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

PhD Thesis

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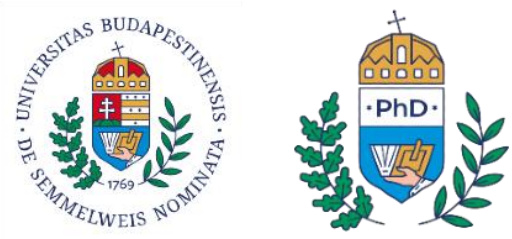

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Budapest, 2025

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. 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 are squamous cell carcinomas. 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. The anatomical location is also of major importance for overall survival. 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. Whereas in the past, HNSCCs were more common, in recent decades there has been a significant increase in the incidence of human papillomavirus (HPV) associated oropharyngeal cancers. In contrast, HPV-negative tumors tend to affect older people and have a significantly worse prognosis than virus-associated malignancies. HPV positivity is a favorable prognostic factor in squamous cell carcinoma of the oral cavity. 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. 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. 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, but also breast cancer.

In patients with head and neck squamous cell carcinoma, 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. 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. However, these factors do not fully account for all cases of SPCs, indicating that genetic susceptibility may also play a role in their development. 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. 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. Better outcomes are predicted by cystic neck node metastases. 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, an oral potentially malignant disorder (OPMD) precedes oral cancers. Leukoplakia (white spot) is a common OPMD of the oral mucosa. Leukoplakia does not often transform into oral cancer, but

significantly increases the risk of developing cancer. 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%. 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. Molecular testing and biomarkers provide a more precise diagnosis and risk assessment on malignant transformation.

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.

3. Methods

At the National Institute of Oncology 432 patients with HNSCC underwent mutagen sensitivity testing. Of the total number of patients, four younger 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. Mutagen sensitivity was tested *in vitro* in lymphocytes by counting chromatid breaks induced by bleomycin. Chromatid breaks were scored in 100 metaphases per sample, and recorded as the mean number 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).

We reviewed the medical records of 253 patients treated for laryngeal or oral leukoplakia at the National Institute of Oncology over a 26-year period. In the computerized institutional database, the number of patients with oral or laryngeal leukoplakia was 221 and 32, respectively. The histological findings were classified as follows: no dysplasia, dysplasia (grade I, II, III) or cancer. 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. 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. Another 75 patients diagnosed with oral leukoplakia had histological samples taken at the Department of Dentoalveolar Surgery, Semmelweis University.

For the statistical analysis, we used the following methods. Intervals to endpoints were examined with Kaplan–Meier method. The log-rank test was used to compare the curves. The impact of potential prognostic factors on event occurrence was assessed using a Cox regression model. Statistical differences in proportions and means were assessed both by the 2-sample t-test and by Fisher exact test. GraphPad Prism (GraphPad Prism version 5.01 for Windows, GraphPad Software, San Diego, CA, USA), Statistica (version 13.5.0.17, TIBCO Software Inc., Palo Alto, CA, USA) and IBM SPSS Statistics for Windows (version 25.0, Armonk, NY, USA: IBM Corp) program packages were used for a data analysis. A p value ≤ 0.05 were regarded as statistically significant.

4. Results

In our research, out of the 432 HNSCC patients (who underwent mutagen sensitivity testing) 124 patients met the criteria mentioned above. Most patients had a stage IV disease (43%). 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%. 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 majority (n=13, 65%) of SPC were HNSCC, the rest of them (n=7, 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. 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). 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 1. The 15-year estimated rate of SPC for not hypersensitive and hypersensitive patients

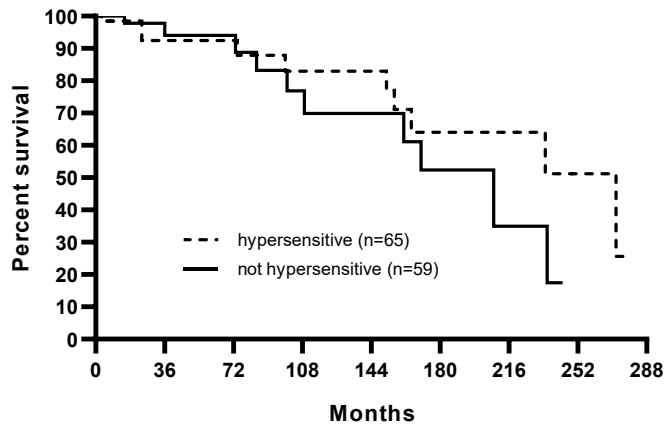

Figure 1. *Second cancer-free survival by mutagen sensitivity.* The 15-year estimated rate of second primary cancer of not hypersensitive or hypersensitive patients was 48 and 36%, respectively (p=0.3743).

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 2). The 45% of SPC was developed after 10 years. 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 $p<0.0001$). The effect of the individual patient characteristics (gender, index cancer site, UICC stage, mutagen sensitivity, radiotherapy) on the risk of SPC was also examined in the Cox proportional hazards model. Results are presented in Table 1. None of the studied variables proved to be a significant predictor of the risk of SPC.

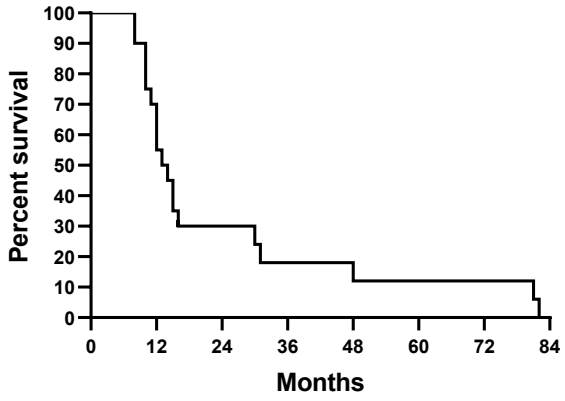

Figure 2. *Cancer-specific survival with second primary cancer (n=20).* The estimated rate of 2- or 3-year survival was 38 and 23%, respectively.

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 (b/c=1.17) was subjected to ipsilateral upper node dissection. The HE staining described well differentiated squamous cell cancer. MRI and panendoscopy examination did not find a primary tumor. Five years later, the neck node metastasis recurred. The histopathologic examination showed

Table 1. *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

squamous cell carcinoma. The molecular pathology examination of neck node metastasis showed HPV16-genotype and p16 over expression by immunohistochemistry. She had distant site metachronous in situ cancers: urothelial cancer and sigmoid colon adenocarcinoma. The patient is alive without relapse (overall survival: 274 months). The second patient (b/c=1.04) had an ipsilateral upper node dissection and one lymph nodes showed squamous cell carcinoma metastasis. The postoperative positron emission tomography/computer tomography (PET/CT) and panendoscopy did not show primary cancer. 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 retrospective molecular pathology of neck node metastasis showed no HPV16 DNA infection or p16 over expression. She is living with a progressive disease (overall survival: 243 months). In the third case (b/c=1.60), both MRI and PET/CT showed bilateral suspect neck nodes. The histopathological examination revealed squamous cell carcinoma metastasis. The panendoscopic examination was also negative. No primary tumor was

found. Tumor cells were p16 positive and the presence of HPV16 was confirmed from tumor DNA. The patient was followed up as an outpatient and after 198 months, there was no evidence of recurrence. In the fourth case (b/c=2.06), the fixed node showed non-keratinized squamous cell carcinoma. The panendoscopic examination did not find primary cancer. He died of a progressive disease. Molecular pathology revealed that no HPV DNA was detected and no p16 stain was seen in the squamous epithelium.

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. 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 average time to the malignant transformation of laryngeal leukoplakia or oral leukoplakia patients

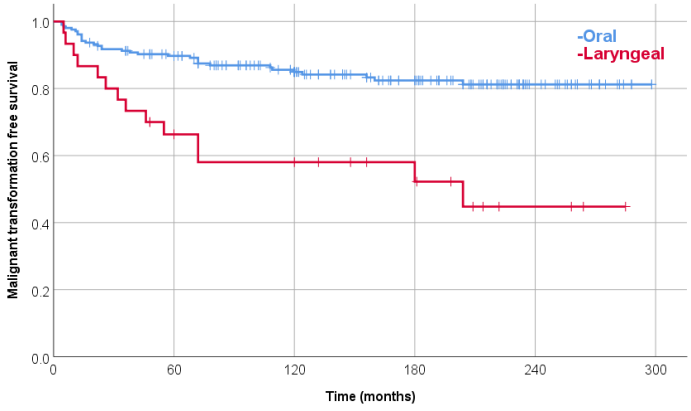

Figure 3. *Malignant transformation-free survival with oral or laryngeal leukoplakia.* The 10-year estimated malignant transformation rate was 15.1% and 42.0%, respectively ($p<0.0001$).

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 ($p < 0.0001$). 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 3). 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. 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). 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 (p=0.016). Of the 75 patients with oral leukoplakia, 32 are smokers and 43 are non-smokers. Smoking in our patient did not significantly increase the

Table 2. *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

incidence of dysplasia (p=0.6208) or the severity of dysplasia (p=0.3256) in leukoplakias. Sixty-one homogeneous (81.3%) and 14 non-homogeneous (18.7%) cases were found. Dysplasia was significantly more frequent in lesions with clinically non-homogeneous leukoplakia (p=0.0088). 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).

5. 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.

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.

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.

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.

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