=0,4272). Betegeink klinikai jellemzői különböztek az idézett szerzők betegeinek jellemzői-

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től. Nálunk a betegek 82%-a előrehaladott, III-IV. stádiumban volt, náluk korai, I-II. stádiumba tartoztak a vizsgált betegek. Továbbá, betegeink valamennyien dohányoztak és idült alkoholisták voltak a daganatos betegségük felismerésekor. Az M. D. Anderson Cancer Center vizsgálatában vagy a holland beteganyagon is (20, 21) csak jelentős változtatásokkal sikerült a korábbi eredményeket validálni: a hiperérzékenységi szintet lecsökkentették (≥0,5 átlagos t/s), az MPR-t és a recidívákat együtt számolták, csak az MPR-t későn (≥36 hónap) kifejlesztő betegek kromatidtörés-átlaga volt viszonylag magas.

Az irodalmi áttekintésből az is megállapítható, hogy az M. D. Anderson Cancer Center eredményeit más központok nem is próbálták érvényesíteni saját beteganyagon, noha számos közlemény foglalkozik az MPR gyakoriságával és kialakulásának okaival fej-nyaki rákos betegeknél (9, 17, 18, 22–24). A New York-i Cornell University tanulmányában a dohányzás és alkoholfogyasztás növelte az MPR kialakulásának kockázatát. A dohányzás abbahagyása után 5 évvel csökkent a kockázat (9). A Tokyo Medical and Dental University betegeinél az MPR leggyakoribb volt szájüregi és algarat index rákkal. Az MPR kialakulása növelte a daganatos elhalálozás kockázatát (23). Valamennyi betegünk dohányzott és fogyasztott alkoholt. A leszokás esetleges volt. Továbbá, az előrehaladott stádium miatt is (korai elhalálozás) a dohányzás felfüggesztésének a hatását a kockázatcsökkenésre nem vizsgáltuk. Az MPR megjelenése betegeinknél is jelentősen rontotta a túlélést. Tizennyolc beteg közül már csak egy van életben, az elhalálozás nyers aránya 94%. MPR megjelenése után az 5 éves túlélés becsült aránya csak 17% volt.

Morris és munkatársai (24) az amerikai SEER program által nyilvántartott 75 087 laphámsejtes fej-nyaki rákos betegnél vizsgálták az MPR gyakoriságát az index rák anatómiai helye szerint. A gyakoriságban helyspecifikusságot illetően változást hozott a HPV-asszociált rákok szaporodása és elkülönítése. Az 1990-es évek előtt a szájgaratrákoknál volt a leggyakoribb az MPR. Most a régió jellemzője a legalacsonyabb kockázati szint, mert a HPV-asszociált daganatban szenvedő betegeknél ritka az MPR kialakulása (25). Ugyanakkor a szájgaratrákok döntő többségét vírus okozza az utóbbi évtizedekben. Keletkezésükben kisebb a szerepe a dohányzásnak és alkoholizmusnak (2). Gan és munkatársai (26) tanulmányában szignifikánsan ritkább volt az MPR kialakulása szájgaratrákos betegeknél, mint a szájüregi, egyéb garat- és gégerákban. Viszont a klasszikus fenotípusok (HPV-eredetű szájgaratrákot nem számolva) vonatkozásában a különbség nem volt szignifikáns. Anyagunkban a gégerákos betegeinknél volt a leggyakoribb az MPR nyers aránya, 35%. A szájüregi rákban szenvedő betegeinknél a nyers arány 13% volt, pedig a t/s hányados náluk volt a legnagyobb, 1,18. Suton és munkatársai (18) jóval nagyobb számú, 183 szájüregi esetet tanulmányoztak. Az MPR gyakorisága szintén 13% volt. Az MPR gyakorisága szériák szerint változik, a követési időtől és a daganatstádiumtól függően. Hosszabb követési idővel és korai stádiummal magasabb az arány. Az eredmények összehasonlításához alkalmasabb a becsült arány (Kaplan–Meier-módszerrel becsült gyakoriság), mint a nyers arány. Cooper és munkatársai a Radiation Therapy Oncology Group (RTOG) regiszterében 928 laphámsejtes fej-nyaki rákos beteget találtak, akiket 1977 és 1980 között sugárterápiával kezeltek. Összesen 110 MPR alakult ki. Így a nyers arány a követés egész idejére 12% volt. A 8 éves becsült arány viszont közel a duplája, 23% volt (betegeinknél a 10 éves becsült arány volt 23%). A primer tumor anatómiai helye nem volt lényeges hatással a gyakoriságra, de a daganatstádium igen. A korai stádium növelte az MPR kialakulásának kockázatát a jobb túlélés miatt (27). Betegeinknél IV. stádiumban egyetlen MPR sem alakult ki. A korai elhalálozás miatt az átlagos követési idő csak 17 hónap volt. Ugyanakkor az MPR-ek 39%-a 10 éves követés után alakult ki.

Tanulmányunkban nem tudtuk megerősíteni, hogy a fokozott mutagénérzékenység emeli az MPR kialakulásának kockázatát fiatal laphámsejtes fej-nyaki rákos betegeknél. A korai stádiumban gyakoribb az MPR a jobb túlélés miatt. A túlélő betegeknél az MPR kialakulása általában halálhoz vezet. Megjelenése tízéves követés után is gyakori. A szenvedélybetegségek (dohányzás és alkoholizmus) eredményes kezelése és hatásos szűrési stratégia eredményezheti a túlélés javulását.

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ORIGINAL ARTICLE

Mutagen sensitivity and risk of second cancer in younger adults with head and neck squamous cell cancer: 15-year results

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Abstract

Purpose To evaluate the mutagen sensitivity phenotype on the risk of second primary cancer (SPC) in patients with head and neck squamous cell carcinoma (HNSCC), and to estimate the long-term rate of SPC and the outcome with SPC.

Methods A survey was made regarding SPC among 124 younger (≤ 50 years) adults with HNSCC who were enrolled in a pretreatment mutagen sensitivity investigation during 1996–2006. Mutagen sensitivity was assessed by exposing lymphocytes to bleomycin in vitro and quantifying the bleomycin-induced chromatid breaks per cell (b/c). Patients were classified as hypersensitive (> 1 b/c) or not hypersensitive (≤ 1 b/c).

Results Mean follow-up time for all patients was 68 months (range: 5–288 months), and the 15-year cancer-specific survival was 15%. Twenty patients (16%) developed a SPC (15-year estimated rate: 41%), and half of them was hypersensitive. The crude rate of SPC for hypersensitive ($n = 65$) or not hypersensitive ($n = 59$) patients were 15 and 17%, respectively ($p = 0.4272$). The 15-year estimated rate of SPC for hypersensitive and not hypersensitive patients was 36 and 48%, respectively ($p = 0.3743$). Gender, UICC stages, anatomical sites of index cancer did not prove to be a significant risk factor for SPC. Forty-five percent of SPC developed after the 10-year follow-up. The 3-year cancer-specific survival was 23% with SPC.

Conclusion According to our findings, mutagen hypersensitivity was not associated with an increased SPC risk in HNSCC patients. Patients are at a lifelong risk of developing a SPC. Survival with SPC is very poor.

Keywords Head and neck squamous cell cancer · Risk of second primary cancer · Survival with second primary cancer · Mutagen sensitivity · Bleomycin test

Introduction

Smoking and excessive alcohol consumption are the main causal factors associated with HNSCC (head and neck squamous cell carcinoma). However, only a small proportion of smokers and drinkers (about 10%) develop HNSCC, suggesting that variations in genetic susceptibility may play an important role in the etiology of cancer [1, 2]. In 1983, the bleomycin assay was suggested by Hsu [3] as a biological marker for the development of environmentally induced cancer. The method is based on the scoring of bleomycin (BLM)-induced chromatid breaks occurring in cultured lymphocytes in vitro in the late G2 phase of the cell cycle. Spitz et al. [4] used this method to investigate the association between mutagen-induced chromosome damage and cancer risk and the interaction of carcinogenic exposures and chromosome damage in healthy and untreated patients with upper aerodigestive tract cancer. The cancer patients showed increased chromosome sensitivity (65.2% of cancer patients had > 0.8 b/c [breaks/cell]) compared to only 23.6% of the control patients had it, and chromosome sensitivity remained a strong and significant risk factor for head and neck cancer after adjustment for potential confounding from age, sex, cigarette smoking, and alcohol con-

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sumption. They concluded that the thesis that chromosome instability and/or defective DNA repair may underlie susceptibility to environmental carcinogenesis is plausible and may present a promising avenue for further research. Individuals with genetic instability may generate more cells with mutations or chromosomal aberrations than those with more stable genomes [5].

Young patients with HNSCC can be treated successfully with surgery and radiotherapy (RT), but they often develop second primary cancer (SPC). The leading cause of morbidity and mortality in these patients is the development of SPC [6]. Impact of smoking status, alcohol consumption, index tumor site, and disease stage on SPC development in patients with cancer of the oral cavity, pharynx, and larynx have been reported previously [7–9]. However, these factors do not account for the development of all SPCs. It is likely that genetic susceptibility also contributes to the development of SPC [10, 11]. Several studies have indicated that mutagen sensitivity may be a valuable biomarker of susceptibility to the development of multiple primary tumors [12–14].

The aim of this study was to determine the predictive value of mutagen sensitivity for the development of SPC in a group of HNSCC patients who were tested for mutagen sensitivity and treated between 1996 and 2006 at the National Institute of Budapest, Hungary [15, 16]. Another aim was to estimate the rate of SPC and the outcome with SPC.

Materials and methods

Between 1996 and 2006, 432 patients with HNSCC underwent mutagen sensitivity assay before treatment. Informed consent was obtained from each study subject before enrollment and study protocol was approved by the institutional review board. The aim of that test was to clarify the usefulness of the bleomycin sensitivity assay elaborated in the USA as a biomarker of HNSCC, to explain the association between HNSCC susceptibility and exposure to carcinogens. The results were published elsewhere [15, 16]. From these patients, 124 met the following conditions: drinker and smoker, age ≤ 50 years at the time of bleomycin test, human papillomavirus (HPV)-negative oral cavity, pharyngeal (except nasopharyngeal) or laryngeal squamous cell cancer, treatment and follow-up were performed at our institute. We reviewed records of patients and compared pretreatment mutagen sensitivity between patients with or without SPC. SPC was partly defined according to the criteria of second primary tumor prevention trial of the M.D. Anderson Cancer Center [11]: the SPC must be diagnosed as malignant, has to be at least 2 cm from site of the index tumor, and has to occur ≥ 4 months after the diagnoses of the index tumor. Mutagen sensitivity was measured in vitro in lymphocytes by counting chromatid breaks induced by bleomycin as described previously [15, 16]. Briefly, blood cultures were incubated for 3 days and then exposed to bleomycin (30 µg/ml) for 5 h. Cells were harvested, and chromatid breaks were scored in 100 metaphases per sample, and recorded as the mean number of breaks per cell (b/c). The patient was classified hypersensitive if the mean number of b/c was > 1. The following survival endpoints were used: any death for overall survival, death from head and neck cancer for cancer-specific survival, death from SPC for survival with SPC, the appearance of SPC for SPC-free survival (time to SPC). Intervals to endpoints were examined with Kaplan–Meier method [17], and the curves were compared with log-rank test. The effect of the possible prognostic factors on the probability of the incidence of a SPC were examined in Cox regression model [18]. Statistical differences in proportions and means were assessed by 2-sample t-test and by Fisher exact test. GraphPad Prism (version 5.01 for Windows, GraphPad Software, San Diego, CA, USA) and Statistica (version 13.5.0.17, TIBCO Software Inc., Palo Alto, CA, USA) program packages were used for data analysis. A p value ≤ 0.05 was considered statistically significant.

Results

Cytogenetic and clinical characteristics of the patients are given in Table 1. Of the four anatomical sites, tumors of oral cavity (34%) represented the largest group. The rate of patients with stage IV disease was high (43%), and few patients ($n = 12$) were treated with chemotherapy alone. Most patients were subjected to surgery and adjuvant RT. Therapy is detailed as follows: 86 patients were subjected to surgery. The number of R0 or R1 resection was 61 and 25, respectively. Of the 86 patients, 84 underwent lymphadenectomy: 20 were node negative and 64 were node positive. Extracapsular tumor extension (ECE) was seen in 14 node-positive patients. Response rate (complete or partial response) for radiochemotherapy alone, definitive RT or chemotherapy alone, was 9/10, 6/7 and 7/12.

Mean follow-up time for all patients and alive patients was 68 months (range: 5–288 months) and 222 months (range: 184–249 months), respectively. Nine patients are still alive, and 115 have died. Crude overall survival rate is 7%. Ten patients died of internal disease. The crude rate of cancer-specific survival is 15%. The estimated rate of 15-year overall or cancer-specific survival is 14.5 and 19%, respectively.

Out of 124 patients, 20 (16.1%) developed SPC. The characteristics of the 20 patients are given in Table 2. The majority ($n = 13$; 65%) of SPC were HNSCC. Seven of them developed outside of head and neck region (esopha-

Table 1 Clinical and cytogenetic characteristics

Characteristics	Patients, *n* (%)
Mean age	
45.8 years (range: 23–50 years)	124 (100)
Mutagen sensitivity	
Hypersensitive (>1 b/c)	65 (52)
No hypersensitive (≤1 b/c)	59 (48)
Anatomical subsite	
Oral cavity	48 (38)
Oropharynx	27 (22)
Hypopharynx	32 (26)
Larynx	17 (14)
Gender	
Male	107 (86)
Female	17 (14)
UICC stage[a]	
I	3 (2)
II	20 (16)
III	48 (39)
IV	53 (43)
Treatment[b]	
Surgery alone	9 (7)
Radiotherapy alone	7 (6)
Surgery + adjuvant radiotherapy	71 (57)
Surgery + radiochemotherapy	6 (5)
Radiochemotherapy	10 (8)
Chemotherapy alone	12 (10)
Palliative therapy	9 (7)

b/c chromatid breaks/cell
[a]UICC Union Internationale contre de cancer, TNM Classification of Malignant Tumours—7th edition
[b]Treatments are detailed in the text

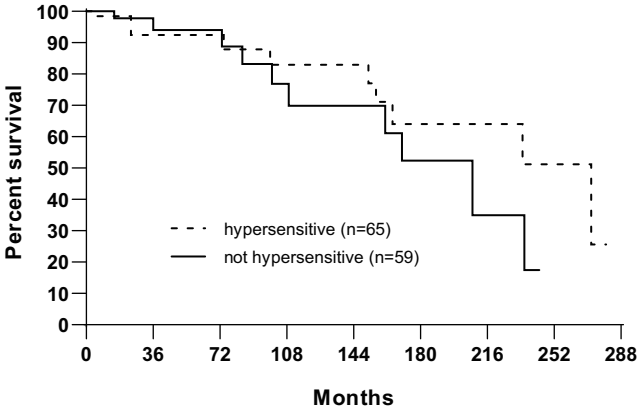

Fig. 1 Second cancer-free survival by mutagen sensitivity. The 15-year estimated rate of second primary cancer of not hypersensitive or hypersensitive patients was 48 and 36%, respectively ($p=0.3743$)

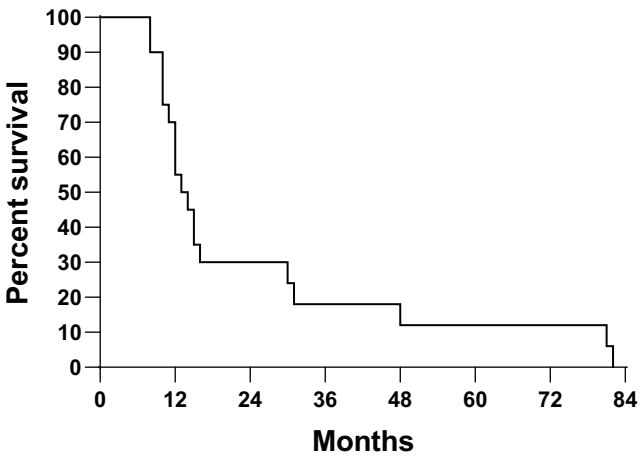

Fig. 2 Cancer-specific survival with second primary cancer ($n=20$). The estimated rate of 2- or 3-year survival was 38 and 23%, respectively

gus, lung, prostate). The mean time to SPC was 118 months (range: 4–272 months). The 10-, 15-, or 20-year estimated rate of SPC was 24, 41 and 65%, respectively. In 3 patients, the index cancer and the SPC occurred in the same subregion (oral cavity, contralateral edge of the tongue). In these 3 cases the time to SPC was 161, 99 and 152 months (more than 5 years), and in every case the distance between the index cancer and SPC was greater than 2 cm. None of the patients with SPC had persistent disease or *percontinuitatem* invasion associated with SPC. The therapy of index cancer for patients with SPC was as follows: surgery alone ($n=2$, R0 resection), surgery and adjuvant RT ($n=17$, R0 or R1 resection 16 and 1), surgery and adjuvant radiochemotherapy ($n=1$, with R1 resection and extracapsular tumor extension).

The number of hypersensitive (>1 b/c) patients were 65 (mean b/c: 1.43 ± 0.39). Ten of them (15%) developed SPC. In the not hypersensitive group ($n=59$, mean b/c: 0.74 ± 0.18), 10 patients (17%) also developed SPC ($p=0.4272$). The mean value of b/c for patients with SPC and without SPC was 1.02 ± 0.37 (range: 0.52–1.6) and 1.12 ± 0.48 (range: 0.35–2.8; $p=0.4062$). The mean value of b/c was separately evaluated for patients with ≥36 months to SPC development. The mean b/c value of patient with "late" (≥36 months) SPC ($n=16$) or patients without SPC ($n=104$) was 1.02 (range: 0.52–1.60) and 1.12 (range: 0.35–2.80), respectively ($p=0.5724$). The 15-year estimated rate of overall survival for hypersensitive or not hypersensitive patients was 16.9 and 11.0%, respectively ($p=0.4164$). The second cancer-free survival curves by mutagen sensitivity are shown in Fig. 1. The 15-year estimated rate of second primary cancer for not hypersensitive and hypersensitive patients was 48 and 36%, respectively ($p=0.3743$). The median and mean survival time with SPC was 23 months (range: 8–82 months) and 15 months. The 2- and 3-year cancer-specific survival with SPC was 38 and 23%, respectively (Fig. 2). The 45% of SPC was developed after 10 years (between 152 and 272 months). The crude

Table 2 Characteristics of the 20 primary cancer patients

	Gender	Index cancer	b/c	UICC stage	Site of SPC	UICC stage	Time to SPC (months)	Histology	Survival with SPC (months)
1	Male	Oral cavity	0.83	II	Oropharynx	II	109	SCC	31
2	Male	Oral cavity	0.97	I	Oral cavity	III	161	SCC	15
3	Male	Oral cavity	1.58	II	Esophagus	III	165	SCC	15
4	Male	Oral cavity	1.27	II	Oral cavity	III	99	SCC	82
5	Male	Hypopharynx	1.02	III	Oral cavity	III	24	SCC	30
6	Male	Hypopharynx	0.59	III	Lung	III	36	SCC	14
7	Male	Hypopharynx	1.51	III	Esophagus	III	24	SCC	10
8	Male	Larynx	0.55	II	Oropharynx	IV	208	SCC	10
9	Male	Larynx	0.64	II	Oropharynx	III	84	SCC	12
10	Male	Larynx	0.52	III	Oropharynx	IV	170	SCC	13
11	Male	Larynx	0.87	III	Oral cavity	IV	236	SCC	8
12	Male	Oral cavity	0.88	II	Lung	III	100	SCC	11
13	Male	Oral cavity	0.72	III	Esophagus	III	73	SCC	8
14	Male	Larynx	0.78	III	Lung	III	15	SCLC	12
15	Male	Oral cavity	1.52	II	Oral cavity	III	152	SCC	48
16	Male	Oropharynx	1.14	III	Oral cavity	III	4	SCC	81
17	Female	Larynx	1.34	III	Oropharynx	III	156	SCC	16
18	Female	Oral cavity	1.60	II	Larynx	III	74	SCC	12
19	Female	Hypopharynx	1.05	III	Oropharynx	II	235	SCC	10
20	Male	Oropharynx	1.45	II	Prostate	III	272	AC	16[a]
Mean	–	–	1.02	–	–	–	118	–	22

b/c chromatid breaks/cell, *UICC* Union Internationale contre de cancer, *TNM* Classification of Malignant Tumours 7th edition, *SPC* Second primary cancer, *SCC* squamous cell cancer, *SCLC* small cell lung cancer, *AC* adenocarcinoma
[a]19 patients died of cancer and 1 died of coronary disease

Table 3 Second primary cancer rate by index cancer site

Index cancer	Patients, *n* (%)	b/c, mean ± SD	Mean FUP time[a], months (range)	Crude rate, % (*n*)
Oral cavity	48 (38)	1.18 ± 0.51	70 (5–240)	13 (6)
Oropharynx	27 (22)	1.10 ± 0.42	75 (6–288)	15 (4)
Hypopharynx	32 (26)	1.10 ± 0.50	49 (6–244)	13 (4)
Larynx	17 (14)	0.90 ± 0.28	84 (7–244)	35 (6)
All	124 (100)	1.10 ± 0.47	68 (5–288)	16 (20)

b/c chromatid-break/cell, *SD* standard deviation
[a]*FUP time* follow-up time; Oral cavity + Oropharynx vs. Hypopharynx + Larynx $p = 0.6253$

Table 4 Second primary cancer rate by UICC stage

Stage	Patients, *n* (%)	b/c, mean ± SD	Mean FUP time[a], months (range)	Crude rate, % (*n*)
I	3 (2)	1.09 ± 0.42	216(176–240)	33 (1)
II	20 (16)	1.20 ± 0.40	145 (21–288)	45 (9)
III	48 (39)	1.01 ± 0.48	82 (11–249)	21 (10)
IV	53 (43)	1.15 ± 0.47	17 (5–77)	0 (0)
All	124 (100)	1.10 ± 0.47	68 (5–288)	16 (20)

b/c chromatid breaks/cell, *SD* standard deviation, *Stage* TNM Classification of Malignant Tumours 7th edition
[a]*FUP time* follow-up time; stage I–II vs. stage III–IV $p = 0.0009$

Table 5 The 15-year estimated rate of second primary cancer by variables (univariate analysis)

Variables	%	*p*-value	RR (95% CI)
Gender	–	0.5071	–
Male	46	–	1.00
Female	24	–	0.6698 (0.2277–1.970)
Site of index cancer	–	0.1049	–
Oral cavity + oropharynx	39.5	–	1.00
Hypopharynx + larynx	44.1	–	2.009 (0.7924–5.084)
UICC stage	–	0.9615	–
Early (I + II)	46.4	–	1.00
Locally advanced (III–IV)	31.9	–	0.9797 (0.4078–2.354)
Mutagen sensitivity	–	0.3072	–
Not hypersensitive	47.6	–	1.00
Hypersensitive	36.1	–	0.6463 (0.2634–1.586)
Radiotherapy	–	0.8767	–
No	28	–	1.00
Yes	43	–	1.120 (0.2427–5.172)

RR relative risk, *CI* confidence interval, *UICC stage* TNM Classification of Malignant Tumours 7th edition

rate of SPC for men and women was 16% (17/107) and 18% (3/17), respectively ($p = 0.9999$), and for irradiated and nonirradiated patients 18% (18/94) and 7% (2/30), respectively (0.1540). It should be noted that 80% ($n = 24$) of the non-irradiated patients had stage IV disease. The rate of SPC by anatomical site of index cancer and UICC stage is given in Tables 3 and 4. The crude rate of SPC was significantly higher among patients with limited disease. However, the majority (62%) of our patients had stage III–IV disease and disease stage had a significant impact on cancer-specific survival. The 15-year cancer-specific survival with stage I, II, III or IV disease was 67, 52, 22 and 0%, respectively (multigroup $p < 0.0001$). All of the patients with stage IV disease died within 77 months. The short survival time might be one of the reasons that none of these patients developed SPC. The effect of the individual patient characteristics (gender, index cancer site, UICC stage, mutagen sensitivity, RT) on the risk of SPC was also examined in Cox proportional hazards model. Results are presented in Table 5. None of the studied variables proved to be a significant predictor of the risk of SPC.

Discussion

Several studies reported that bleomycin-induced mutagen sensitivity, reflecting latent genetic instability, is a significant predictor of SPC. HNSCC patients with SPC have higher b/c scores than SPC-free individuals [12–14, 19–21]. Schantz et al. [12] from the M. D. Anderson Cancer Center (Houston) used the bleomycin test first to estimate the risk of SPC in patients with HNSCC. The rate of SPC was significantly higher in the hypersensitive (b/c > 1) than in the not hypersensitive group (33% vs. 8%), and hypersensitivity increased the risk by 4.4 times. A weakness of their study was the short follow-up time: median < 2 years (range, 4–31 months). They extended the study to include 278 patients, and the results were published in 1994. Mutagen hypersensitivity (b/c > 1) also increased the risk of developing SPC. The mean b/c value for patients with SPC was 1.17 (±0.54) compared with 0.98 (±0.44) for SPC-free patients ($p = 0.044$). The follow-up time was again very short. The mean time from the diagnosis to the development of the SPC was only 10.5 months [13]. The mean time to SPC for our patients was more than 10 times longer (118 months). Cloos et al. [19] examined the mutagen sensitivity as a biomarker of SPC in Dutch HNSCC patients ($n = 218$). Nineteen of them (8.7%) developed SPC. The follow-up time was relatively short (median: 4.5 years). In this prospective study there was no difference between the groups (with or without SPC) with respect to mutagen sensitivity. They also found that patients who developed SPC ≥ 3 years after the index cancer had a significantly higher mean b/c value compared to patients with early SPC. In our study, the mean b/c value of patient with "late" (≥ 36 months) SPC ($n = 16$) or without SPC ($n = 104$) was not significantly different. Minard et al., from the M. D. Anderson Cancer Center studied the risk of SPC in patients with early stage HNSCC, who were enrolled in a placebo-controlled chemoprevention trial of low-dose 13-*cis*-retinoic acid to reduce the occurrence of SPC. Of the 1080 participants, a sample of 303 Caucasian patients was potentially available for their analysis. Overall, 50 of 303 patients (16.5%) developed SPC. Data on average follow-up time were not provided. The bleomycin-induced chromatid breaks were not associated with an increased risk. The lack of association between mutagen hypersensitivity and SPC was partly attributed to early UICC stage (I–II)

of the enrolled patients [20]. A half year later, they published their experience with 991 patients. The mean follow-up time for living patients was 7 years, and the b/c cutoff value for mutagen sensitivity was 0.50. Among patients with SPC, the hypersensitive ($b/c \geq 0.5$) and not hypersensitive ($b/c < 0.5$) rate was 82% ($n = 243$) and 18% ($n = 55$), respectively, $p = 0.036$ [21].

Our patients with HNSCC before treatment underwent bleomycin sensitivity assay and the method was not suitable for the assessment of individual index cancer risk due to overlapping of b/c values with those of controls [15, 16]. Here, we present our experience with the association between SPC and mutagen sensitivity in younger adults with HNSCC with the longest published follow-up time: mean time for all patients and alive patients was 5.8 and 18.5 years, respectively. The long follow-up time has to be emphasized, as patients are at high risk of developing SPC even 10 years after initial treatment. In our series, 45% of SPC developed after 10 years. In the current analysis, mutagen hypersensitivity was not significantly associated with an increased risk of developing SPC. The 15-year estimated rate of SPC for hypersensitive ($b/c > 1.0$) and not hypersensitive ($b/c \leq 1.0$) patients was 36 and 48%, respectively. Cloos et al. [14] found significantly higher break/cell values in patients with multiple cancer (mean, 1.2), than in patients with a single cancer (mean, 0.96; $p = 0.025$). Our comparable values were 1.02 and 1.10 ($p = 0.4062$), respectively. The investigators from the M. D. Anderson Cancer Center changed the experimental parameters several times. First, the cutoff value for b/c was 1.0, and later it was decreased to 0.5. Furthermore, in their large-scale study, the SPC was counted together with local relapse [21]. In our series, the number of patients with $b/c \leq 0.5$ was only nine and local relapse was not included in the analysis. Metastasis or local relapse was not considered as SPC.

Several studies investigated the risk of SPC and its impact on survival independently of mutagen sensitivity, and published long-term (more then 10-year) results [22–24]. In a large multicentric study (99,257 patients), the 20-year cumulative rate of SPC was 36%, and smoking and alcohol drinking increased the risk of developing SPC. Increased risks of SPCs persisted 10 years after diagnosis of the first primary [22]. Our patients also had a lifelong risk of developing SPC. All of our patients were drinkers and smokers, and the majority of them did not stop smoking and drinking. Otolaryngologists from Oslo studied 2063 head and neck patients [23]. The crude rate of SPC was 17%. The mean time to SPC was more than 4 years, and the median survival time with SPC was 12 months. SPC was most common in patients with limited (stage I/II) disease. Patients with a poor prognosis did not live long enough to develop SPC. In our series, none of the patients with stage IV disease developed SPC, and the crude rate of SPC was 16%, close to their rate. The survival with SPC was also very poor. The median and mean survival time was 23 and 15 months, respectively. In the study of Tiwana et al. from British Columbia, Canada [24], the follow-up time was 25 years (median time for alive patients: 23.2 years), and the crude incidence of SPC was 27%. Oral cavity and oropharyngeal index cancer were more likely to develop SPC. The estimated 5-year overall survival with SPC was 15%. According to our analysis the anatomical subsite had no significant impact on the risk of SPC. In a German observation study [25], 118 patients (close to our sample) with HNSCC were selected in a SPC survey. The crude rate of SPC was 18%. Interestingly, 52% of SPC were diagnosed within 2 years. At our patients, only 2 SPCs were diagnosed within 2 years (4 and 15 months). Bugter et al. [26] from the Erasmus Cancer Institute, Rotterdam studied the risk factor of SPC in a Cox model. The crude rate of SPC was 15.6%. Smoking and alcohol consumption, comorbidity, and the oral cavity subsite were risk factors for SPCs. All of our patients were drinkers and smokers; therefore, we did not investigate these potential risk factors. In a current, population-based study from the United States, the smoking-related cancers were studied. Among 10 smoking-related cancer sites head and neck cancer patients had the highest risk of developing a SPC [27]. We studied the following risk factors of SPC in a Cox model: gender, UICC stage, site of index cancer, RT, and mutagen sensitivity (all of our patients were smoker and drinker). None of them proved to be a significant predictor of SPC development. Arie et al. [28] from Israel assessed the incidence of SPCs in patients with head and neck malignancies, according to treatment modality. Neither RT nor chemotherapy was associated with SPC development. Patients with an advanced-stage cancer had less time to develop SPC compared to early stage patients. Lifelong follow-up has to be emphasized because of permanent risk of SPC. Boakye et al. [29] examined the incidence and sites of SPCs stratified by a first HPV-associated HNSCC compared with non-HPV-associated HNSCC. Incidence of SPCs was higher among those with non-HPV-associated HNSCC than from potentially HPV-associated HNSCC. Among 109,512 patients with first HNSCC, 13,517 (12.3%) developed SPC (9.6% for patients diagnosed with a first potentially HPV-associated HNSCC and 14.0% for patients with a first non-HPV-associated HNSCC). All of our patients had HPV-negative cancer.

Chemoprevention of SPC in HNSCC patients are discussed in several publications [4, 5, 12, 19, 21]. In a large multicentric trial from the United States, the conclusion was that further prevention trials are needed to find an appropriate compound. In this trial, the low-dose 13-isoretinoic acid for 2 years did not decrease the incidence of SPC. Mutagen sensitivity as a biomarker was not involved in this study to define patients with high risk of SPC development

[30]. Earlier, the same compound was used in the MD Anderson chemoprevention trial [20, 21]. Bhatia et al. stated: "Our findings did not confirm findings from the pivotal MD Anderson trial that used high-dose, short-term isotretinoin in patients with stage I–IV HNSCC. There were no statistically significant benefits in either OS or SPT" [30]. According to the published data, in clinical practice we have no appropriate biomarker to define risk of SPC development or compound to prevent its development in patients with HNSCC. Both former and current publications [31, 32] suggest long-term follow-up and early detection to improve outcome of patients with SPC. Patients (distinctively smokers and drinkers) have lifelong risk of SPC development.

Conclusion

HNSCC survivors had an increased lifelong risk of developing SPC. The risk of developing SPC was higher in patients with less advanced cancer. Its incidence rate is high even after the 10-year follow-up. Survival is poor with SPC. Our results show that mutagen hypersensitivity does not increase the risk of SPC development. Therefore, mutagen sensitivity cannot be used as a biomarker to predict which patients will develop SPC.

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Declarations

Conflict of interest B. Bukovszky, J. Fodor, G. Székely, S.Z. Kocsis, F. Oberna, T. Major, Z. Takácsi-Nagy, C. Polgár and Z. Jurányi declare that they have no competing interests.

Ethical standards For this article no studies with human participants or animals were performed by any of the authors. All studies mentioned were in accordance with the ethical standards indicated in each case.

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Rákos és rákmegelőző állapotok története: szájüregi leukoplakia definíciók és klasszifikációk változása

History of cancer and precancerous disorders: changing in definition and classification of oral leukoplakia

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Abstract

The description of cancers and precancerous disorders goes back to Hippocrates. Each historic age had its own specialities, thus polyhistorians, medical doctors, anatomists and pathologists described diseases and the treatment options according to their own body of knowledge. For a long time, researchers were interested in the development of cancer and its related changes in the human body. Later on, diagnostic and therapeutic solutions came to the fore. Actually, cancer research is a dynamically evolving discipline, development of which is essential, since the conditions are typically incurable, fatal diseases. The importance of precancerous disorders was also highlighted through historic ages. Early diagnosis of these lesions is critical for avoiding potentially developing malignancies. Oral leukoplakia is one of the most common precancerous disorders. In oral precancerous disorders, there are several changes in definitions and terms in the national and international literature as well. The main diagnostic and therapeutic criteria have also changed over the years thanks to the latest discoveries. It is advisable to follow the current literature about accepted definitions and protocols, however, it is useful to study the differences among grouping and individual classifications since these are just calling our attention to diagnostic

Kulcsszavak: leukoplakia, dysplasia, rákmegelőző, potenciálisan malignus

Key words: leukoplakia, dysplasia, precancerous, potencially malignant

Bevezetés

A rák, mint betegség elnevezése és leírása az ókori görög korig, Hippokratészig vezethető vissza. **Hippokratész** (Kr.e. 460 - Kr.e. 377) a jóindulatú elváltozásokat onkosznak nevezte, ami duzzanatot jelent görögül. A rák görög elnevezése karcinosz, ezzel a névvel illette a rosszindulatú elváltozásokat. Az elnevezés feltehetőleg a rosszindulatú daganat rákhoz való (nyúlványok) hasonlóságából ered. Az elnevezésben fontos szerepe volt **Aulus Cornelius Celsus** (Kr.e. 25 - Kr.u. 50), római orvosnak, aki a latin fordítást, „cancer" használta. Az onkológia kifejezés **Claudius Galénus** (129-216) nevéhez köthető, aki általánosságban az onkosz szóval jellemezte a tumoros elváltozásokat [1,2].

A rákos megbetegedésekkel kapcsolatos történelem egyik kiemelkedő alakja tehát – mint a legtöbb orvostudományi jelenségé – Hippokratész. Hippokratész nem csak a saját megfigyeléseivel és gyakorlati tevékenységével foglalkozott a rák vonatkozásában, hanem az ókori egyiptomi feljegyzéseket is alaposan

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figyelembe vette. Munkássága során leírta, hogy a rák egy különálló entitás a betegségek között. Legfontosabb jellemzőinek a gazdag vérellátottságot, kapilláris rendszert és infiltrációs képességet tekintette. Ezek a mai napig helytálló felismerések voltak. Mint sok mindent, a rák patogenezisét is a négy testfolyadék (a vér, a nyálka-nyirok, a sárga epe és a fekete epe) teóriája alapján vizsgálta. Az egészséges állapot fenntartásához a négy folyadék egyensúlyára van szükség. Ha valamelyik csökken vagy növekszik, az az egyensúly felborulásához, és így betegségek kialakulásához vezet. A rákos állapotokban Hippokratész szerint a fekete epe mennyisége növekszik, és ez vezet a súlyos megbetegedéshez. **George Stahl** (1659-1734) és **Frederick Hoffman** (1660-1742) elmélete szerint a nyirokváladékkal hozható összefüggésbe a rák, amivel később **John Hunter** (1728-1793) is egyetértett sebészi tevékenysége során szerzett tapasztalatai alapján (erről ma már tudjuk, hogy például a nyirokcsomó áttétek magyarázhatták is a tapasztalataikat). Ezek az elvek ma már csak teoretikusok, de sok feljegyzés és gondolat bír valóságalappal, amelyek megalapozták a rákos megbetegedésekkel kapcsolatos tudásunkat. Hippokratész foglalkozott a rákos betegek gyógyításával is. A súlyos, előrehaladott rákkal bíró betegeket palliatív kezelésben részesítette, amely a mai modern elvek szerint is hasonlóan zajlik. Hangsúlyozta a korai felismerés, és a minél korábbi terápiás beavatkozás fontosságát. Orvosetikai elveit a rákos betegek gyógyításával kapcsolatosan is leírta, amelyek a mai napig érvényesek, és az orvosi eskünk kötelez betartásukra [1,2].

A reneszánsz idején a tudósok a világ, a természet megismerését tartották fontosnak. Az emberi test megismerése is fontos lett. **Galileo Galilei** (1564-1642) és **Isaac Newton** (1642-1727) tanulmányait felhasználva elkezdhették alaposabban tanulmányozni a betegségeket, és **William Harvey** (1578-1657) elvégezhette az első boncolásokat. Korábban a boncolások vallási okból történő tiltása sokáig hátráltatta az orvostudomány fejlődését. 1761-ben a padovai **Giovanni Battista Morgagni** (1682-1771) volt az első, aki boncolási tevékenysége során komoly összefüggéseket írt le a halált okozó betegségek és a valós patológiás elváltozások között. Ezzel megalapozta a tudományos onkológia és a rák tanulmányozásának alapjait. A már említett John Hunter, skót sebész, javaslatokat tett, hogy mely esetekben érdemes a daganatokat műtéti úton eltávolítani. Egy évszázaddal később, az érzéstelenítés bevezetése után, a sebészeti beavatkozások száma drasztikusan nőni kezdett. Például ekkor vezették be az emlőrák gyógyításában a radikális mastectomiát. Természetesen az onkológiában az egyik legnagyobb áttörést és fejlődési lehetőséget a modern mikroszkópok megjelenése jelentette. **Rudolf Virchow** (1821-1902), a celluláris patológia atyja, lerakta a rák modern szövettani vizsgálatának alapját. Vizsgálta, hogy a rákos sejtek miben mások, mint a normális sejtek, és ez hogyan függ ez össze a rákos megbetegedés súlyosságával. Ezeket az elveket már korábban tanítója, **Johannes Peter Müller** (1801-1858), német patológus is leírta. Virchow összefüggésbe hozta a rák kialakulását a krónikus irritációval is. A történelem során a rák keletkezésében sok tényezőt vizsgáltak: trauma, fertőzés, vegyületek, sugárzás, stb. Például a családon belül halmozódó emlőrákot fertőzésnek is tekintették. Ma már tudjuk, hogy genetikai oka van. Azonban azt is tudjuk, hogy bizonyos vírusok és baktériumok elsődleges etiológiai faktorként szerepelnek daganatoknál, és ebben az értelemben a fertőzés igen jelentős gondolat. Az 1970-es években írták le az onkológia szempontjából a két legfontosabb géncsaládot: onkogének és tumorszupresszor gének. Ezután a kutatások fő célpontjába a rák kialakulásában résztvevő etiológiai faktorok felkutatása, a diagnózis módjai és a terápiás beavatkozások kerültek. Mind a mai napig dinamikusan fejlődő tudományágról van szó, és mivel a daganatos megbetegedések sok esetben gyógyíthatatlanok, így szükség is van a folyamatos fejlődésre és a nyitott kérdések kutatására [1,2,3,4].

Definiciók

A rákmegelőző állapot olyan kóros sejtekkel jellemezhető elváltozás, amely a rák kialakulásának fokozott kockázatával jár, szemben az ép szövetekkel. Ez klinikailag számos olyan elváltozást foglal magába, amelyeknél fennáll a rák kialakulásának kockázata. Patológiai szempontból számos típust

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különböztethetünk meg, amelyek a rákmegelőző állapotokhoz tartoznak. Benignus neoplasiak, különböző fokú dysplasiák, egyes klasszifikációk az in situ carcinomát is ide sorolják, ami azonban erősen vitatható. A rákmegelőző állapotok alapos ismerete, nevezéktanuk változása és fejlődésének követése nélkülözhetetlen, hiszen potenciálisan malignus folyamatok alakulhatnak ki belőlük.

A szájüregi rákmegelőző elváltozások definíciója folytonos változáson ment keresztül. A WHO 2005-ben javasolta a premalignus és precancerosus nevek helyett a potenciálisan rosszindulatú kifejezés használatát. Továbbá a korábbi *potenciálisan rosszindulatú léziók* (potentially malignant lesions) ill. *potenciálisan rosszindulatú állapotok* (potentially malignant conditions) megnevezés helyett a *potenciálisan rosszindulatú rendellenességek* (potentially malignant disorders) kifejezés használata mellett döntöttek. A szájüregben ezek közül a leukoplakia és az erythroplakia a leggyakoribbak. Ezeknek a diagnosztizálása elsősorban az egyéb fehér / vörös elváltozások kizárásával történik. Emellett a lichen planus és a submucosus fibrosis, valamint egyéb potenciálisan rosszindulatú betegségekről számol be az irodalom. Bizonyos klasszifikációk egyébként megtartották a potenciálisan rosszindulatú állapotok és léziók elkülönítését. Előbbi csoportba (*állapotok*) olyan generalizált, általános állapothoz kapcsolódó elváltozások tartoznak, amelyekben a malignus elfajulás gyakorisága szignifikánsan magasabb, mint az egészséges egyének esetében. Ide sorolható például a lichen planus, a submucosus fibrosis, a sideropenia, a discoid lupus erythematosus, a cheilitis actinica chronica, a cornu cutaneum, az epidermolysis bullosa, a xeroderma pigmentosum és az AIDS. A másik csoport (*léziók*) olyan lokálisan kialakuló, morfológiailag átalakult szöveteket jelentenek, amelyekben a daganatos elváltozás kialakulásának valószínűsége nagyobb, mint a normál szövetekben. Klasszikus példája az orális leukoplakia, az orális erythroplákia és a proliferatív verrucosus leukoplakia [5,6].

Az egyik legjelentősebb tanulmány a leukoplakiákkal kapcsolatosan egy magyar orvosnak, **Bánóczy Jolán** (1929-2016) Professzor Asszonynak köszönhető [7]. Bánóczy Jolán 1929-ben született Budapesten. Orvosdoktori diplomáját is itt szerezte, de munkássága során számos külföldi úton vett részt. Egyetemi tanár és a Fogorvostudományi Kar dékánja is volt. Rákmegelőző elváltozások témájában 1967 és 1977 között WHO kollaborációs centrumot vezetett Koppenhágával együttműködésben. Az orvostudományok kandidátusa és az orvostudomány doktora fokozatot is leukoplakiával kapcsolatos témájával nyerte el. Mind a hazai mind a nemzetközi irodalomban számos publikációja született a témában, megalapozva ezzel a széleskörű kutatás lehetőségét. Kutatási eredményeinek komoly nemzetközi visszhangja lett. Bánóczy Jolán felhívta a figyelmet a rákos megbetegedések esetén a korai diagnózis és korai kezelés fontosságára. Úgy vélte, hogy nem csak a rákos elváltozásoknál fontos a mielőbbi kezelés megkezdése, hanem a rákmegelőző elváltozásoknál is. 1982-es tanulmányában említi, hogy az azt megelőző 10-20 évben egyre alaposabban foglalkoztak az orális leukoplakia kialakulásával, klinikai lefolyásával és terápiájával. Magyarország kiemelkedő szereppel bírt ebben. Maga a leukoplakia kifejezés is egy magyar bőrgyógyász orvostól, **Schwimmer Ernő**től (1837-1898) származik [8]. Schwimmer Ernő 1837-ben született Budapesten, és Bécsben avatták orvosdoktorrá. Egyiptomi útja után visszatért Budapestre, ahol főorvos, majd egyetemi tanár lett. Számos tanulmánya jelent meg hazai és nemzetközi folyóiratokban, elsősorban bőrgyógyászati témában. A leukoplakiák leírásával kapcsolatos két legfontosabb publikációja a „Leukoplakia buccalis” (1878) és a „Die ideopatischen schleimhautplaques der Mundhöhle” (1878). A 20. század közepén, **Balogh Károly** (1895-1973) stádiumbeosztást hozott létre a leukoplakiák osztályozására. Két csoportba sorolta az elváltozásokat: reverzibilis és irreverzibilis leukoplakiák. Tanulmányai szerint, a korai stádiumban felismert leukoplakiák spontán is meggyógyultak, ha az irritáló tényezőket megszüntették. Az orális leukoplakia incidenciájára vonatkozó első tudományos publikáció szintén Magyarországhoz köthető, **Bruszt Pál**nak (1906-1979) köszönhetően, aki 3,6%-os incidenciáról számolt be. A leukoplakiákkal kapcsolatos incidencia adatok egyébként a mai napig igen széles skálán mozognak, akár az előfordulási gyakoriság, akár a

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malignizációs hajlam vonatkozásában. Ez alátámasztja a téma fontosságát és a további vizsgálatok szükségességét [7].

A leukoplakia definíció tehát Schwimmer Ernő nevéhez köthető (1877). A szó jelentése fehér folt (görögül: leukos = fehér, plakia = folt), amely a klinikai megjelenésre utal. A leukoplakia definiálásának történetében az egyik fő nehézséget az adta, hogy egyes leírásokban mint klinikai fogalmat, más esetekben pedig mint szövettani diagnózist használták. Ennek elkerülése érdekében hozta létre a WHO 1978-ban a leukoplakia definícióját (ami mind a mai napig a leginkább elfogadott definíció): azok a fehéres, nem letörölhető elváltozások nevezhetők leukoplakiának, amelyek klinikailag és patológiailag más betegségcsoportba nem sorolhatók be. 1983-ban **Tony Axell** (1939-), svéd fogorvos, egyetemi tanár módosította a definíciót: fehéres folt vagy plakk, amely klinikailag és patológiailag más betegségcsoportba nem sorolható be, és se fizikai se kémiai irritatív tényezővel nem hozható összefüggésbe, kivéve a dohányzás. 2007-ben **Saman Warnakulasuriya** angol fogorvos, egyetemi tanár leírása szerint a leukoplakiák bizonytalan, kérdéses elváltozások, amelyek esetén minden más, fokozott rákos kockázattal járó betegség kizárandó. **Vagish Kumar Shanbhag**, indiai fogorvos, egyetemi tanár 2017-ben kiegészítette az eredeti WHO definíciót azzal, hogy az elváltozás általában dohányzással, bételdióval és alkohollal hozható összefüggésbe, de idiopathiás esetek is előfordulnak. Ezeken kívül is számos definíció látott napvilágot, de leginkább az eredeti WHO szerinti definíciót használják a tudományos irodalomban [9].

Bánóczy Jolán és **Sugár László** (1944-) 1969-es tanulmányukban 324 beteget vizsgáltak, akiknél az azt megelőző 20 év során alakult ki leukoplakia. Eredményeik alapján etiológiai faktorként szerepelt a dohányzás, alkoholfogyasztás, mechanikai irritáló tényezők és a szájban kialakuló elektromos potenciálkülönbség. Fokozott kockázatot jelentett több faktor együttes fennállása. Az enyhe tünetekkel járó esetekben általában a kiváltó ok eliminálásával az elváltozás és javult vagy megszűnt, míg a súlyosabb esetek gyakrabban alakultak át rákká, és gyakrabban volt szükség műtéti beavatkozásra [10].

A nemzetközi irodalomban is nyilvántartott egyik legrégibb tanulmány 1898-ban került közlésre [11]. **J. S. Marshall** definíciója szerint a leukoplakia egy krónikus, felszíni nyálkahártyát érintő (elsősorban nyelven, szájpadláson és fogínyen kialakuló) gyulladásos elváltozás, amely fehéres felszínnel jellemezhető. Nomentklatúra vonatkozásában ide sorolja a psoriasis linguet, herpes zostalt, leucomat, dohányos foltot, leucokeratosist és superficialis glossitist. Kétféle leukoplakiát különböztet meg. Az egyik típus a tejszerű, opálos, olykor szürkés színű, foltos leukoplakia, ami a szifilisz második stádiumára jellemző elváltozás. A másik típus a nem-szifiliszes leukoplakia, amely egy fehéres, gyöngyházfényű folt. A szerző Schwimmer-féle leukoplakiának is nevezi ez utóbbi típust, utalva a betegség magyar leírójára. A második csoportba sorolja a Hutchinson-féle leukomát is. A tanulmányban felhívják a figyelmet a korai diagnózis fontosságára, és arra, hogy a fogorvosi és fül-orr-gégészeti vizsgálatokon a kezelőorvos alapos vizsgálódása szükséges, a fennálló leukoplakiák korai diagnosztizálása érdekében. A lichen planust is említik, mint leukoplakiához hasonló elváltozást. Bőrbetegségként írják le, amelynek szájüregi tünetei is lehetnek, elsősorban fehéres, megvastagodott nyálkahártyakiemelkedésként. Ezt **Wilson**, **Hutchinson**, **Crocker** és **Kaposi Mór** (1837-1902) írták le. Az irodalomban több közlemény is olvasható a szifilisz és a leukoplakia kapcsolatáról, osztályozásáról. Egy 1923-as cikkben ún. leukoplakia buccalis esetet mutatnak be [12]. Ebben az esetben a kemény- és lágyszájpadra kiterjedő, súlyosabb destrukciókat is okozó elváltozásról van szó, amely egyértelműn szifilisz-asszociált megbetegedés volt. Egy másik, 1926-ban publikált esetben [13], 40 leukoplakiás pácienst vizsgáltak. A legtöbb elváltozás a nyelv területén alakult ki, és döntően férfiakban. A tanulmányban úgy vélték, hogy egyik esetben sem volt összefüggésbe hozható a leukoplakiás elváltozás szifilisszel, így nem-szifiliszes leukoplakiákként írták le őket. A vizsgálatba bevont páciensek 65%-ában volt igazolt szifilisz, Wassermann-reakcióval és / vagy egyéb szifiliszes manifesztációkkal.

Az idők során számos összefoglaló tanulmány készült (és folyamatosan készül is), amelyekben a leukoplakiás elváltozásokat definiálták, nomenklatúrákat és klasszifikációkat hoztak létre. Ezek a

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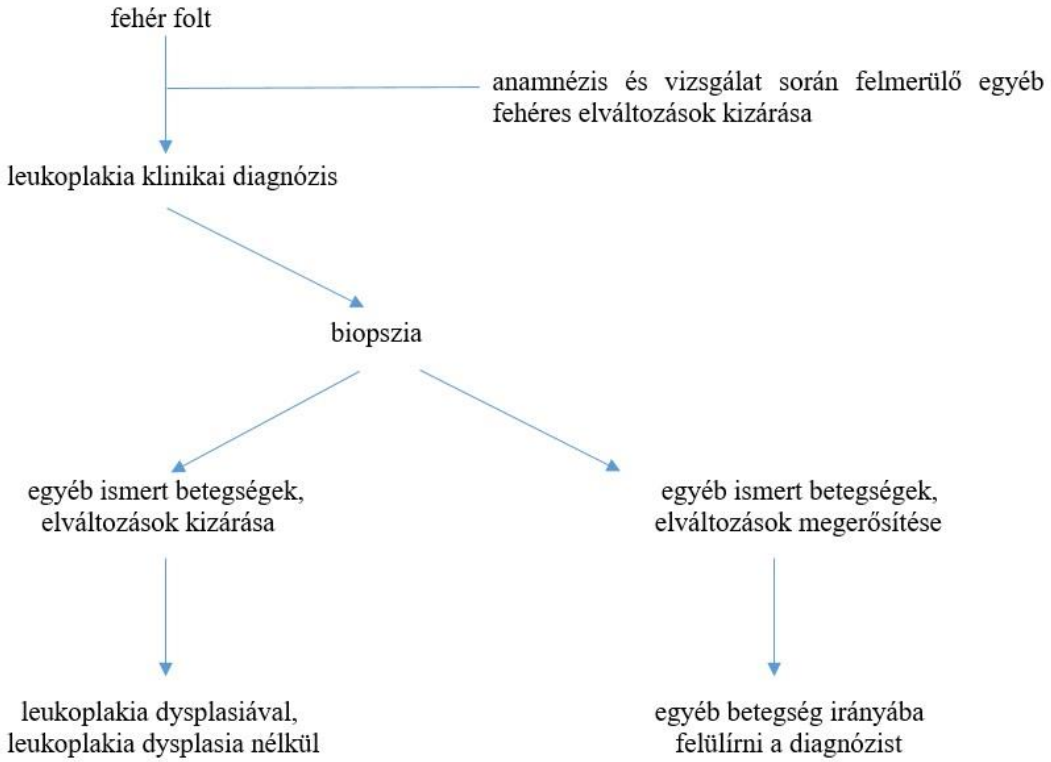
tanulmányok bizonyos dolgokban egyetértenek, de kisebb eltérések is előfordulnak a különböző definíciók és klasszifikációk között. 2005-ben Londonban ülésezett egy nemzetközi munkacsoport, kollaborálva a WHO-val, ahol megvitatták a téma sarkalatos pontjait: alapfogalmak, terminológia, patológiai vonatkozások, molekuláris markerek, diagnosztikus és terápiás javaslatok [14]. A tanulmányban leírták, hogy miért is nevezhetők ezek az elváltozások rákmegelőzőnek. Követés után a leukoplakis elváltozások egy részéből egyre súlyosabb dysplasia majd laphámrák alakul ki. Egyes esetekben a rákos elváltozásokkal párhuzamosan, általában azok széli részén szintén gyakran jelenik meg leuko- és/vagy erytrho-leukoplakia. Megfigyelték, hogy bizonyos morfológiai és citológiai jellemzők mind a daganatokban, mint a leukoplakiákban megfigyelhetők. Bizonyos molekuláris, kromoszómális jellemzők, amelyek kifejezetten a rosszindulatú daganatokra jellemzők, esetenként a leukoplakiákban is fellelhetők. Végsősoron ez a publikáció is a potenciálisan rosszindulatú rendellenességek kifejezés használatát javasolja, ugyanis nem mindegyik esetben alakul át rosszindulatú folyamattá, még súlyosabb esetben sem, de nagyobb az esélye a malignizálódásnak az egészséges szövetekhez viszonyítva. Ugyanakkor a tanulmány megtartotta és javasolja a fentebb említett „rákmegelőző elváltozás” és „rákmegelőző állapotok” elkülönítését. Ezen kívül a két fő típust is említi, amely a legelterjedtebb csoportosítást adja ma is: homogén és nem-homogén leukoplakiák. Felhívja a figyelmet azokra az elváltozásokra, amelyektől el kell különíteni a leukoplakiát a diagnózis szempontjából. Ezeket szemlélteti az 1. táblázat. Egy sematikus ábrát is közöltek, ami szemlélteti, hogyan kell eljárni a fehér foltos elváltozások diagnosztizálása során (1. ábra). A végső leukoplakia diagnózisnál fontos különbséget tesz abban, hogy dysplasia jelen van e, vagy sem [14].



1. ábra Szájüregi fehér foltok diagnosztikai folyamata

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Betegség	Diagnosztikus jellemzők
white sponge naevus	fiatal életkor, családi anamnézis, nagy kiterjedés, nemi szervek nyálkahártyájának érintettsége
mechanikai keratosis	fogak occlusiójával / fogpótlásokkal összefüggésbe hozható elhelyezkedés, trauma fennállása, trauma megszűnésével általában javul / elmúlik
morsicatio buccarum	szaggatott vonalszerű megjelenés, occlusiós síknak megfelelően, habituális rossz szokások, bucca rágása
kémiai sérülés	anamnézisből ismert sérülés, fájdalmas, gyors lefolyású
akut pseudomembranosus candidiasis	letörölhető elváltozás, amely után apró, pontszerű erythémák jelennek meg
leukoedema	kétoldali elváltozás, nyújtásra „eltüntethető”, bizonyos rasszokban gyakoribb
lichen planus	lichen planus egyéb tünetei, diagnosztizálása
lichenoid reakció	gyógyszerszedés, amalgám tömés az elváltozás közelében
DLE (discoid lupus erythematosus)	középső, kör alakú erythemás elváltozás, amelyből fehér vonalak sugároznak
hairy leukoplakia	kétoldali nyelv keratosis, EBV (Epstein-Barr vírus), immunszuprimált állapot
leukokeratosis nicotina palate	szürkés-fehéres szájpadlás, ismert dohányzás az etiológiában

1. táblázat Leukoplakia diagnózis felállításakor kizárandó egyéb elváltozások

Az erythoplakia definiálására is kitértek az átfogó tanulmányban. Az elváltozás legfontosabb jellemzője, hogy nagyon magas, szinte kivétel nélküli malignizációs hajlammal bír. A definíció nem sokat változott az idők alatt, ma is a WHO 1978-as meghatározását használják széleskörben: vörös, tűzvörös folt a szájüregben, amely klinikailag és patológiailag más betegségcsoportba nem sorolható be. Az erythroplakia önmagában ritkán fordul elő, leggyakrabban leukoplakiával együtt, ún. erythro-leukoplakia formájában van jelen. Az erythroplakiát, definíciójának megfelelően, el kell különíteni a többi, vöröses szájüregi elváltozástól. Ezek közül a leggyakoribbakat szemlélteti a 2. táblázat [14].

Egyéb vöröses állapotok	Diagnózis
gyulladásos és immun eredetű betegségek	desquamativ gingivitis erythematous lichen planus DLE (discoid lupus erythematosus) pemphigoids hiperszenzitív reakciók Reiter-kór
infekciók	erythémás candidiasis histoplasmosis
hamartoma és neoplasma	haemangioma Kaposi sarcoma

2. táblázat Erythroplakia differenciáldiagnosztikája

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Klasszifikációk

Számos publikáció készült a szájüregi leukoplakiák klasszifikációjával kapcsolatosan. A 3. táblázat szemléleti idősorrendben a legelterjedtebb csoportosításokat [9].

Klasszifikáció (évszám)	Csoportok	Jellemzők
Bánóczy-féle (1977)	1. típus: leukoplakia simplex	egyenletes, kissé elemelkedő fehér plakkok, szabályos szélekkel
	2. típus: leukoplakia verrucosa	kiemelkedő, lekerekített, fehér és / vagy vörös színű, olykor szemcsés-csomós megjelenéssel
	3. típus: leukoplakia erosiva	verrucosus proliferáció a nyálkahártya felszínén
Amagasa-féle (1977)	1. típus	lapos, fehér folt, vörös komponens nélkül
	2. típus	lapos, fehér folt, vörös komponenssel vagy erózióval
	3. típus	kissé kiemelkedő fehér folt
	4. típus	markánsan kiemelkedő fehér folt
WHO (1980)	1. típus: homogén	egyenletesen lapos, fehér folt, alacsony malignizációs hajlam
	2. típus: non-homogén	fehér és / vagy vöröses, szabálytalan felszínű, nodularis vagy exophytikus megjelenésű, magas malignizációs hajlam
Axell-féle (1996)	1. típus: homogén	
	- lapos, sima	sima, egyenletes felszín
	- hullámos	hullámos megjelenés
	- habkőszerű	finom vonalszerű mintázat
	- redőzött	száraz, repedezett felszín
	2. típus: non-homogén	
	- verrucosus	lassú növekedés, papilláris, erősen elszarusodó megjelenés
	- nodularis	erythémás alapon, fehér nodulusok
	- ulceratív	fehér foltok, vöröses szegéllyel
	- erythroleukoplakia	leukoplakia és erythroplakia együttes jelenléte
Warnakulasuriya-féle (2007)	1. típus: homogén	egyenletesen lapos felszín, alacsony malignizációs hajlam
	2. típus: non-homogén	szabálytalan felszín, nagyobb malignizációs hajlam
	- pettyes / foltos	keverten fehér és vörös, de dominál a fehér szín
	- noduláris	polipoid, nodularis megjelenés, kevert színnel
	- verrucosus	redős vagy hullámos felszín

3. táblázat Leukoplákiák klasszifikációi

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2009-ben jelent meg egy tanulmány, ami hasonlóan bemutatja a diagnosztikus kritériumokban és klasszifikációkban létrejött változásokat [6]. Kiemeli, hogy a szövettani diagnózis során mely csoportokba érdemes besorolni az elváltozást. Manapság is ez alapján történik leggyakrabban az osztályozás (4. táblázat).

Csoport		Jellemző
1.	laphám hyperplasia	a tüskés sejtes rétegben (acanthosis) és/vagy a bazális sejtrétegben jön létre, szabályos sejtek, atypia nélkül
2.	enyhe dysplasia	a hám alsó harmadában zajlik, szövettanilag atypia
3.	mérsékelt dysplasia	a hám középső harmadáig terjed, fokozódik az atypia mértéke és mennyisége
4.	súlyos dysplasia	a hám több mint kétharmadát érinti, kifejezettebb atypia
5.	in situ carcinoma	teljes vastagságban, kifejezett atypia

4. táblázat Szövettani diagnózis szerinti csoportosítás

Egy másik, 2019-es tanulmány szintén összefoglalta a leukoplakiák csoportosítására vonatkozó javaslatokat [15]. Úgy vélték, hogy áttekinthetőbb és egyértelműbb diagnózist tesz lehetővé, ha két csoportot, homogén és non-homogén leukoplakiákat különböztetünk meg, amelyek vagy túlnyomórészt fehérek, vagy vegyesen fehérek és pirosak lehetnek. Ez nagyjából tükrözi a WHO 1980-as klasszifikációját, néhány módosítással. Egyik előny, hogy a rosszul definiálható proliferatív verrucosus leukoplakia így nem alkot külön csoportot. Javaslatot tettek a hairy leukoplakia átnevezésére is, amely EBV-asszociált, általában HIV pozitív egyéneken jelentkező betegség: EBV-pozitív fehér nyelvi elváltozás.

Összefoglalás

A szájüregi rákmegelőző állapotok esetén számos, definícióban és terminológiában történő változást láthatunk mind a hazai mind a nemzetközi irodalomban. A fő diagnosztikai és terápiás kritériumok szintén változnak az évek során, köszönhetően az újabb kutatási eredményeknek. Habár sokféle nomenklatúrát használ az irodalom, és ezek mindegyike helytálló, a legtöbb szerző azokat a definíciókat javasolja, melyeket nemzetközi tanácskozásokon az ezzel foglalkozó szakemberek (és a WHO) is jóváhagytak. Célszerű azonban szem előtt tartani a többi nomenklatúrát és klasszifikációt is, hiszen sok esetben épp ezek a csoportosítások azok, amelyek segíthetik akár a diagnózis-terápia sikerességét, akár a további kutatási eredmények fejlődését. Habár sok tanulmány készült a leukoplakiákkal kapcsolatosan, még mindig sok hiányosság és kevés tapasztalat van bizonyos területeken. A témában továbbra is számos nyitott kérdés van, mely egyaránt érinti az etiológiát, a diagnosztikát és a terápiás javaslatokat. A téma fontosságát nem lehet eléggé hangsúlyozni, hiszen egy rutin fogorvosi vizsgálat során is könnyen felismerhető elváltozásról van szó, amely felismerése felhívhatja a figyelmet az etiológiára, a háttérben már zajló, vagy potenciálisan kialakuló súlyosabb folyamatokra is. Ehhez elengedhetetlen az irodalom folyamatos kutatása, a fejlődés és a változások nyomon követése.

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Malignant Transformation and Long-Term Outcome of Oral and Laryngeal Leukoplakia

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Abstract: Background: Oral or laryngeal leukoplakia has an increased risk for malignant transformation but the risk of the two anatomical sites has not been compared to each other yet. Materials and Methods: Clinical data of 253 patients with leukoplakia (oral = 221 or laryngeal = 32) enrolled from January 1996 to January 2022 were analyzed. One hundred and seventy underwent biopsy and 83 did not. The mean follow-up time was 148.8 months. Risk factors for the malignant transformation of leukoplakia were identified using Cox proportional hazard models. Results: In the oral or laryngeal group, the rate of cancer was 21.7% and 50% ($p = 0.002$), respectively. The 10-year estimated malignant transformation was 15.1% and 42% ($p < 0.0001$), respectively. The laryngeal group had an increased risk of malignant transformation ($p < 0.0001$). The 5-year estimated survival with leukoplakia-associated cancer for the oral or laryngeal group was 40.9% and 61.1% ($p = 0.337$), respectively. Independent predictors of malignant transformation in the oral group were dysplasia and the grade of dysplasia of the leukoplakia, and in the laryngeal group, dysplasia had a significant impact. The malignant transformation rate was low for oral patients without biopsy or with no dysplasia, 3.9% and 5.1%, respectively. The malignant transformation occurred over 10 years. Conclusions: Patients with dysplastic leukoplakia have an increased risk of malignant transformation, but the risk is higher with laryngeal than with oral leukoplakia. There is no significant difference between the groups regarding survival with leukoplakia-associated cancer. Oral patients with no dysplastic lesions have a low risk of malignant transformation. A complete excision and long-term follow up are suggested for high-risk patients to diagnose cancer in an early stage and to control late (over 10 years) malignant events.

Keywords: oral and laryngeal leukoplakia; risk of malignant transformation; survival with cancer

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1. Introduction

Leukoplakia is the most common potentially malignant disorder of head and neck cancer. The range of malignant transformation from leukoplakia to squamous cell carcinoma amounts from 0.13% to 34% for oral leukoplakia and from 0% to 64.7% for laryngeal leukoplakia [1–3]. This large variation in the malignant transformation rates is in part caused by the criteria applied for a leukoplakia diagnosis, a geographical location, differences in study populations, possible etiological factors and length of follow up [3]. A prediction of the malignant potential is traditionally based on the histologically determined severity of the dysplasia. An increase of the grade of dysplasia increases the risk of malignant transformation into squamous cell carcinoma [2–4]. Moderate/severe dysplasia bears a much higher risk of cancer evolution than mild dysplasia [5,6]. A clinical examination

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does not accurately predict the risk of malignancy. More than half of the reported leukoplakia lesions with biopsies showed no dysplasia [5,7]. Since 2007, leukoplakia has been limited only to a clinical diagnosis defined by the exclusion of other white lesions such as oral lichen planus, white sponge nevus, nicotine stomatitis, leukoedema, etc. [8]. To date, therapy strategies in the management of leukoplakia focus on "watch-full waiting", a non-surgical application of local therapeutics and photodynamic therapy, serial incision biopsies or excision biopsy and laser vaporization depending on the determined grade of dysplasia and clinically apparent extension of the leukoplakia [9–11]. The aim of this study was to examine the association between the risk of malignant transformation and dysplasia in oral or laryngeal leukoplakia and to highlight the importance of the time course of the disease and follow-up strategies for these patients.

2. Materials and Methods

A chart review of all patients treated at the National Institute of Oncology Budapest, Hungary with laryngeal or oral leukoplakia over a 16-year period (January 1996 to January 2022) was performed. In the computerized institutional database, the number of patients with oral or laryngeal leukoplakia was 221 and 32, respectively. The histopathology of leukoplakia from the biopsy material was classified as no dysplasia, dysplasia and carcinoma. Dysplasia was classified as mild, moderate and severe (grade I–II–III). The pathology department in our institution uses the World Health Organization (WHO) classification for oral and laryngeal dysplasia [12,13]. Initial management consisted of biopsy, surgical excision, CO_2 laser vaporization or observation only. Re-biopsy was performed if there was any deterioration in symptoms (such as the voice quality, swallowing or pain) or appearance of the lesion (endoscopically for laryngeal and to the naked eye in the case of oropharyngeal lesions). The histological findings were classified as follows: no dysplasia, dysplasia (grade I–II–III) or cancer. The treatment consisted of surgery with the removal of the lesion, CO_2 laser treatment or observation. First, and before any treatment, we always took at least one biopsy before laser vaporization. In addition, surgical excisions were performed for dysplastic leukoplakia with a histological examination of the entire lesion. Regular follow up (two visits/year) was suggested for patients.

To assess the associations between the different study variables and the risk of developing cancer, a survival analysis was performed using the Kaplan–Meier [14] method. The number of patients progressing towards malignancy was evaluated in each of the groups. The follow-up period was defined as the interval from the time of a leukoplakia clinical diagnosis to death or the last follow up. The following survival endpoints were used: death from leukoplakia-associated cancer for cancer-specific survival, and the time to an appearance of cancer for malignant transformation-free survival. The survival curves were compared with a log-rank test. The effect of the possible prognostic factors on the probability of the incidence of leukoplakia-related cancer was examined in a Cox regression model [15]. Statistical differences in proportions and means were assessed with a Fisher-exact test and chi-squared test. All tests were two-sided, and p values of ≤ 0.05 were accepted for statistical significance. GraphPad Prism (GraphPad Prism version 5.01 for Windows, GraphPad Software, San Diego, CA, USA) and IBM SPSS Statistics for Windows (version 25.0, Armonk, NY, USA: IBM Corp) program packages were used for a data analysis.

3. Results

The mean or median follow-up time was 148.8 months and 144 months (range: 14–328 months), respectively. Seventeen patients presented with in situ or invasive cancer, and half of the invasive cancers had III–IV stages. In 47 patients, the cancer was developed during the follow-up time, between 6 and 204 months (mean time: 53.6 months). Six patients developed cancer after 120 months. The early-stage cancer (stage 0–I–II) rate was 48.4% (31 of 64). In total, 11 of the 64 cancer patients (in situ cancers are also included) are currently alive, 40 died of leukoplakia-associated head and neck cancer, 3 died of second primary cancer and 10 died of internal diseases. The average survival time with cancer was

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64.3 months (10–221 months). The 5-year estimated survival with leukoplakia-associated cancer for patients with oral or laryngeal leukoplakia was 40.9% and 61.1% ($p > 0.337$), respectively. The number of biopsy/patients (prior to malignant transformation) was 1/148, 2/17, 3/4 and 4/1. Dysplasia progressed in 20 of 22 patients with multiple biopsies (oral, 13; laryngeal, 7). The average time to the malignant transformation of laryngeal leukoplakia or oral leukoplakia patients was 55.6 months (range: 6–204 months) and 52.7 months (range: 6–204 months), respectively ($p = 0.913$). The grade of dysplasia had a significant effect on the time to malignant transformation with oral leukoplakia. The mean metastasis transformation-free survival with a low grade or with a high grade was 88.0 and 11.3 months, respectively ($p < 0.0001$). In the laryngeal group, the difference between the two grades was not significant ($p = 0.982$). The crude rate of malignant events and the 10-year estimated malignant transformation rate using characteristics are shown in Table 1.

Table 1. Results of all patients using characteristics.

Characteristic	Malignant Events *n* (%)	*p*	10-Year MTFS % (±SD)	*p*	Univariate Cox HR (CI 95%)	*p*
All patients	64/253 (25.3%)		81.5 ± 2.6			
Gender						
female	29/138 (21%)	0.11	86.0 ± 3.2	0.061	1	0.065
male	35/115 (30.4%)		76.3 ± 4.3		1.72 (0.97–3.06)	
Age (years)						
≤60	38/152 (25%)	>0.999	79.8 ± 4.7	0.546	1	0.548
>60	26/101 (25.7%)		81.9 ± 3.3		1.20 (0.66–2.16)	
Smoking						
never	5/34 (14.7%)	0.0004	87.8 ± 5.7	0.004	1	0.009
past and present	47/96 (49%)		62.6 ± 5.6		4.01 (1.42–11.30)	
unknown	12/123 (9.8%)					
Oral vs. laryngeal						
oral	48/221 (21.7%)	0.002	84.9 ± 2.6	<0.0001	1	<0.0001
laryngeal	16/32 (50%)		58.0 ± 9.4		3.13 (1.71–5.72)	
Lesion type						
homogenous	53/236 (22.5%)	0.0004	83.2 ± 2.6	0.003	1	0.005
non-homogenous	11/17 (64.7%)		53.8 ± 13.8		3.46 (1.46–8.18)	
Biopsy						
no	4/83 (4.8%)	<0.0001	95.9 ± 2.4	<0.0001	1	<0.0001
yes	60/170 (35.3%)		74.0 ± 3.7		6.48 (2.33–18.05)	
Histology						
no dysplasia	5/88 (5.7%)	<0.0001	95.3 ± 2.3	<0.0001	1	<0.0001
grade I dysplasia	7/29 (24.1%)		79.3 ± 7.5		4.76 (1.51–15.03)	
grade II dysplasia	16/20 (80%)		18.3 ± 10.3		32.62 (11.51–92.44)	
grade III dysplasia	15/16 (93.8%)		18.8 ± 9.8		29.79 (10.73–82.73)	
in situ cancer	5					
invasive cancer	12					
Dysplasia						
no	5/88 (5.7%)	<0.0001	95.3 ± 2.3	<0.0001	1	<0.0001
yes	38/65 (58.5)		45.9 ± 6.4		14.58 (5.71–37.24)	
Dysplasia						
low grade (I)	7/29 (24.1%)	<0.0001	79.3 ± 7.5	<0.0001	1	<0.0001
high grade (II, III)	31/36 (86.1%)		18.3 ± 7.0		6.78 (2.94–15.63)	

MTFS, malignant transformation-free survival; SD, standard deviation; HR, hazard ratio; CI, confidence interval.

The 10-year estimated malignant transformation rate of leukoplakia for all (253) patients was 18.5%. The laryngeal leukoplakia patients have a significantly increased risk of malignant transformation compared with oral patients (univariate Cox Hazard Ratio (HR): 3.13). The 10-year estimated malignant transformation rate was 42.0% and 15.1%, respectively (Figure 1).

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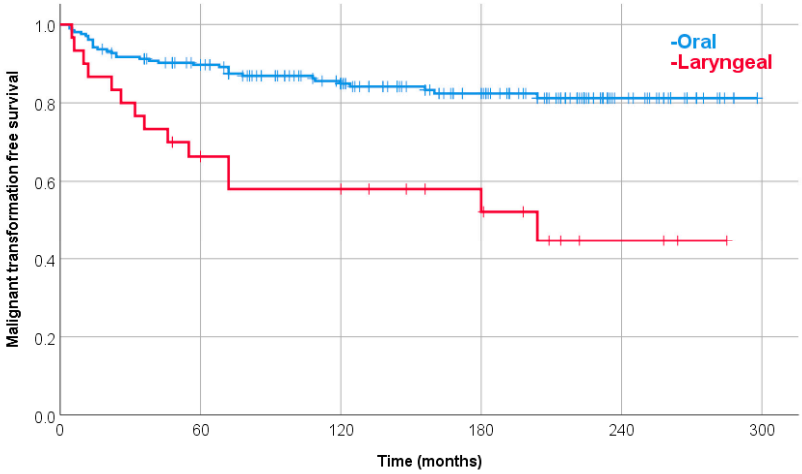

Figure 1. Malignant transformation-free survival with oral or laryngeal leukoplakia. The 10-year estimated malignant transformation rate was 15.1% and 42.0%, respectively ($p < 0.0001$).

The results of the multivariate Cox regression model, run for all patients, are shown in Table 2. The non-homogenous lesion and higher grade of dysplasia remained independent negative predictors of malignant transformation-free survival.

Table 2. Multivariate model for all patients.

Characteristic	Multivariate Cox HR (CI 95%)	*p*
Smoking		
never	1	0.142
past and present	2.49 (0.74–8.44)	
Lesion type		
homogenous	1	0.014
non-homogenous	3.74 (1.31–10.09)	
Dysplasia		
no	1	
low grade (I)	4.23 (1.11–16.16)	<0.0001
high grade (II, III)	12.25 (4.10–36.66)	

HR, hazard ratio; CI, confidence interval.

A separate analysis of patients with oral leukoplakia is shown in Table 3. The mean follow-up time was 149.9 months (14–328 months). Smoking, the lesion site, the lesion type, dysplasia and the grade of dysplasia had significant effects on malignant transformation-free survival. In the multivariate Cox regression model, only dysplasia and the grade of dysplasia remained independent predictors of malignant transformation-free survival (Table 4). Four patients with oropharyngeal leukoplakia developed cancer. None of them had an HPV-positive histology with a p16 immunohistochemistry examination.

The mean follow-up time for laryngeal patients was 140.9 months (36–285 months). To separately evaluate the patients with laryngeal leukoplakia, some of the subgroups have underpowered materials regarding the number of patients and malignant events (Table 5). The rate of malignant events was significantly higher only for patients with dysplastic leukoplakia: no or yes, 11.1% and 80%, respectively ($p = 0.002$). In total, 73% (11 of 15) of patients with dysplastic leukoplakia were smokers. The 10-year estimated malignant-free survival was 88.9% and 30.5%, respectively ($p = 0.002$). The presence of dysplasia significantly increased the risk of malignant transformation (HR: 12.43; 95% Confidence Interval: 1.59–97.38; $p = 0.016$). Furthermore, seven dysplastic lesions progressed to a higher grade over the follow-up time.

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Table 3. Results of patients with oral leukoplakia using characteristics.

Characteristic	Malignant Events *n* (%)	*p*	10-Year MTFS %, SD	*p*	Univariate Cox HR (CI 95%)	*p*
All patients	48/221 (21.7%)		84.9 ± 2.6			
Gender						
female	26/128 (20.3%)	0.621	85.8 ± 3.3	0.756	1	0.756
male	22/93 (23.7%)		83.9 ± 4.1		1.12 (0.56–2.23)	
Age (years)						
≤60	25/126 (19.8%)	0.51	86.2 ± 3.2	0.235	1	0.239
>60	23/95 (24.2%)		81.9 ± 4.8		1.52 (0.76–3.02)	
Smoking						
never	4/33 (12.1%)	0.0004	90.6 ± 5.2	0.004	1	0.009
past and present	35/73 (48%)		63.4 ± 6.5		4.93 (1.48–16.38)	
unknown	9/115 (7.8%)					
Lesion site *						
tongue	14/60 (23.3%)	0.003	82.6 ± 5.4	0.007	2.89 (0.90–9.20)	0.003
sublingual	6/24 (25%)		76.7 ± 9.2		3.67 (0.98–13.66)	
palate	3/8 (37.5%)		71.4 ± 17.1		5.00 (0.92–27.35)	
buccal	6/60 (10%)		96.5 ± 2.4		1	
gingiva	6/13 (46.1%)		52.4 ± 15.7		7.17 (1.93–26.74)	
lips	0/27 (0%)		100		0	
oropharyngeal	4/10 (40%)		75.0 ± 15.3		4.24 (0.78–23.13)	
multifocal	9/19 (47.4%)		73.3 ± 11.4		5.47 (1.47–20.39)	
Lesion type						
homogenous	37/203 (18.2%)	0.0002	87.0 ± 2.6	<0.0001	1	0.001
non-homogenous	11/18 (61.1%)		53.8 ± 13.8		4.57 (1.88–11.13)	
Biopsy						
no	3/77 (3.9%)	<0.0001	96.9 ± 2.2	<0.0001	1	<0.0001
yes	45/144 (31.3%)		77.9 ± 3.8		6.60 (2.01–21.63)	
Histology						
no dysplasia	4/79 (5.1%)	<0.0001	96.1 ± 2.2	<0.0001	1	<0.0001
grade I dysplasia	4/25 (16%)		88.0 ± 6.5		3.43 (0.86–13.75)	
grade II dysplasia	12/15 (80%)		22.2 ± 12.2		40.71 (11.91–139.11)	
grade III dysplasia	10/10 (100%)		0		54.33 (15.15–194.84)	
in situ cancer	4					
invasive cancer	11					
Dysplasia						
no	4/79 (5.1%)	<0.0001	96.1 ± 2.2	<0.0001	1	<0.0001
yes	26/50 (52%)		50.0 ± 7.4		13.79 (4.79–39.72)	
Dysplasia						
low grade (I)	4/25 (16%)	<0.0001	88.0 ± 6.5	<0.0001	1	<0.0001
high grade (II, III)	22/25 (88%)		11.3 ± 7.1		14.63 (4.28–50.03)	

MTFS, malignant transformation-free survival; SD, standard deviation; HR, hazard ratio; CI, confidence interval; * p16 immunohistochemistry was negative for oropharyngeal patients.

Table 4. Multivariate Cox model for patients with oral leukoplakia.

Characteristic	Multivariate Cox HR (CI 95%)	*p*
Dysplasia		
no	1	
low grade (I)	2.47 (0.41–15.03)	<0.0001
high grade (II, III)	18.19 (4.71–70.25)	
Lesion type		
homogenous	1	0.063
non-homogenous	2.79 (0.95–8.24)	
Smoking		
never	1	0.098
past and present	3.51 (0.79–15.51)	

HR, hazard ratio; CI, confidence interval.

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Table 5. Malignant events of patients with laryngeal leukoplakia using characteristics.

Characteristic	Malignant Events *n* (%)	*p*
All patients	16/32 (50%)	
Gender		
female	3/10 (30%)	0.252
male	13/22 (59.1%)	
Age (years)		
≤60	13/26 (50%)	>0.999
>60	3/6 (50%)	
Smoking		
never	1/1 (100%)	>0.999
past and present	12/23 (52.2%)	
unknown	3/8 (37.5%)	
Site		
unilateral	9/20 (45%)	0.717
bilateral	7/12 (58.3%)	
Lesion type		
homogenous	16/32 (50%)	>0.999
non-homogenous	0/0	
Biopsy		
no	1/6 (16.7%)	0.172
yes	15/26 (57.7%)	
Histology		
in situ cancer	1	
invasive cancer	1	
Dysplasia		
no	1/9 (11.1%)	0.002
yes	12/15 (80%)	
Dysplasia		
low grade (I)	3/4 (75%)	>0.999
high grade (II, III)	9/11 (81.8%)	

4. Discussion

In the present study, we evaluated the malignant transformation rate of patients with oral or laryngeal leukoplakia. The 10-year estimated rates were 15.1% and 42.0%, respectively ($p < 0.0001$). The mean time to malignant transformation was longer with 3 months for laryngeal leukoplakia compared to an oral lesion, but the difference was not significant (laryngeal or oral: 55.6 months and 52.7 months, respectively). The relative risk for the malignant transformation of leukoplakia was more than three times higher for patients with laryngeal cancer. The dysplasia of leukoplakia significantly increased the malignant transformation rate in both groups. The grade of dysplasia also had a significant effect on the malignant transformation ($p < 0.0001$). Up until now, no study compared the malignant transformation risk of leukoplakia of the two anatomical sites. The survival with leukoplakia-associated cancer was similar in the two groups.

We made a detailed analysis for patients with oral leukoplakia because of the appropriate number of patients ($n = 221$) with a long follow-up time (mean: 149.9 months). The 10-year estimated rate of the malignant transformation of oral leukoplakia was 15.1%. A smoking habit (never vs. ever), the lesion type (homogenous vs. non-homogenous), dysplasia (yes vs. no) and the grade of dysplasia had a significant effect on malignant transformation-free survival. The presence of dysplasia and high-grade dysplasia remained independent negative predictors of malignant transformation-free survival in a multivariate Cox model. In an earlier study from China, the mean follow-up time was 5.3 years and the malignant transformation rate was 17.9%. High-grade epithelial dysplasia proved to be an independent predictor of malignant transformation-free survival, but a smoking habit did not [16]. Two years later, also from Shanghai [17], a multivariate analysis revealed that four factors including a patient age of >60 years, a lesion located on the lateral/ventral tongue, a non-homogenous lesion and high-grade dysplasia were significant

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independent indicators for the malignant transformation of oral leukoplakia. Sequential biopsies were suggested for high-risk patients for the early detection of a malignant event. In a study by de Vincente et al. [18], histopathological grading was also significantly associated with oral cancer risk and was found to be a significant independent predictor in the multivariate analysis. In a review study (11,423 patients) by Warnakulasuriya and Ariyawardana, the malignant transformation rate for oral leukoplakia had a wide range between 0.13% and 34.0%. Significant determinants of the malignant transformation of oral leukoplakia included an advanced age, the female sex, leukoplakia exceeding 200 mm^2, the non-homogeneous type and higher grades of dysplasia. They concluded that the determinants exposed in the review require further investigation [3]. In another review and meta-analysis of the last 5 years, 16,604 patients with oral leukoplakia were involved. The proportion of malignant transformation varied between 1.1% and 40.8%. The female sex, non-homogeneous clinical type and presence of epithelial dysplasia were significantly related to malignant transformation. Other risk factors previously suggested did not show significant results [19]. In a Swedish study [20] of the 234 included patients, with a median follow up of 9 years, 27 (11.5%) developed oral squamous cell carcinoma. Among the clinicopathologic factors investigated, non-homogeneous oral leukoplakia, leukoplakia with dysplasia and leukoplakia localized to the tongue showed statistically significant increased rates of malignant transformation in the multivariate Cox regression analysis. In our patients, oral leukoplakia with dysplasia or non-homogenous lesions were also independent predictors of malignant transformation, but the most common site of malignant transformation was the gingiva. The mean follow-up time of our patients was longer than 9 years—148.8 months. Malignant transformations (n = 6) were seen even over 10 years after the diagnosis of leukoplakia. Our long-term follow-up period could contribute to the increased rate of malignant transformation. The longest time to the malignant transformation of oral leukoplakia was observed in a Spanish study [21]—15 years and 2 months. In our patients, the longest time to the malignant transformation of leukoplakia was 204 months, which is longer with 22 months. The severe dysplasia also significantly increased the rate of malignant transformation in their patients. Furthermore, the rate of patients with early-stage cancer was much better than in our patients, 19.2% and 48.4%, respectively. The majority of the patients of Jäwert et al. [22] had 3–6 month follow-up intervals conducted by a specialist and the oral potentially malignant disorder-associated cancers were diagnosed in an early stage, which improved the survival with cancer significantly. A large cohort study from Northern California included 4886 oral leukoplakia patients with 4.62 mean years of follow up. The risk of progression to oral cancer significantly increased with the grade of dysplasia. One of their key observations was that a large proportion of oral cancer arose from leukoplakias diagnosed initially as non-dysplastic lesions. They suggested a biopsy of all clinically diagnosed leukoplakias because of the modest accuracy of the decision to biopsy leukoplakia [23]. In our cohort study, 77 patients with oral leukoplakia were not subjected to biopsy and only 3.9% of them developed oral cancer. The oral cancer rate of patients with a non-dysplastic histology was also low—5.1%. Therefore, we do not suggest routine biopsy of all leukoplakias. They need close (two times/year) monitoring for signs of early cancer.

To separately evaluate the patients with laryngeal leukoplakia, some of the subgroups have underpowered materials regarding the number of patients and malignant events. The presence of dysplasia increased the risk of malignant transformation in our patients. In a Chinese study [24], 215 patients were analyzed. The rate of no dysplastic leukoplakia was very high—54.4%. In our study, this rate was only 11.1%. The multivariate analysis showed that the pathological classification of moderate to severe dysplasia was the independent risk factor for the recurrence and malignant transformation of laryngeal leukoplakia ($p < 0.05$). In a study from Israel [25], severe dysplasia at the initial diagnosis and heavy smoking were risk factors of malignant transformation. In our patients, the malignant transformation rate was high even with grade I–II leukoplakia. However, initial dysplasia progressed over the follow-up period, and 73% of our patients with dysplasia were

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smokers. A study by Zhang et al. [26] included 32 patients with laryngeal leukoplakia. The malignant transformation rates for mild, moderate and severe dysplasia were 33%, 75% and 75%, respectively. Our patients with dysplasia also had high malignant transformation rates. Leduchowska et al. [27] used endoscopic (plaque morphology) and stroboscopic (mucosal wave assessment) examinations to estimate the degree of dysplasia in vocal fold leukoplakia. The rate of low- or high-grade dysplastic leukoplakia was 61.8% and 38.2%, respectively. Their findings can be used to guide a decision regarding immediate biopsy or watchful waiting. Our patients had high (75%) malignant transformation even with grade I dysplasia.

5. Conclusions

Patients with oral or laryngeal dysplastic leukoplakia have an increased risk of malignant transformation, but the risk is about three times higher for patients with laryngeal leukoplakia. There is no significant difference between the groups regarding survival with leukoplakia-associated cancer. Patients with non-dysplastic lesions have a low risk of malignant transformation especially in the oral group. An immediate surgical complete excision and strict and long-term follow up are suggested for high-risk patients to diagnose cancer in an early stage and to control late (over 10 years) malignant events.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of Medical Research Council (protocol code BM/5034-1/2023).

Informed Consent Statement: Patient consent was waived due to it was a retrospective study.

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Case Report

Neck Node Squamous Cell Metastasis from Unknown Primary and Mutagen Sensitivity: A Case Series

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Keywords
Neck node metastasis · Unknown primary carcinoma · Mutagen sensitivity · HPV+ or cystic squamous cell cancer

Abstract
Most of the neck node metastases from cancer of unknown primary (CUP) are squamous cell carcinomas (SCCs). The majority of which are human papillomavirus (HPV)-related, frequently show cystic morphology referring to Waldeyer's ring origin. Here, we report four cases of neck node SCCs metastases from CUP. In our institute, 432 patients with head and neck (HN) SCC underwent pretreatment mutagen sensitivity (MS) assay between 1996 and 2006. Among them, 4 patients ≤50 years of age had metastatic cervical nodes from CUP. The primary treatment was cervical node dissection ± radiotherapy. All patients had elevated (>1.0 chromatid break/cell) MS. One male patient died of progressive neck metastasis within 3 years and the 3 female patients are still alive more than 15 years after initial treatment of HPV+ (two) or cystic (one) SCC. Two female patients developed second and third distant site metachronous primary cancers. HPV+ or cystic HNSCC from CUP with elevated MS indicates good outcome. Distant site metachronous cancers of different histologic origins cannot be explained by field cancerization. The clinical significance of elevated MS in neck node SCC metastasis from CUP requires further investigation.

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Introduction

Cancer of unknown primary (CUP) is a metastatic disease defined by the absence of a clinically identified primary malignancy at the time of diagnosis, despite appropriate diagnostic work-up. CUP is a relatively frequent cancer type causing incomparable difficulties in pathological diagnosis as compared to other tumor types [1]. The primary may even remain unknown at autopsy due to microscopic size or previous regression. Confirmed CUP accounts for 2–5% of all cancers. Most of the neck node metastases from CUP are squamous cell carcinomas (SCCs). The rate of head and neck (HN) cancers with unknown primary can be reduced to less than 3% after appropriate investigation [pan-endoscopy, computer tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET)/CT examination [2]. The majority of neck lymph node SCC metastases in CUP originate from the HN site. A substantial number of primary tumors identified in squamous cell CUP (SCCUP) patients are found in the oropharynx and are human papillomavirus (HPV)-related, with HPV16 being the predominant high-risk subtype. The incidence of oropharyngeal SCC is increasing, due to the epidemic emergence of HPV-mediated oropharyngeal SCC. Besides tobacco and alcohol consumption, HPV is an accepted risk and prognostic factor for oropharyngeal SCC. In addition, patients with HPV-positive oropharyngeal SCC have a much better clinical response to therapy than patients with HPV-negative oropharyngeal SCC and other HN cancers, but site, stage, and smoking are also significant prognostic factors [3–5]. The predominant pathology in HPV-related SCCUP is the non-keratinizing SCC [6]. A non-keratinizing morphology of neck node metastasis of CUP suggests a tonsillar or base of the tongue localization. HPV-related metastatic carcinoma may correlate with the following morphologic characteristics: large size, cystic nature, and limited extracapsular extension [6]. Cystic neck node metastasis also predicts better outcomes [7].

It is well-known that only a fraction of all individuals exposed to environmental carcinogens (tobacco and alcohol) will develop HN cancer. Deficiencies in DNA (deoxyribonucleic acid) repair capacity are thought to be associated with the risk HN cancer in smoking and drinking patients [8]. SCC of the upper aerodigestive tract has a high propensity to develop second primary malignancy [9]. An explanation for this phenomenon was proposed by Slaughter, who gave the concept of “condemned mucosa” developing after chronic carcinogenic exposure [10]. It is an unresolved issue whether mutagen sensitivity (MS) has a causative role in the development of squamous cell neck node metastases from CUP with or without multiple distant site primary cancer.

Here, we report 4 cases of younger (≤50 years) adults with HN node-positive SCC from CUP who were involved in the MS assay before initial treatment. In our institute, 432 HN cancer patients with HNSCC underwent pretreatment MS assay between 1996 and 2006. The aim of MS assay measured by bleomycin test was among others to determine the MS of HNSCC patients, alcoholic patients, healthy nonsmokers and nondrinkers, and nondrinking smokers. MS was significantly elevated in HNSCC patients as compared with the healthy controls [11]. Among 432 patients with HNSCC four younger (≤50 years) patients were found with SCC of neck lymph nodes from CUP and 124 patients had primary HPV-negative HNSCC. MS was measured by determining the mean number of chromatid-breaks per peripheral lymphocyte after in vitro bleomycin exposure. The long-term results of patients with known primary cancers have been published recently [12]. The Eighth Edition of TNM system [13] was used to classify metastases of neck nodes of CUP patients. We review the literature based on our cases and discuss the challenging diagnostic and treatment aspects. The CARE Checklist has been completed by the authors for this case report and is included as online supplementary material (for all online suppl. material, see https://doi.org/10.1159/000533708).

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Case Series

Patient 1

A 44-year-old female smoker patient presented with cystic mass measuring 5 cm on the right side of neck in 1999. The patient had a history of treatment for TisN0M0 urothelial cancer of urinary bladder. In 1997, she underwent transurethral excision and BCG installation at another hospital, but the details are unknown. She was subjected to ipsilateral upper node dissection. The largest size of SCC metastasis was 25 mm in the metastatic node. Hematoxylin and eosin stain described well differentiated squamous cell cancer without extracapsular tumor extension. Ten nodes were negative (T0pN1M0). MRI and panendoscopy examination did not find primary tumor. Pretreatment value of MS assay by bleomycin test was 1.17 b/c (break/cell). Observation was recommended. Five years later (2004), the neck node metastasis recurred. Four lymph nodes were dissected, and two of them had SCC metastasis. The size of the largest deposit was 20 mm with capsular invasion, but no extracapsular tumor extension was observed. Histopathologic examination showed SCC. Biopsies from the tonsils were negative. The patient underwent unilateral epi- and oropharyngeal and regional (upper neck nodes) radiotherapy (to 50 Gy, 25 fractions). In 2011, she underwent sigmoid polypectomy (pTisN0M0 adenocarcinoma). In 2021, molecular pathology examination of neck node metastasis (from a regional relapse in 2004) showed HPV16-genotype and p16 over expression by immunohistochemistry. She had distant site metachronous in situ cancers: urothelial cancer and sigmoid colon adenocarcinoma. In situ cancer does not give metastasis and her neck node metastasis was SCC both in 1999 and 2004. The patients are alive without relapse. The overall survival is 274 months.

Patient 2

A 43-year-old female smoker presented with enlarged (55 mm) right submandibular lymph node of the neck in 2000. An ipsilateral upper node dissection was performed and 1 out of 19 lymph nodes showed poorly differentiated non-keratinizing-cystic SCC (Fig. 1) metastasis without extracapsular extension (T0pN1M0), which may indicate tonsillar cancer, but histologic findings of tonsils biopsies were negative. Postoperative positron emission tomography/computer tomography (PET/CT) and panendoscopy did not show primary cancer. Postoperative radiotherapy was given (epi- and oropharynx and ipsilateral upper neck nodes to 50 Gy, 2 Gy fraction). The pretreatment value of the bleomycin test for MS was 1.04 b/c. She was subjected to right-side colon cancer surgery at another hospital on April 9, 2014: adenocarcinoma, pT3pN0M0. On September 3, 2014, she underwent left-side nephrectomy: clear cell cancer, pT1apN0M0. She developed abdominal lymph node metastasis from colon cancer in 2015 and was treated with chemotherapy. In 2019, hepatic and pulmonary metastases were diagnosed. In 2022, the retrospective molecular pathology of neck node metastasis showed no HPV16 DNA infection or p16 over expression. She is living with a progressive disease. The histology of her distant site metachronous invasive cancers was adenocarcinoma and clear cell kidney cancer but the neck node metastasis was SCC. The overall survival is 243 months.

Patient 3

A 34-year-old nonsmoking female patient presented with cervical lymph node enlargement in 2006. Both MRI and PET/CT showed bilateral suspect neck nodes and increased glucose metabolism was found in the base of the tongue by PET/CT. She underwent pretreatment MS examination. The bleomycin test value was 1.60 b/c. She underwent bilateral cervical lymph node dissection and excisional biopsy from base of the tongue. Fifty-two cervical lymph nodes were dissected and the histopathological examination revealed poorly

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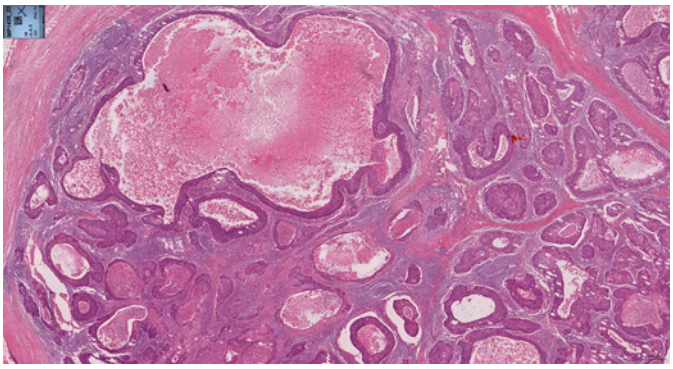

Fig. 1. Extensive cystic changes can be seen in tumor cell nests. Stained with hematoxylin and eosin.

differentiated SCC metastasis in five nodes (three on the right side and two on the left side) (Fig. 2a). The largest node size was 55 mm, and the largest metastasis was 30 mm. No extracapsular tumor extension was detected (T0pN2 M0). The biopsy specimen from the base of the tongue was free of cancer. The panendoscopic examination was also negative. No primary tumor was found. After surgery, radio-chemotherapy was given (Cisplatin 6 × 152 mg; 1.8 Gy/fraction, neck lymph nodes (bilateral) and hypopharynx to 55.8 Gy; epi- and oropharynx to 66.6 Gy). Molecular pathology was performed in 2020. Tumor cells were p16 positive and the presence of HPV16 was confirmed from tumor DNA (Fig. 2b). The patient was followed up as an outpatient and after 198 months, there was no evidence of recurrence.

Patient 4:

In 2001, a 50-year-old male smoker and alcoholic presented with bilateral fixed metastatic neck nodes (T0N3M0). Biopsy of a fixed node showed non-keratinized SCC. Panendoscopy examination did not find primary cancer. The pretreatment value of MS was 2.06 b/c. Radio-chemotherapy resulted in a partial response. He died of a progressive disease in 2003. Overall survival was 30 months. Molecular pathology was performed in 2022: no HPV DNA was detected and no p16 stain was seen in the squamous epithelium.

Discussion

Various methods are used to identify the primary cancer in neck node SCC metastasis of CUP. Aro et al. [14] emphasize the use of panendoscopy including tonsillectomy. Standardized diagnostic workup including PET/CT imaging, bilateral tonsillectomy followed by neck node dissection, and risk-factor adapted therapy improves survival of patients with neck node HPV-positive SCC metastasis from unknown primary malignancy [15]. The appropriate treatment for HNSCC patients with CUP has not been determined. Primary-specific therapy has to be consistent with that of an equivalent known primary tumor. Primary treatment generally consisted of neck node dissection and radio- or radio-chemotherapy. In operable cases, the 5-year overall survival is around 70% with neck nodes dissection followed by radiotherapy with or without chemotherapy. The majority (80%) of patients have HPV-positive cancer [16, 17]. Patients with HPV-positive tumors, particularly in CUP in the HN region, tend to have better clinical outcomes than those with HPV-negative tumors [18, 19]. In the Danish study [19] 60 cases were analyzed. Thirteen of them were HPV-positive and ten of this group had cystic morphology. It is noteworthy that only 1 patient was female but our 3 patients with HPV-positive or cystic histology were women. In the Danish study [19] HPV positivity was an

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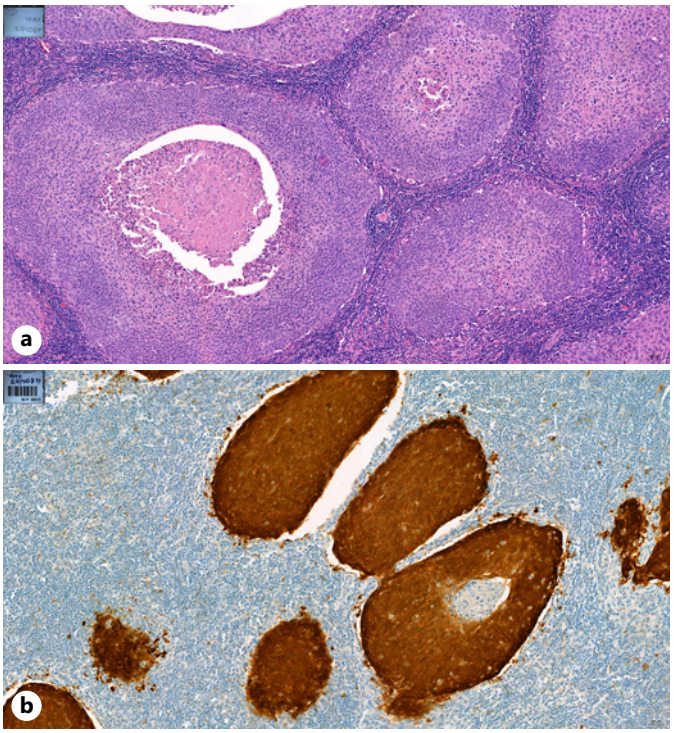

Fig. 2. a Lymph node metastasis of squamous cell carcinoma. Tumor cells show mainly basaloid morphology. Tumor cell nests show extensive central necrosis. Stained with hematoxylin and eosin. **b** Diffuse intensive p16 expression suggests HPV association. Immunohistochemistry for p16.

independent predictor of better overall and disease-free survival. The favorable outcome was related to the fact that the HPV-positive cancers responded better to treatment. It was suggested in the Danish study that the presence or absence of HPV/p16 should be determined early in the diagnostic work-up of CUP patients, as their status may influence treatment decisions. Two of our patients had HPV-positive SCC, but HPV infection was diagnosed more than 15 years after initial presentation. Rassy et al. [6] from the Institute Gustave Roussy emphasized that if a cervical metastasis is considered HPV-positive, the primary lesion is likely to be in the oropharynx, and further diagnostic interventions such as tonsillectomy seem to be mandatory. It remains a matter of debate whether the optimal treatment for extra-nodal extension or advanced lymph node stages is neck node dissection followed by adjuvant radiotherapy or concomitant radio-chemotherapy, or definitive radio-chemotherapy followed by neck node dissection (in the case of positive fluorodeoxyglucose F 18-PET/CT). The clinical significance of gen-expression profiling-based site-specific treatment for CUP patients is also under debate [20]. On the basis of our experience, we believe that neck node HPV-positive or cystic metastases from CUP can be effectively treated with standard multimodality treatment: neck node dissection + radiotherapy + platinum-based chemotherapy. In our patient with bilateral neck node metastasis, the PET/CT showed a suspect area in the base of the tongue but the biopsy did not find cancer. She was treated with bilateral radical neck node dissection followed by radio-chemotherapy and alive without relapse. Her primary cancer site has not been confirmed to date.

Investigation of MS would provide more detailed information on HPV-related cancer risks. The activation of DNA damage repair factors in HPV-positive oropharyngeal cancers has been studied recently. In the cervix, the activation of DNA damage repair pathways is critical for viral replication, but little is known about their role in oropharyngeal (OP) SCC.

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HPV-related OPSCC exhibits increased activation of the ataxia telangiectasia mutated-dependent DNA-related pathway as compared to HPV-negative lesions or normal epithelia in marginal regions. The results suggest that members of these pathways may be important in HPV-induced disease in the oropharynx [21].

Several studies [22–24] have discussed the association between oropharyngeal HNSCC and cystic neck node metastasis. Most cases of cystic SCC metastasis in the upper neck nodes are associated with HPV-positive oropharyngeal primary cancer [23]. In the majority of CUP patients with cystic SCC lymph node metastasis of the HN region, occult primary cancers are localized in the oropharynx [24]. In our female patients, the biopsy of tonsils or base of the tongue did not show cancer. At the Pennsylvania State Medical Center, 20 cases of cystic neck node metastases were studied. Seventeen of these patients had primary tumors arising in the palatine or lingual tonsil. Three were "unknown primary" [23]. Lateral solitary cystic masses in adults often represent occult primary cancers arising from the epithelium within Waldeyer's ring. The poor histological differentiation and the absence of transitions from benign epithelium to malignant carcinoma in lymph node metastases are indicators for metastases of SCC of Waldeyer's ring origin rather than a primary branchiogenic carcinoma. The survival is good with cystic lymph node metastasis [7, 22]. Our patient with cystic morphology is alive more than 15 years after the initial presentation but has progressive distant metastases from a second primary (colon) cancer. The other 2 female patients with HPV-related cancer are still cancer-free.

SCC of the upper aerodigestive tract has high propensity to develop multiple primary malignancies. An explanation for this phenomenon was proposed by Slaughter, who gave the concept of a "condemned mucosa" developing after chronic carcinogenic exposure [10]. However, metachronous three primary malignancies of different histology and distant site origin (as in our 2 cases) cannot be explained by field cancerization. There may be a possible association with genetic and/or immunologic alterations. Vikesa et al. [25] from the University of Copenhagen found that CUP was characterized by chromosomal instability leading to DNA double-strand breaks. Tobacco use is an exogenous risk factor to develop HN cancer and urinary bladder or colon malignancy too. Due to variations in individual susceptibility, only a fraction of all individuals exposed to environmental carcinogens will develop cancer. Endogenous risk factors are also involved in the multifactorial genesis of HNSCC. Individual MS and DNA repair capacity are likely to be candidates affecting an individual's susceptibility to cancer [26, 27]. MS (intrinsic risk factor) plays a role in developing urothelial and colorectal cancers [28, 29]. In our patients with known primary cancer, the majority (19 of 20, 95%) of the metachronous SPC was located in the upper aerodigestive tract [12]. Two of our patients with CUP developed distant site primary cancers: bladder and colon or kidney and colon cancer. Our patient with in situ bladder and in situ colon cancer is living without relapse. The other patient is living with a progressive disease. Patients with HNSCC are at high risk of developing multiple cancers in the upper aerodigestive region, but distant site new primary cancer is a rare event [30, 31]. In our above-mentioned study [12] the number of hypersensitive or not hypersensitive patients was 65 (52.4%) and 59 (47.6%), respectively. In the present study, all patients had elevated MS (b/c > 1.0). The association between MS and CUP has not been previously studied.

Conclusion

We conclude that neck node SCC from CUP is characterized by elevated MS which indicates decreased DNA repair capacity. The clinical significance of MS in CUP requires further examination. HPV positivity or cystic morphology of neck node metastasis from CUP signifies good outcome and can be treated effectively with conventional site-specific therapy. HPV examination should be performed before treatment of CUP.

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Statement of Ethics

This study protocol was reviewed and approved by ETT TUKEB (Medical Research Council), approval code: 19098/2016/EKU (0556/16), approval date: March 30, 2016. Ethics statement (study approval statement and a consent to publish statement): the patients has provided written informed consent to the publication of this case report and accompanying images. Written informed consent was obtained from the next of kin of the patients for publication of the details of their medical case and any accompanying images.

Conflict of Interest Statement

The authors declare no conflict of interest.

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Author Contributions

Bukovszky B. participated in the study design, analysis of clinical events, and drafting the manuscript. Fodor J. conceived the study, participated in its design and coordination, and helped draft the manuscript. Székely G. participated in the bleomycin assay. Tóth E. carried out the immunoassays and histology. Major T. contributed to drafting the manuscript. Oberna F. participated in the study design. Takácsi-Nagy Z. participated in the study design. Polgár C. participated in the study design and helped draft the manuscript. All authors contributed to the article and approved the submitted version.

Data Availability Statement

Clinical and treatment characteristics and ethics statements of the patients can be found in the database of the National Institute of Oncology Budapest, Hungary. The data that support the findings of this study are available through Dr. Botond Bukovszky or Dr. János Fodor.

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Semmelweis Egyetem, Patológiai és Kísérleti Rákkutató Intézet**

Szájüregi leukoplakiák előfordulása és hisztopatológiai vizsgálata

DR. BUKOVSZKY BOTOND*, DR. SIMONFFY LÁSZLÓ*, DR. GYULAI-GAÁL SZABOLCS*,
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Bevezetés: A szájüregi potenciálisan rosszindulatú elváltozások közül az egyik leggyakoribb lézió a leukoplakia. Fontos tisztázni, hogy a klinikailag leukoplakiának diagnosztizált esetek mögött milyen hisztopatológiai diagnózis áll, amely döntően meghatározza a prognózist és a szükséges terápiát.

Anyag és módszer: 2021. április és 2024. április között 75 szájüregi leukoplakiával diagnosztizált betegnél történt szövettani mintavétel a Semmelweis Egyetem Orális Diagnosztikai Tanszék Dento-alveolaris Sebészeti Osztályán. A szövettani minták hisztopatológiai elemzése hematoxilin-eozin festett minták alapján történt a Semmelweis Egyetem Patológiai és Kísérleti Rákkutató Intézetében, bizonyos esetekben immunhisztokémiai vizsgálattal kiegészítve.

Eredmények és megbeszélés: A 75 betegnél a leukoplakiák előfordulási helye, gyakoriság szerinti csökkenő sorrendben: gingiva/fogatlan állcsontgerinc (n = 26), bucca (n = 16), szájfenék (n = 12), nyelv (n = 11), szájpad (n = 6), ajak (n = 2). Ritka esetben multifocalis megjelenés is előfordult (n = 2). A szövettani mintavétel általában próba excisio volt, nem történt meg az elváltozás teljes kimetszése. A hisztopatológiai eredmény 51 esetben hyperkeratosis (dysplasia nélkül), 19 esetben enyhe dysplasia, 5 esetben mérsékelt dysplasia volt. Súlyos dysplasiás eset nem volt. Klinikai homogenitást tekintve 61 esetben homogén és 14 esetben non-homogén volt a leukoplakia. A nem dysplasiás és dysplasiás betegcsoportokat tekintve szignifikánsan gyakrabban állt dysplasia az elváltozás mögött, amennyiben klinikailag non-homogén megjelenést mutatott a leukoplakia ($p = 0,0088$). A vizsgált beteganyagon 75 beteg közül 32 dohányzik, 43 nem dohányzik. Eredményeink alapján a dohányzás nem volt szignifikáns hatással a dysplasia jelenlétére és súlyosságára. A betegek követése folyamatos (félévenkénti kontrollvizsgálat). Átlagos követési idő: 17,4 hónap (tartomány: 1–38 hónap).

Következtetés: A szájüregi leukoplakiával diagnosztizált betegek esetében szövettani mintavétel szükséges a hisztopatológiai diagnózis felállításához, valamint hosszú távú követés javasolt a késői rosszindulatú átalakulás elkerülésére.

Kulcsszavak: szájüregi rákmegelőző állapot, leukoplakia, dysplasia

Bevezetés

A rákmegelőző állapot olyan kóros sejtekkel jellemezhető elváltozás, amely a rák kialakulásának fokozott kockázatával jár, szemben az ép szövetekkel. Ez klinikailag számos olyan elváltozást foglal magában, amelyeknél fennáll a rák kialakulásának kockázata, és patológiai szempontból is több típust különböztethetünk meg. Elkülöníthetjük egymástól a praecancerosus állapotokat és léziókat. Praecancerosus állapot esetén olyan generalizált, általános állapothoz kapcsolódó elváltozásokról beszélünk, amelyekben a malignus elfajulás gyakorisága szignifikánsan magasabb, mint az egészséges egyének esetében (pl. lichen planus, submucosus fibrosis, sideropenia, discoid lupus erythematosus, cheilitis actinica chronica, cornu cutaneum, epidermolysis bullosa, xeroderma pigmentosum, AIDS). A praecancerosus léziók olyan lokálisan kialakuló, morfológiailag átalakult szöveteket jelentenek, amelyekben a daganatos elváltozás kialakulásának valószínűsége nagyobb, mint a normál szövetekben (pl. orális leukoplakia, orális erythroplakia, proliferatív verrucosus leukoplakia). A klinikai gyakorlatban a szájüregi potenciálisan rosszindulatú elváltozások közül gyakran találkozunk leukoplakiával. Fontos tisztázni, hogy a leukoplakia egy klinikai diagnózis, csak a szövettani mintavételt követően állítható fel hisztopatológiai diagnózis. Ez utóbbi jelentősen befolyásolhatja a prognózist és a szükséges terápiát. A szájüregi leukoplakiák esetén a malignizációs ráta 0–64,7% közé esik. A széles tartomány hátterében állnak többek között különbségek a leukoplakia diagnózisának kritériumrendszerében, a lehetséges etiológiai faktorokban, geográfiai elhelyezkedésben, valamint a követési idő hosszában. Malignus átalakulás hosszú, 10 éves követés után is előfordulhat, mely a követés fontosságát támasztja alá [1, 2].

A leukoplakiák kialakulásának háttere sokszor nem tisztázott, melyeket úgynevezett idiopathiás leukoplakiának is nevezünk. Más esetekben oki/rizikó tényezőként szerepel a dohányzás és/vagy alkoholfogyasztás az anamnézisben. Ez a két káros szenvedély szinergista, egymást erősítő hatásáról is beszámolnak az

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irodalomban, különös tekintettel a rákos átalakulásra vonatkozóan. A dohányzás abbahagyása esetén a leukoplakiák sok esetben javulást mutatnak. Emellett krónikus irritáció is okozhatja, súlyosbíthatja az elváltozásokat. A Candida mint etiológiai faktor szerepe vitatott a szájüregi leukoplakiák esetén [3, 4].

A szájüregi leukoplakiák malignus átalakulásának előrejelzésére több paramétert is vizsgálnak, azonban biztos, megbízható marker még nem áll rendelkezésre. A vizsgálatok alapján az elváltozásból vett szövettani minta és hisztopatológiai eredmény (dysplasia jelenléte, dysplasia mértéke), valamint a klinikai megjelenés (homogén vs. non-homogén) azok, amik leginkább segíthetnek a malignus átalakulás előrejelzésében. Ezenkívül felmerül a toluidinkék festés, a Candida albicans jelenléte, és számos molekuláris marker, mint például a p16, Ki-67, p53. Ez utóbbi markerek vizsgálatával számos nyitott kérdés van az irodalomban [2, 4].

A szájüregi leukoplakiák kezelésével kapcsolatban szintén számos a nyitott kérdés. Amennyiben történt szövettani mintavétel, úgy a hisztopatológiai diagnózisnak megfelelően történik a kezelés. Ha nem volt dysplasia az elváltozásban, úgy observatio és hosszú távú követés javasolt, szükség szerint ismételt mintavétellel kiegészítve. Súlyos dysplasia esetén az elváltozás teljes kimetszése javasolt. A sebészi eltávolításkor figyelembe kell venni az anatómiai és funkcionális szempontokat. Lézerterápia szintén szerepel a potenciális kezelések között. Konzervatív terápiaként mind a leukoplakiák kezelésében, mint a szájüregi rákok kialakulásának megelőzésében felmerül különböző lokális hatóanyagok használata, mint például A-vitamin származékok, COX inhibitorok, adenovirus, bleomycin. Ezek hatásosságával és mellékhatásprofiljával kapcsolatban nincsenek egyértelmű eredmények, további vizsgálatokra van szükség. Szisztémás gyógyszeres kezelésről is beszámolnak az irodalomban, azonban ezekben az esetekben sem írtak le egyértelmű kedvező hatást, és ez esetben figyelembe kell venni a szisztémás mellékhatásokat is. A leukoplakiás elváltozások recidívájával szinte minden kezelési mód esetén számolni kell, így az elváltozások teljes eltávolítása esetén is kiemelten fontos a hosszú távú követés [2, 4, 6]. Kutatásunk fő célja a klinikai gyakorlatban előforduló leukoplakiák szövettani hátterének vizsgálata és elemzése. Beteginket hosszú távon követjük, az így született eredmények feldolgozása szintén célkitűzésünk.

Anyag és módszer

A Semmelweis Egyetem Orális Diagnosztikai Tanszék Dento-alveolaris Sebészeti Osztályán 2021. április és 2024. április között 75 szájüregi leukoplakiával diagnosztizált betegtől történt szövettani mintavétel. A hisztopatológiai vizsgálatra a Semmelweis Egyetem Patológiai és Kísérleti Rákkutató Intézetében került sor. A szövettani minták elemzése hematoxilin-eozin (HE) festett minták alapján történt, mely bizonyos esetekben immunhisztokémiai vizsgálattal (p53, p16, ki67) egészült ki. A dysplasiák osztályozása a hazai és nemzetközi protokollnak megfelelően enyhe, közepes és súlyos dysplasia (azaz grade I, II, III) osztályokba történt. Megjegyzendő, hogy más klasszifikációkban low grade és high grade besorolás szerint osztályozzák ezen elváltozásokat [7]. A statisztikai elemzéshez Fisher-exact tesztet használtunk, a $p \leq 0,05$ eredményt tekintettük szignifikánsnak.

Eredmények és megbeszélés

A szájüregi leukoplakiákkal kapcsolatosan számos tanulmány készült mind hazai, mind nemzetközi vonatkozásban, azonban a témában továbbra is sok a nyitott kérdés. A leukoplakiákkal kapcsolatosan kiemelten sok kutatás egy magyar orvoshoz, Bánóczy Jolánhoz köthető [8]. A leukoplakia kifejezés is egy magyar orvostól, Schwimmer Ernőtől származik, melynek jelentése „fehér folt" (görögül: leukos = fehér, plakia = folt), amely a klinikai megjelenésre utal [9]. Habár a szájüregi leukoplakia definíciója és klasszifikációja számos változáson ment keresztül, továbbra is elmondható, hogy ezen elváltozások a szájüregi nyálkahártya fehéres, nem letörölhető elváltozását jelentik, amik más betegségcsoportba nem sorolhatók [10]. A szájüregi fehér foltok esetén kiemelten nagy jelentősége van a differenciáldiagnózisnak. Az *1. kép* szemlélteti a szájüregi fehér foltok diagnosztikájának sémás folyamatát.

Jelen tanulmány a szájüregi leukoplakiák klinikai és szövettani elemzésével foglalkozik. Az *1. táblázat* foglalja össze a 75 beteg jellemzőit. Saját eredményeink alapján nőknél kissé gyakrabban fordul elő szájüregi leukoplakia (nők: 53,5% vs. férfiak: 46,7%), melyet más vizsgálatok is alátámasztanak [11]. A dysplasia jelenlétét tekintve a nemek között nem volt szignifikáns különbség ($p = 0,8052$). Az átlagos életkor 59,5 év volt (tartomány: 23–88). Gyakrabban találkoztunk leukoplakiával idősebb (>50 év) betegeknél (57/75, 76%). Az életkornak nem volt szignifikáns hatása a dysplasia gyakoriságára ($p = 0,5648$). A 75 beteg közül 32 páciens dohányzik, 43 nem. Beteganyagunkon a dohányzás nem növelte szignifikánsan sem a dysplasia gyakoriságát ($p = 0,6208$), sem a dysplasia súlyosságát ($p = 0,3256$) leukoplakiás elváltozások esetén. Azonban a dohányzást jelentős rizikófaktorként tartja számon mind a hazai, mind a nemzetközi irodalom. A leukoplakiák rákos átalakulására szignifikáns hatása van a dohányzásnak [2].

Az anatómiai elhelyezkedés szerinti megoszlást mutatja a *2. kép*. Leggyakrabban a gingiva/fogatlan állcsontgerinc területén találkoztunk leukoplakiával (26/75, 34,7%), ezt követte a bucca (16/75, 21,3%), a szájfenék (12/75, 16%), a nyelv (11/75, 14,7%), a szájpad (6/75, 8%) és az ajak (2/75, 2,7%). Multifocalis (azaz egyidejűleg több anatómiai régióban jelenlevő)

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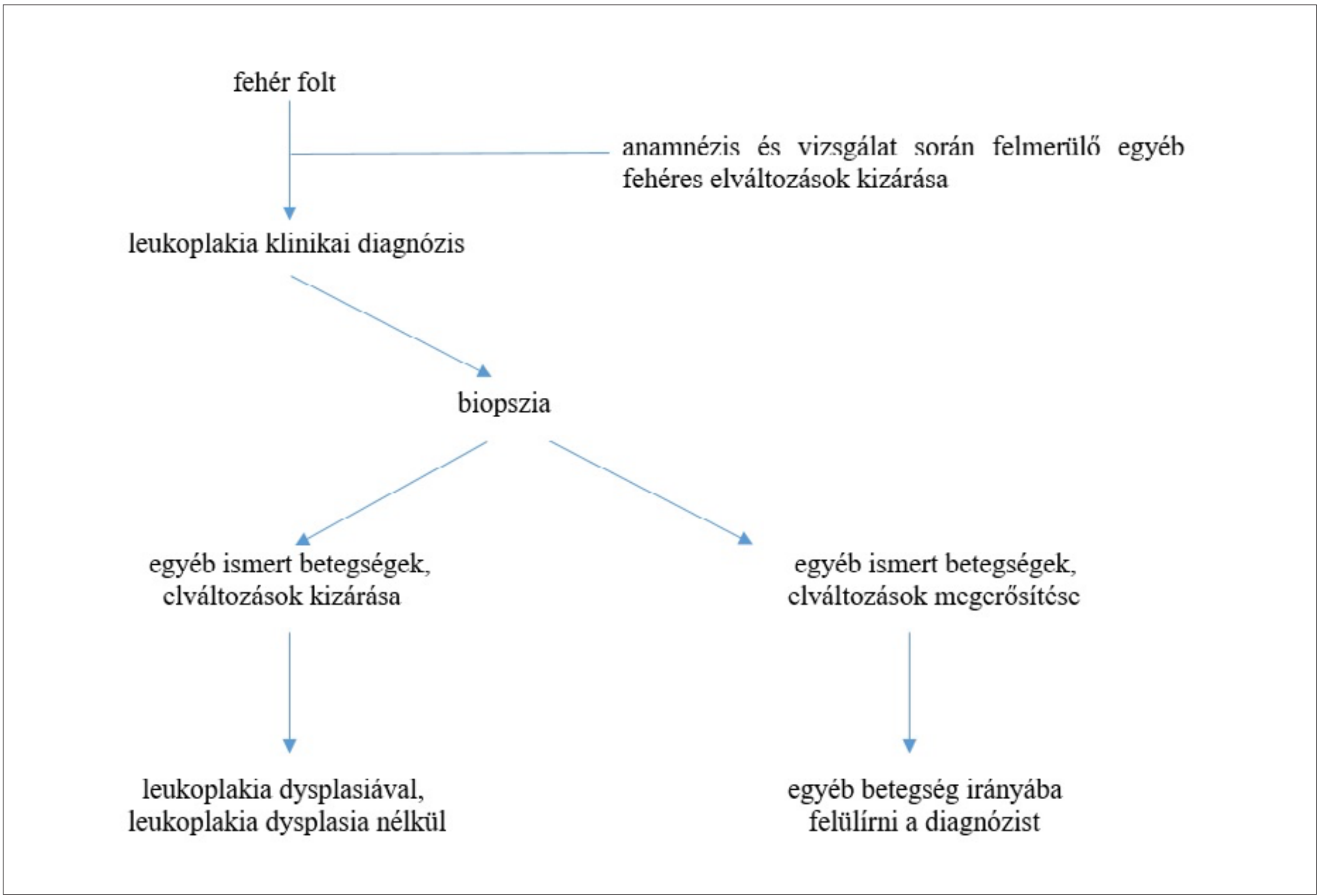
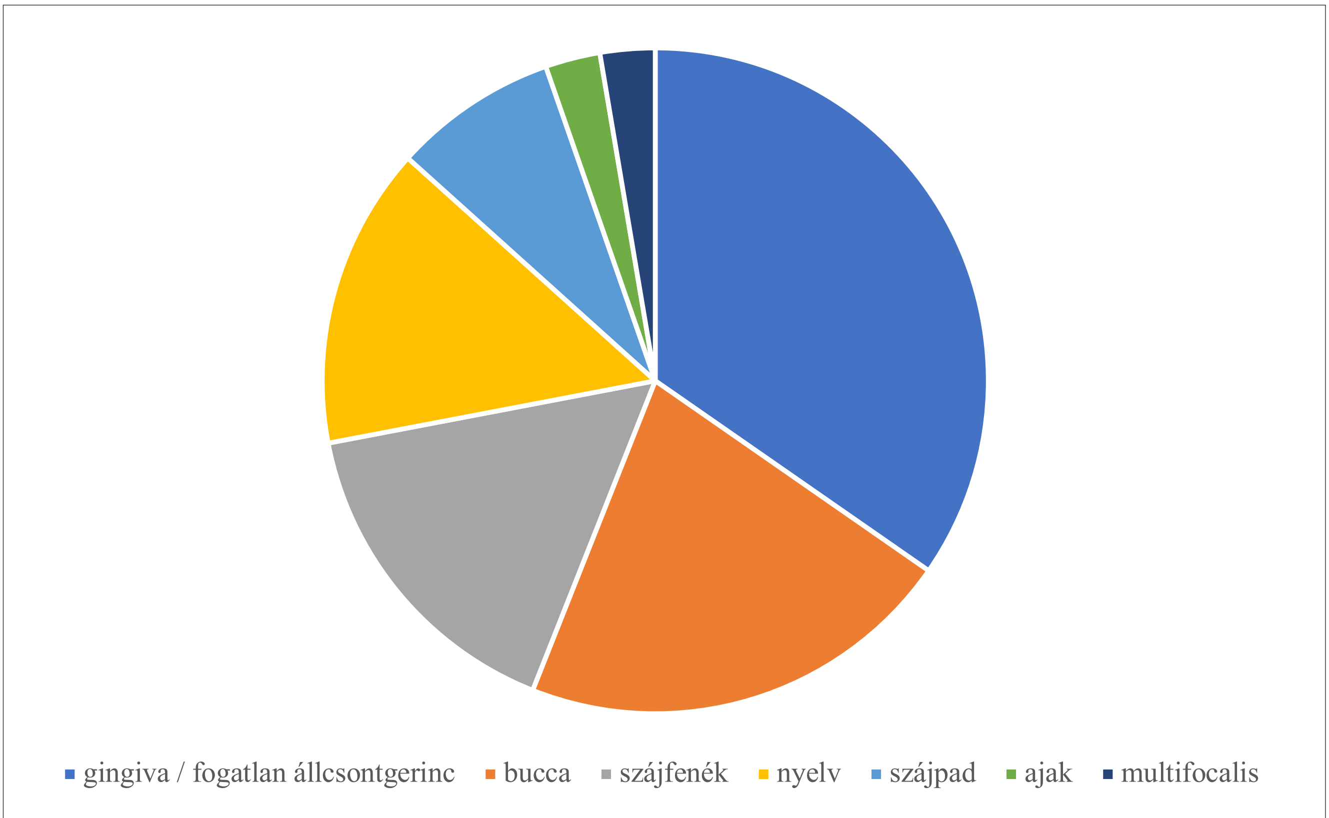


1. kép: Szájüregi fehér foltok diagnosztikus folyamata



2. kép: Leukoplakiák megoszlása anatómiai lokalizáció szerint

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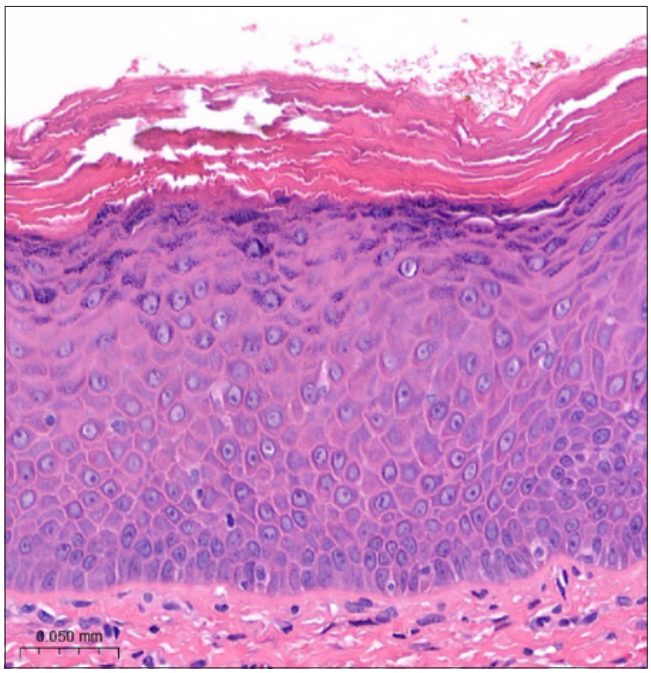
leukoplakia 2 esetben fordult elő (2/75, 2,7%). Egy 2020-as tanulmányban 412 leukoplakiás esetet dolgoztak fel, melyben hasonló eredményekről számoltak be az anatómiai lokalizációk tekintetében [11]. Leggyakrabban esetükben is a gingiva (168/412, 40,8%) területén fordult elő leukoplakia. Ezt követte a nyelv (138/412, 33,5%), a bucca (130/412, 31,6%) és a szájfenék (46/412, 11,2%). Ritkább esetben írtak le leukoplakiát az ajak (19/412, 4,6%) és a szájpad (36/412, 8,7%) területén. Bilateralis megjelenéssel 52 esetben találkoztak (52/412, 14,3%). Az anatómiai lokalizáció befolyásoló tényező lehet a rákos átalakulásra vonatkozóan is. Egy másik beteganyagunkon végzett kutatásunk alapján a leggyakrabban a gingiva területén volt jellemző a malignus transzformáció (46,1%). Megjegyzendő, hogy a fej-nyaki régióban a gége területén levő leukoplakiák esetén a leggyakoribb a malignus transzformáció (50%), azonban ezt a területet nem soroljuk a szájüregi leukoplakiák közé. Multifocalis megjelenés esetén szintén szignifikánsan gyakoribb a rákos átalakulás (47,4%) [2].

1. táblázat

Jellemzők	***n (%)***
Összes beteg	75
Nem	
férfi	35 (46,7%)
nő	40 (53,3%)
Életkor	
≤ 50	18 (24%)
> 50	57 (76%)
Dohányzás	
igen	32 (42,7%)
nem	43 (57,3%)
Lézió típusa	
homogén	61 (81,3%)
non-homogén	14 (18,7%)
Lokalizáció	
gingiva / fogatlan állcsontgerinc	26 (34,7%)
bucca	16 (21,3%)
szájfenék	12 (16%)
nyelv	11 (14,7%)
szájpad	6 (8%)
ajak	2 (2,7%)
multifocalis	2 (2,7%)
Hisztopatológia	
hyperkeratosis	51 (68%)
enyhe dysplasia	19 (25,3%)
mérsékelt dysplasia	5 (6,7%)
súlyos dysplasia	0 (0%)

A leukoplakiák klinikai megjelenése szerint megkülönböztetünk homogén és non-homogén formákat, melyek közül a homogén esetek gyakoribbak. Tanulmányunkban 61 homogén (81,3%) és 14 non-homogén (18,7%) esettel találkoztunk. Rubert és munkatársai nagy esetszámú vizsgálatában meglehetősen hasonló eredményekről számoltak be: homogén esetek 81,6% és non-homogén esetek 18,4% [11]. Egyes tanulmányok szerint a leukoplakia megjelenési formája (homogén vs. non-homogén leukoplakia) szerint eltérő lehet a gyakoriság az anatómiai lokalizációban, a homogén formák gyakoribbak a nyelv és szájfenék területén, míg a proliferatív formák a bucca és gingiva területén fordulnak elő gyakrabban [12]. Saját eredményeink alapján homogén esetek leggyakrabban a gingiva területén, míg non-homogén esetek leginkább a bucca és nyelv területén fordultak elő. A nem dysplasiás és dysplasiás betegcsoportokat tekintve szignifikánsan gyakrabban állt dysplasia az elváltozás mögött, amennyiben klinikailag non-homogén megjelenést mutatott a leukoplakia ($p = 0,0088$).

A szájüregi nyálkahártyából vett minták esetén normális esetben többrétegű el nem szarusodó laphámmal találkozunk. A szájüregi leukoplakiák esetén sok esetben találkozunk ettől eltérően, hyperkeratosist / orthokeratosist / parakeratosist véleményező leletekkel. Amennyiben atypiát mutató sejtek is jelen vannak a fokozott elszarusodást mutató sejtek mellett, úgy dysplasiáról beszélünk. Attól függően, hogy a hám rétegének mekkora területét érintik a dysplastikus sejtek, megkülönböztetünk enyhe, közepes (mérsékelt) és súlyos dysplasiás eseteket. Amennyiben a kóros sejtek a hám teljes vastagságát érintik, azonban nem törik át a basal membránt, in situ carcinomáról beszélünk. Invazív laphámrák esetén a basal membránt is áttörik a rákos sejtek. Saját beteganyagunkon a hisztopatológiai eredmény 51 esetben hyperkeratosis (dysplasia nélkül) *(3. kép)*, 19 esetben enyhe dysplasia *(4. kép)*, 5 esetben mérsé-



3. kép: Dysplasia nélküli szövettani kép, hyperkeratosis (HE festés, 40× nagyítás)

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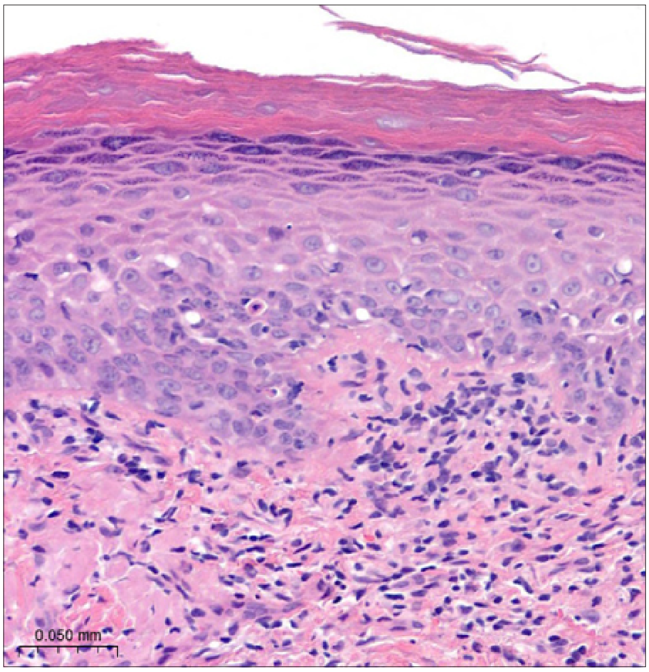
kelt dysplasia volt *(5. kép)*. Súlyos dysplasiás esettel nem találkoztunk. Beteganyagunkon a hisztopatológiai vizsgálatok során bizonyos esetekben immunhisztokémiai vizsgálat is történt (p53, p16, ki67). A kis esetszámú vizsgálat miatt jelenleg nem áll rendelkezésre elég információ ahhoz, hogy következtetéseket tudjunk levonni, azonban ezen markerek elemzése fontos lehet az elváltozások prognózisának, terápiájának megítélésében, így ezekkel kapcsolatosan további vizsgálatokat tervezünk.

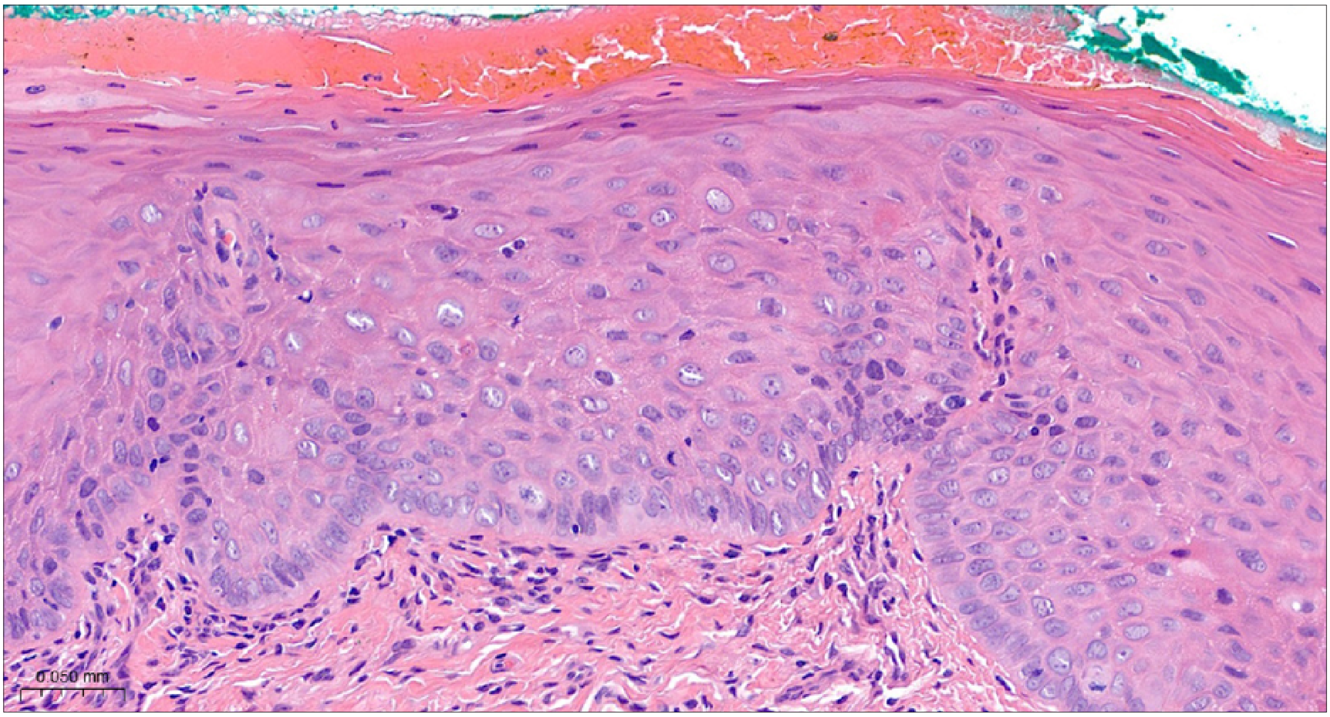


4. kép: Enyhe dysplasiát mutató szövettani kép (HE festés, 40× nagyítás)

Betegeinket folyamatosan követjük féléves kontrollvizsgálatokkal. Átlagos követési idő: 17,4 hónap (tartomány: 1–38 hónap). Amennyiben az elváltozás klinikai megjelenése gyanússá válik, illetve dysplasia volt jelen az első alkalommal vett mintában, úgy ismételt szövettani mintavételt végzünk. Teljes remisszió csak az elváltozások teljes eltávolítása esetén volt, recidíva ezekben az esetekben nem alakult ki. Egy esetben (nyelvszélen levő, enyhe dysplasiát mutató leukoplakia) 2 éves követés után az elváltozás klinikai megjelenése változott, nagyobb területen vöröses komponenseket is mutatott (erythroplakia), így ismételt mintavétel történt, melynek eredménye in situ carcinoma lett. Erythroplakia esetén a szövettani eredmény nagyon gyakran nem csak dysplasia, hanem in situ vagy invazív carcinoma, így az elváltozás teljes eltávolítása szükséges [13].

Következtetés

A fogorvosi–szájsebészeti klinikai gyakorlatban gyakran találkozhatunk leukoplakiás elváltozásokkal, mely esetekben fontos a megfelelő szakrendelésre irányítás, a szövettani mintavétel, a hisztopatológiai eredménynek megfelelő terápia, valamint a hosszú távú követés, szükség szerint ismételt szövettani mintavétellel kiegészítve. A klinikailag non-homogén esetek nagyobb rizikót jelentenek mind a dysplasia jelenlétére, mind a malignus átalakulás valószínűségére. A dsyplasia jelenléte és foka emelkedett rizikót jelent a rákos átalakulásra.



5. kép: Mérsékelt dysplasiát mutató szövettani kép (HE festés, 40× nagyítás)

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Original article

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Incidence and histopathological examination of oral leukoplakia

Introduction: Leukoplakia is one of the most common lesions of potentially malignant lesions of the oral cavity. It is important to clarify the histopathological diagnosis underlying cases clinically diagnosed as leukoplakia, which is crucial in determining the prognosis and the required therapy.

Material and Methods: 75 patients diagnosed with oral leukoplakia were histologically sampled between April 2021 and April 2024 at the Department of Oral Diagnostics, Department of Dento-alveolar Surgery, Semmelweis University. Histopathological analysis of the histopathological samples was performed on the basis of hematoxylin-eosin staining and in selected cases immunohistochemical analysis was performed at the Department of Pathology and Experimental Cancer Research.

Results and discussion: The 75 patients were classified according to the location of leukoplakia in decreasing order of frequency: gingiva/ edentulous jaw ridge (n = 26), buccal (n = 16), floor of the mouth (n = 12), tongue (n = 11), palate (n = 6), lip (n = 2). Multifocal appearance was also seen (n = 2). Histological sampling was usually a partial and not total excision of the lesion. Histopathological findings were hyperkeratosis (without dysplasia) in 51 cases, 19 cases with mild dysplasia, 5 cases with moderate dysplasia. There were no cases with severe dysplasia. In 61 cases were homogeneous and in 14 cases non-homogeneous leukoplakia. Dysplasia was significantly more frequent in clinically non-homogeneous leukoplakia ($p = 0.0088$). 32 of the 75 patients were smokers and 43 were non-smokers. Our results showed that smoking had no significant effect on the presence and severity of dysplasia. Patients were followed up continuously (6 months follow-up). Average follow-up time: 17.4 months (range: 1–38 months).

Conclusion: Patients diagnosed with oral leukoplakia require histopathological sampling for histopathological examination and long-term follow-up is recommended to prevent late malignant transformation.

Keywords: potentially malignant lesions, leukoplakia, dysplasia