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PhD THESIS

**IMPACT OF MOLECULAR PROFILE ON ORAL
CANCER TREATMENT AND PREVENTION**

SUMMARY

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Tumors of the oral cavity and pharynx have an increasing incidence from one year to another. Early detection of these pathologies by initiating and implementing a national screening program, to be carried out at the level of dental offices and family doctors would be an important step in the prevention and early detection of various malignant and premalignant conditions located in the oral cavity.

Oral cancers are cancers of the oral cavity, salivary glands, larynx, pharynx and are commonly referred to as squamous cell carcinomas of the head and neck (HNSCC). This cancer is the sixth most common male cancer in the developed world, and the eighth most common in the world. Squamous cell carcinoma is the most common histopathological form of head and neck cancer, accounting for 90% of all oral cancers. Squamous cell carcinomas of the head and neck (HNSCC) are the fifth leading cause of death worldwide from neoplastic disease. Currently, the only known screening method is the visual examination performed by dentists during patient visits or by otolaryngologists.

The aim or *main objective* of personal research is to analyze the molecular profile of tumor cells, and to identify a method for individualizing the treatment of oral cancer, but also an effective screening method in the early detection of oral cancer.

A scientific contribution that my project will make is a molecular classification of oral cancer, which would allow new therapeutic regimens and the proposal of an algorithm for early detection of this pathology, included in a screening program.

Specific objectives:

- Immunohistochemical and molecular characterization of premalignant and malignant squamous cell lesions at the oral level.
- Multimodal evaluation of premalignant and malignant lesions in the oral cavity by applying specific diagnostic and laboratory methods, followed by correlating the results obtained with clinical, morphological, and especially molecular criteria.
- Comparative analysis of the molecular profile of squamous cells in the oral cavity and saliva in patients with diagnosed oral cancer and in a group of patients with risk factors for oral cancer.
- Autofluorescence screening analysis of patients presenting to a Dentistry office through VELscope Vx.

The general part addresses known epidemiological data of head and neck cancer, along with its histopathological and molecular classification. Microangiogenesis and its mechanisms in the development of oral cancer are also described with the latest developments in the field and the involvement of podoplanin in oral cancer therapy.

In the *specific part* we started the research from the premise that malignant tumors of the oral cavity have a growing incidence, as we showed in the general part. Most of these tumors are squamous cell carcinomas, clinically detected in various stages of natural evolution. Despite efforts in early

diagnosis and the contributions of conventional adjuvant therapy, the long-term prognosis of these tumors remains unfavorable, and survival and disease-free survival have not changed significantly in the last two decades.

Recently, numerous molecular markers have been evaluated in order to characterize new prognostic markers and to identify new therapeutic targets. This approach was generated by clinical observations, which reported different incidence of recurrences and different durations of survival for cases with the same histopathological form and degree of differentiation. The vast majority of these studies focused only on the molecular characteristics of tumor cells and took less or don't even study the microenvironment, which is essential for local progression, lymph node and systemic metastasis. Perhaps for this reason the results obtained were not convincing in terms of oral squamous cell neoplasia. On the other hand, markers that have been shown to be useful in *in vitro* and *in vivo* experiments could not be extrapolated to humans, and clinical trials based primarily on tyrosine kinase receptor inhibitors have been disappointing.

One of the peculiarities of tumor progression and metastasis in general and oral squamous cell carcinomas in particular is the formation of new blood and lymph vessels, which are the support for local and distant progression of the tumor. Angiogenesis and lymphangiogenesis are relatively little studied in oral squamous cell carcinomas and precursor lesions, and there is currently a controversy debate about the prognostic value and the existence of real therapeutic targets in these lesions. Thus, the prognostic value of microvascular density is controversial, the types of tumor-associated vessels are not studied, the therapeutic value of VEGF expression and specific receptors is uncertain, and data on lymphangiogenesis are incomplete.

Taking these into account, we set some objectives related to the molecular profile of these tumors in terms of protein expression, exploring angiogenesis and lymphangiogenesis, also benefiting from the fact that unlike malignant tumors developed in other organs, tumors of the oral cavity form a group. morphologically homogeneous, in which the criteria of histopathological diagnosis and gradation are well standardized and accepted. In this regard, we aimed to evaluate: the prognostic value of blood and lymph vascular microdensity, and the role in predicting lymph node metastases. Evaluation of the types of blood vessels associated with the tumor, as a potential therapeutic target, together with the elucidation of the origin of the neoformation vessels. We studied some of the growth factors that stimulate the formation of new vessels, but at the same time there are already specific inhibitors. Last but not least, we made an epidemiological analysis of the prevalence and incidence of squamous cell carcinoma in the head and neck area, emphasizing the peculiarities of our geographical area.

We hope that the results obtained by us during this research will generate answers to these questions and will contribute to a better understanding of the molecular biology of oral squamous cell carcinomas, opening new perspectives for adjuvant therapy in these patients.

STUDY I. EPIDEMIOLOGICAL ANALYSIS OF THE INCIDENCE OF ORAL CANCER IN CENTRAL AND EASTERN EUROPE

Oral cavity, laryngeal and pharyngeal cancer

Oral cavity, laryngeal and pharyngeal cancer accounted 686328 new cases in 2012 and caused 375665 deaths in the same year. The highest incidence was in West of Europe where ASR incidence/100000 people was 13, and in more developed countries the incidence was higher than in the less developed countries, but the mortality was higher in the less developed countries. The incidence of oral, laryngeal, and pharyngeal cancer of 11.5 is higher in CEE than the global incidence 9 or than incidence for the more developed countries 10.4. The mortality from oral and pharyngeal cancer is higher in CEE than the other studied regions.

Lip and oral cavity cancer

300373 (4) of new lip and oral cavity cancer was diagnosed in the world, 199550 (3.7) in the less developed countries, 100823 (4.7) in more developed countries and 23765 and incidence 5 from CEE. The global incidence of lip and oral cavity at men 5.5 is higher than the woman 2.5. In CEE the incidence at men is 9.1 and is the third highest incidence from the regions on the world after Melanesia 22.9 and South- Central Asia 9.9. At woman in CEE the incidence is 2, which is the fourteen places from the world regions, and is smaller than West of Europe 3.2, more than in developed regions 2.8 or than less developed regions 2.5. From the countries of CEE, the highest incidence is in Hungary 9.7, followed by Slovakia 6.5. In Romania in 2012 was diagnosed 1847 (5.4) of new cases of lips and oral cavity and died 1001 (3). 145323 people died worldwide because of lip and oral cancer that represent a mortality/100000 people of 1.9, in CEE died 12516 people (2.6). The mortality was also higher for men than the woman, in the world 2.7:1.2, in CEE 5.1: 0.7. The highest mortality/100000 people in countries from CEE was found in Hungary 4.4, followed by Slovakia and Belarus 3.2. Hungary has the highest mortality rate from the CEE countries 23.2, followed by Romanian men 19.4 people and Slovakia men 18.4 people. For woman, also the highest mortality was found in Hungary 8.4 people, followed by woman from Poland 4.2 people and Slovakia 3.9 people.

Pharyngeal cancer

229078 (3.1) new pharyngeal cancer was diagnosed in the world in 2012 and 146936 (1.9) died because of it. In CEE were diagnosed 13216 (3) people and died 9159 (2). The incidence of pharyngeal cancer in CEE, was lower than the incidence found in West of Europe 4.8, more developed regions incidence 3.1 and less developed regions incidence 3.2, but the mortality was higher than West of Europe 1.7, more developed regions 1.3 and lower than less developed regions 2.2. The same as in lips and oral cavity cancer Hungarian men are the second most affected people from the world (14.1), Romanian men are on the fifth positions. From the CEE countries, the highest incidence was found in Hungary 7.8, followed by Romania 6.1 and Slovakia 5.5. The highest incidence of pharyngeal cancer was found at Hungarian men

14.1, followed by Romanian men 12.1 and Slovakian men 10.7. At woman, the highest incidence was found at Hungarian woman 2.4, followed by Czech woman 1.5 and Moldavian woman. The mortality from pharyngeal cancer in CEE countries was found in Hungary 4.9, followed by Slovakia 4.1 people and Romania 3.8. The mortality was highest at the Hungarian men 9.2, followed by Slovakian men 8.3 and Romanian man 7.4. The mortality at women in the studied countries was low, but the highest was found in Hungary 1.3, Bulgaria 0.8, Romania 0.6, and Slovakia 0.6.

Larynx cancer

156977 (2.1) new cases of larynx cancer were diagnosed on the world and 83376 (1.1) people died because of it. In CEE countries were diagnosed 16494 (3.6) people and died 2.2, this region being the fourth most affected region from the world after Caribbean (4.2), Southern Europe (3.7) and Western Asia (3.6). The incidence and mortality for larynx cancer is highest in CEE than the West of Europe 2.7/ 0.8, more developed countries 2.7/1.1 and less developed countries 1.9/1.1. The Hungarian men has the five incidences from the world 12.3 and the Romanian men with seven incidences 10.4 and on the first place is Cuba with 10.4. Like at lips and oral cavity cancer and at pharyngeal cancer in Hungary are the highest incidence/ 100000 people from the CEE countries 6.3 (12.3 for men and 1.4 for woman). On the second place is Republic of Moldova 5.4 (11.4 for men and 0.3 for woman) and on the third place is Romania 5 (10.4 men and 0.4 woman). For woman, the second affected country is Poland 0.9, and third is Czech Republic 0.5. The mortality/100000 people are highest and Moldavian men 7.6 followed by Bulgarian men 6.7, Hungarian and Romanian men 6.2.

Partial conclusions. The incidence of OSCC has an increasing trend for pharynx cancer, plate of mouth and a decreasing for larynx cancer and lip cancer. OSCC incidence and mortality in Central and East Europe has variable differences between countries, Hungary, Romania, and Slovakia experiencing the highest rates from this region. The differences between sexes are also extremely high, males from CEE are very affected, being the third most affected males from the world, but the women are on the fourteen places. The most affected people from this region are Hungarians, especially men. The alcohol consumption and tobacco is still the main risk factors for OSCC in this region, and most of the countries implemented the WHO recommendation for public anti-tobacco strategy and anti-alcohol consuming. The extremely high mortality because of the OSCC in CEE can be reduce by a preventive national or regional strategy with health education for population with informed by the OSCC early symptoms, and by screening on the dentistry, or improve the diagnosis methods.

STUDY II. HISTOPATHOLOGICAL AND IMMUNOHISTOPATHOLOGICAL STUDY OF PREMALIGNANT AND MALIGNANT SQUAMOCELLULAR INJURIES AT THE ORAL LEVEL

The present study included 56 patients in whom the histopathological diagnosis was squamous cell carcinoma with varying degrees of differentiation, located in the oral cavity. The specimens came from the Oral and Maxillofacial Surgery Clinic, the ENT and Oral Surgery Clinic of the University of Medicine and Pharmacy in Timișoara, during 2017-2020.

This study has three directions of research:

- Morphological evaluation and degree of differentiation
- Podoplanin expression and lymphatic microvascular density in tumor cells
- Molecular classification

RESULTS

In all cases included in the study, the resection margins showed normal tissue, including the covering epithelium and the lamina propria. The epithelium of the oral mucosa consisted of cells arranged in layers, organized basally, intermediate, and superficial, and as seen under normal conditions, occasionally with areas of parakeratosis. The lamina propria was formed of loose and densely disordered connective tissue, with rare mobile connective cells and numerous blood and lymphatic vessels with a regular, relatively wide lumen (*fig.1 a*). The epithelial component reveals the organization of cells in distinct layers, the most numerous being those of intermediate type, with medium dimensions, relatively equal, polygonal, which form adhesion junctions between them. In all cases we noticed the presence of the granular layer (*fig.1 b*). In the basal part of the epithelium we noticed the presence of isolated cells with chromophobic pericellular halo and intensely colored nuclei.

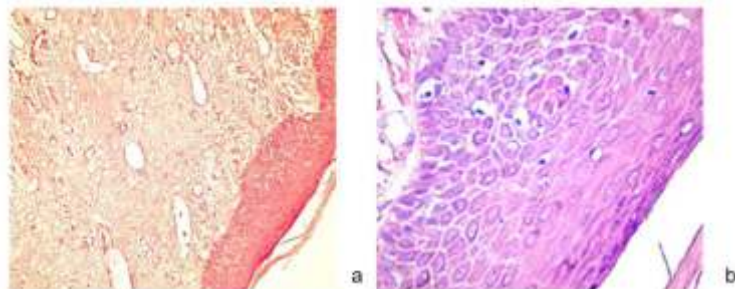


Figure 1. Normal oral epithelium, overall appearance with areas of parakeratosis and vessels in the lamina propria (a, x100). Detail on the normal covering epithelium, which reflects the organization of the cells in the form of ordered layers and the presence of the granular layer (b, x400). Hematoxylin-eosin staining.

We note focal epithelial hyperplasia in 14 of the 56 cases in the epithelium adjacent to malignant proliferation. This lesion, which has been shown for many years to be induced by the human papilloma virus, can also occur as an isolated nodular lesion. In our cases it was characterized microscopically by the numerical increase of the cells of the basal layer, which is thicker, the cells being arranged in several rows. The intermediate layer consisted of numerous rows of ballooned cells with pale colored or chromophobic cytoplasm (*fig. 2*). In the basal part of the hyperplastic

epithelium we found the presence of small conjunctival papillae with dilated blood vessels containing blood figurative elements. This aspect supports the early onset of angiogenesis during the initiation of carcinogenesis and the natural evolution of oral squamous cell neoplasia, an aspect reported by other authors. The lamina propria corresponding to focal epithelial hyperplasia showed collagen fibers arranged in thick bundles and increased density of inflammatory infiltrate compared to normal mucosa.

Tumors diagnosed with squamous cell carcinoma showed diffuse proliferation of tumor cells located in the form of lobes, cords, nests, or compact, large beaches of different sizes, invading the stroma. All carcinomas included in this study were invasive, and of these, the tumor exceeded 5 mm depth in 33 of the patients.

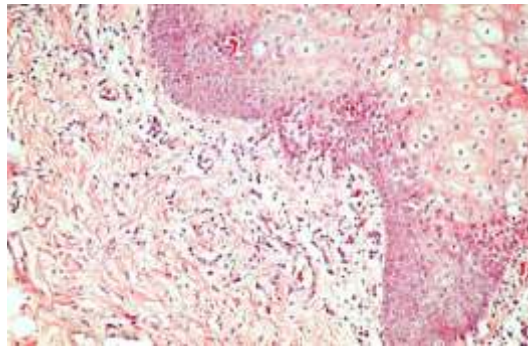


Figure 2. Focal epithelial hyperplasia characterized by increased number of basal cells and ballooning of cells in the intermediate layer. Hematoxylin-eosin staining, x400.

Intracellular and extracellular keratinization is one of the characteristics of squamous cell carcinoma, which we observed in 29 and 36 of the cases included in the study, respectively. The defining aspect of this process is represented by the keratosis and parakeratosis pearls, which we observed in all well-differentiated cases and only in one of the poorly differentiated cases. The parakeratosis and keratosis beads had different dimensions, being formed by cells arranged concentrically, with various degrees of degeneration in the central area, which in some cases had a microcystic appearance (*figure 3a*). Sometimes the cavity was bounded by apparently viable tumor cells (*figure 3b*). In well-differentiated tumors, these areas often present as intensely acidophilic, amorphous masses, occupying large areas within the proliferation (*figure 3c*).

The individual tumor cells were characterized by aspects of cellular-nuclear pleomorphism (moderate and severe), but it drew attention to the relative uniformity of the nuclei, round or oval with euchromatic appearance, pale colored, with reduced basophilia. Hyperplasia and hypertrophy of the nucleoli, to which anisonucleolosis is added, have been constantly observed, especially in cases with poor or undifferentiated differentiation. Only in certain situations and focally I did notice the existence of apoptotic cells, with intensely colored nucleus and homogeneous acidophilic cytoplasm. The nucleo-

cytoplasmic ratio, although altered, is rarely supraunitary. We observed intracytoplasmic keratinization in 29 of the 56 cases and it was characterized by intense and homogeneous acidophilia which sometimes tends to mask the contour of the nucleus (*figure 3d*). Even under these conditions, at the nuclear level there are nucleoli enlarged in volume, multiple and different in size for the same nucleus, an important criterion for assessing cellular malignancy.

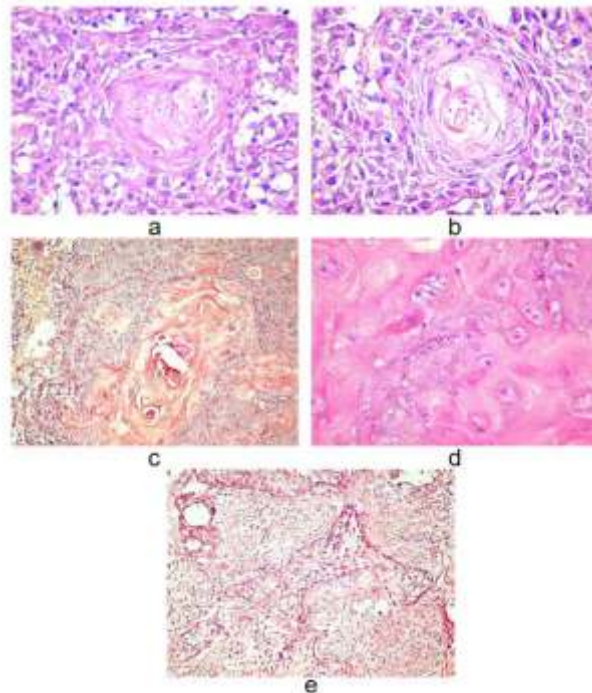


Figure 3. Keratotic pearl (a). Microcystic transformation delimited by tumor cells (b). Massive accumulation of keratin in the central area of the tumor (c). Massive intracellular keratinization, intense and homogeneous acidophilia (d). Extensive and irregular ranges of tumor cells, the peripheral ones being morphologically different from those in the central area (e). Hematoxylin-eosin staining.

Podoplanin expression and lymphatic and tumor cell microvascular density

In order to evaluate the above listed elements, we studied the expression of podoplanin / D2-40 in the 56 cases of squamous cell carcinoma of the oral sphere, in which we took biopsies from the level taken from the lip (n = 36), the oral mucosa (n = 5), tongue (n = 9) and pharynx (n = 6). We monitored the presence, morphology and density of lymphatic vessels, the presence of lymphovascular invasion and D2-40 expression in tumor cells. Of the cases included in the study, 19 had regional lymph node metastases.

Podoplanin / D2-40 expression in tumor cells has been reported for several human tumors, including squamous cell carcinomas of the head and neck. On our material, the immunoreaction was positive with varying degrees of intensity in 41 of the 56 cases (73.21%). In evaluating the positive immunoreaction, we considered the distribution pattern of the final reaction

product and the percentage of positive tumor cells. In this way we identified two subgroups of positive tumors, aspects correlated with the degree of differentiation. The positive reaction in most tumor cells, a model of expression that we called diffuse, was identified in moderately and poorly differentiated tumors, the highest intensity being observed at the proliferation front and in the immediate vicinity of the lymphatic vessels (*figure 4a*). In these cases, the final reaction product was located predominantly membranous, but also diffuse in the cytoplasm of isolated tumor cells (*figure 4b*). The second distribution pattern, often associated with G1 tumors, is also heterogeneous, but mimics the basal cell layer in the normal epithelium as a localization. In these cases, the reaction was restricted to the membrane, and in very well differentiated cases, with extensive keratinization, the reaction was very weak in the tumor cells, limited to a fine membrane lysate.

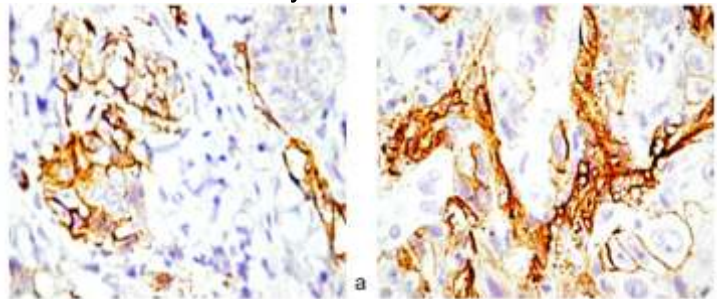


Figure 4. Expression pattern of D2-40 in diffuse and heterogeneous tumor cells at the invasion front, in the vicinity of a lymphatic capillary (a). Membrane and cytoplasmic pattern in isolated cells (b). X400.

Partial conclusions. The study of 56 cases of oral squamous cell carcinoma using the podoplanin / D2-40 immunoreaction revealed the following aspects: D2-40 positive lymph vessels were present in the tumor and peritumor area, but their morphology is different. LMVD is higher for intertumoral vessels than for peritumoral vessels, and for both values were higher than for normal mucosa. The D2-40 method is particularly useful for the detection of lymphovascular invasion, not identified on hematoxylin-eosin stained sections. Tumor cells express D2-40 in 73.21% of cases, an aspect correlated with the degree of differentiation and local invasion.

Molecular classification

In the present study we investigated retrospectively 42 of the cases included in the study, selecting a homogeneous group in which the main inclusion criterion was the histopathological form, respectively squamous cell carcinoma with different degrees of differentiation. The 42 cases had the following location: larynx 27 cases, pharynx 12, and oral cavity 3. Biopsies were included in paraffin for morphological and molecular evaluation at the protein level. From each case and block were made serial sections with a thickness of 3 μ m, glued on slides for immunohistochemistry. The initial sections were stained with hematoxylin-eosin, the standard variant, based on which the histopathological form, the degree of differentiation and the

extension of the well-differentiated squamous component (incidence and extent of keratosis and parakeratosis beads) were reanalyzed. The other sections underwent a fully automated immunohistochemical procedure (Bond Max, Leica Microsystems, Milton Keynes, UK). The selection of tumor markers was made based on existing data and their prognostic value in the evolution of malignant tumors and the impact they have in shaping the therapeutic attitude.

The antibodies used in this part of the study were: epidermal growth factor receptor (EGFR, Novocastra), cytokeratin 5, Bcl-2 and E-cadherin. In cases where cytokeratin 5 and EGFR were positive, methods for p53 protein and stem cell growth factor receptor, CD117 or c-kit, were performed to identify cells with stem element potential. The immunohistochemical procedure used a biotin-free working and visualization system (Bond Refine Detection System, DAB, Leica, Microsystems), and the final reaction product was stained brown at nuclear level (for p53 and p63), cytoplasmic (for cytokeratin 5 and CD117) and membrane (for EGFR and E-cadherin). Co-localization of p53 with EGFR, and p63 with CD117 was performed by standard double immunohistochemistry to which Bond Refine Detection System Red was added.

For microscopic evaluation we applied the accepted interpretation criteria for these markers, based on the intensity of the reaction and the percentage of positive tumor cells. Correlations of immunohistochemical results with tumor stage, degree of differentiation, TNM were performed to anticipate the prognostic and potential therapeutic role of these investigations, in cases with HNSCC.

RESULTS

All cases included in the study were squamous cell carcinomas with or without the presence of keratosis and parakeratosis beads. Of the cases that constituted this group, 64% were in the larynx, 28.5% in the pharynx and 7.3% in the oral cavity, more precisely in the tongue. Most of the differentiation in the included cases was G2, representing 73.8%. The incidence of patients with G1 was 7.14%, and with G3 19%. Among the clinic-pathological parameters with prognostic relevance, we analyzed the T element, most cases being T3 (45.2%) and T2 (38%). Only one case was Q1 (2.3%), signaling once again the deficient early detection, and Q4 6 cases (14.5%). The tumors aggressivity also results from the distribution of cases according to the N element: 64.28% cases with N1, 26.19% with N2 and 9.52% with N3. Remote metastases were noted in four cases, 90.47% being classified as M0.

In the first stage we evaluated the immunohistochemical expression of the above-mentioned markers EGFR, Bcl2, cytokeratin 5, p53 and E-cadherin. EGFR was expressed in many cases, the final reaction product being exclusively membrane or cytoplasmic with significant membrane intensification. Given the type of marker, the membrane reaction product was considered essential for interpretation (*figure 5a* and *figure 5b*).

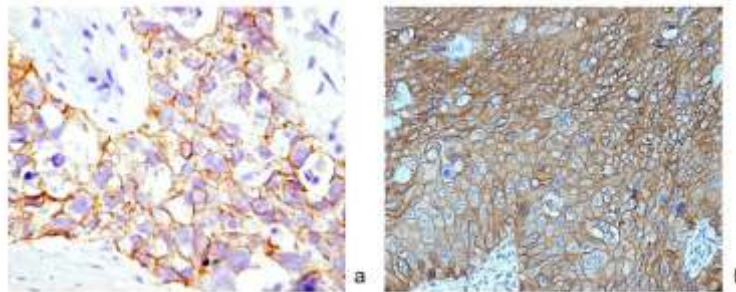


Figure 5. Immunoreaction for EGFR. Restricted membrane (a) and cytoplasmic restricted reaction final product (b). x400.

When reporting the results, we signal the great variability of expression of the markers mentioned and exemplified above. In the general evaluation of cases with HNSCC we obtained statistically significant correlation between the degree of differentiation and Bcl2 expression ($p = 0.033$), between the parameter M and EGFR expression ($p = 0.039$), and between EGFR expression and p53 ($p = 0.001$). p53a was expressed in a representative number of tumor cells in half of the total number of cases studied. All p53 positive cases expressed EGFR, but on the other hand, not all EGFR positive cases expressed p53. We did not identify other significant differences between positive / negative p53 cases with the other markers investigated.

EGFR expression in cases with HNSCC revealed a high percentage of positive cases, respectively 85.71% showed intense or moderate reaction. Half of the EGFR-positive cases co-expressed p53. In EGFR positive cases we observed the highest number of 5 cytokeratin positive cases. EGFR-cytokeratin 5 co-expression was noted in 61% of all cases and this combination draws attention to a particular subtype. It is well known that the normal stratified epithelium in the head and neck region expresses EGFR restricted to basal cell cells. In HNSCC, EGFR overexpression was frequently associated with cytokeratin 5, suggesting that this subgroup would be a basal cell derivative by activating potential progenitor cell factors. Next, we considered it useful to characterize this subgroup by investigating the expression p63 and c-kit, CD117. Co-expression CD117 and p63 was identified in 75% of positive cytokeratin 5 / EGFR cases, i.e. 42.28% of the total cases investigated. Based on these aspects we can try to propose the basal-like subtype of HNSCC, defined as EGFR positive - cytokeratin 5 positive, CD117 positive and p63 positive. For this group we found a statistically significant correlation between G and the expression p63 ($p = 0.01$) and between T and the expression CD117 ($p = 0.04$).

Despite the small number of negative EGFR cases reported in the current study, they form a particular subtype, the cases being negative for all markers studied. Occasionally, cytokeratin 5 and E-cadherin were positive in 50% of cases in this group, but a separate subtype cannot be established

based on them. Given the small number of cases in this group, we did not evaluate statistical parameters for EGFR-negative cases. Bcl2 and p53 appear to be expressed with high frequency in EGFR-positive cases and are constantly negative in those with EGFR-negativity. Correlating the data obtained based on the expression p53, Bcl2 and EGFR, we can define three distinct subtypes: EGFR + / p53– / bcl2–, EGFR + / p53 + / bcl2– and EGFR + / p53 + / bcl2 +. From the statistical analysis of the EGFR + / p53 + / bcl2– subtype, it appears that in this group, EGFR expression correlates with lymph node status ($p = 0.04$) and CD117 / p63 expression ($p = 0.04$). Cytokeratin 5 was positive in 59.5% of cases, the most common co-expression being observed with EGFR and CD117 / p63. For these cases we note the inverse statistical relationship between the T element and p53 / EGFR expression ($p = -0.03$) and the positive correlation between T and CD117 / p63 expression in tumor cells ($p = 0.01$). Lymph node status was significantly correlated with Bcl2 expression ($p = 0.04$). Due to the small number of cases with metastases we did not statistically evaluate element M.

Partial conclusions. Our results support the need for molecular classification of HNSCC only based on tumor marker expression and gene analysis. By immunohistochemical methods we identified with certainty the subtype EGFR + / cytokeratin 5+, subsequently sub qualified according to the expression p53, Bcl 2, and CD117. We consider that studies on large series of patients are needed to allow the application of a combination therapy of targeted type.

STUDY III. SCREENING OF ORAL CANCER BY THE VELSCOPE VX METHOD

To carry out this study we performed the conventional direct visual examination and the comparative examination with VELscopeVx which allows the visualization of an area with loss of fluorescence, of premalignant lesions encountered in a dental office.

The Velscope cannot diagnose cancer directly, it is done only with the help of biopsy and histopathological examination, but it is an adjunct to oral examination and is considered by specialists in the field a fantastic non-invasive screening device for early detection of premalignant lesions. or malignant, which are not visible to the naked eye.

The use of the VELscope is based on the early detection of dyspathic cells, which have a different absorption capacity than normal cells, thus highlighting the smallest early tissue changes.

The VELscope Vx system used allowed: real-time examination of the oral cavity; identification and diagnosis of oral lesions that require further treatment; malignant and premalignant lesions by highlighting local loss of fluorescence; the camera, which allowed the documentation and evaluation of the lesions.

Oral cancer is usually detected after conventional direct visual examination by a doctor.

RESULTS

Characteristics of subjects from the study

Patient no.	Age	Gender	Lesion location	Optical clinical examination	Intensity and the size of the loss of visual fluorescence	Histopathological
1	34	F	lateral face of tongue	Erythematous area well demarcated	Well defined dark area	
2	43	F	lateral face of tongue	1 cm tumor, erythematous area	Well defined dark area	
3	48	M	Right antero-lateral face of tongue	Partly erythematous partly demarcated	Well defined dark area	
4	53	M	Palatine mucosa	Partly erythematous well demarcated	Well defined dark area	
5	76	F	Vermilion tongue	Partly demarcated, ulcerous, erythematous area	Well defined dark area	Scuamo celular carcinoma
6	68	M	Upper left gum	Partly erythematous well demarcated	Well defined dark area	
7	51	F	The soft and hard palace	Partial demarcated, erithematous area	Well defined dark area	
8	46	M	Dorsal face tongue	Partly erythematous well demarcated	Well defined dark area	
9	73	M	Upper anterior gum	Partly erythematous well demarcated	Well defined dark area	
10	82	F	Hard palace	Partly erythematous well demarcated	Well defined dark area	
11	68	M	Lower right gum	Partly erythematous well demarcated	Well defined dark area	
12	64	F	The floor of the mouth	Partly erythematous well demarcated	Well defined dark area	

Detection of premalignant oral lesions before they progress to malignancy is necessary to improve survival rates for oral cancer. Several

studies have shown that VELscope Vx is a simple, non-invasive and inexpensive test of the oral mucosa that can help the experienced clinician find pre-malignant oral / malignant lesions in the early stages and the correct location for biopsies in the altered mucosa.

In the literature, the use of VELscope Vx is considered to have limited ability to discriminate high-risk lesions from malignancy from those without risk of malignancy. In any case, conventional visual inspection under normal incandescent light, followed by biopsy of suspicious lesions, will remain the gold standard for the immediate future. Future approaches to optical imaging could involve a real-time quantitative assessment to determine a diagnosis of oral mucosal lesions, rather than simply highlighting the presence of abnormalities, making the possibility of "optical biopsy" a clinical reality.

CONCLUSIONS AND OWN CONTRIBUTIONS

1. The incidence of head and neck cancer has an increasing trend for pharyngeal and oral floor cancer and decreasing for laryngeal and lip cancer.
2. The incidence and mortality from head and neck cancer in Central and Eastern European countries are different depending on the country, with Hungary, Romania and Slovakia having the highest disease and mortality rates in the region.
3. The gender gap is also large, with men in Central and Eastern Europe being severely affected, being the third most affected region in the world and women in fourth place. The most affected by head and neck cancer are the people of Hungary, especially men.
4. Alcohol and tobacco use are the most important risk factors for men in Central and Eastern Europe, and most countries in the world have so far implemented WHO recommendations for year-round public health policies against smoking and alcohol consumption.
5. High mortality from head and neck cancer in Central and Eastern Europe can be reduced through a national or regional strategy combined with health education for the entire population with information on the early symptoms of head and neck cancer, introduction of screening for oral cancer at the family doctor and / or dentist, or by improving diagnostic methods.
6. The study of 56 cases of oral squamous cell carcinoma using the podoplanin / D2-40 immunoreaction revealed the following aspects: - D2-40 positive lymphatic vessels were present in the tumor and peritumor area, but their morphology is different.
7. LMVD is higher for intratumoral vessels than for peritumoral vessels and for both values were higher than for normal mucosa.
8. The D2-40 method is particularly useful for detecting lymphovascular invasion, unidentified on hematoxylin-eosin stained sections. Tumor cells express D2-40 in 73.21% of cases, an aspect correlated with the degree of differentiation and local invasion.

9. The VELscope VX offers the possibility of presumptive diagnosis of higher accuracy, which can assess potentially malignant lesions, which after performing the biopsy and histopathological examination help guide the patient to various methods of treatment, be they surgical, radioactive or chemotherapy.
10. The vision of detecting precancerous or even cancerous lesions is a positive and desirable one, but patients' reluctance sometimes speaks for itself. Fear of receiving bad news, or a serious diagnosis, prevents many people from choosing to perform these tests.

Own contributions

Our results support the need for molecular classification of HNSCC only based on tumor marker expression and gene analysis. By immunohistochemical methods we identified with certainty the subtype EGFR + / cytokeratin 5+, subsequently sub qualified according to the expression p53, Bcl 2, and CD117.

Future directions of research

We consider that studies on large series of patients are needed to allow the application of a combination therapy of targeted type.