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DOCTORAL THESIS

HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF INVASIVITY IN SQUAMOS CARCINOMAS OF THE TONGUE

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CONTENT

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squamos carcinomas, tongue, pathogenesis, progression, invasivity, prognosis, histopathology, immunohistochemistry

INTRODUCTION

Oral squamous carcinoma is the most common form of cancer of the head and neck and, at the same time, it is also a major cause of morbidity and mortality due to cancer affecting worldwide, in 2016, more than 1 million people. Oral localisation of squamous and especially lingual carcinoma is, by far, associated with the highest risk of death and this is due both to the morphofunctional features of these areas and to the advanced loco-regional stage in the moment of presentation of these patients to the doctor.

Due to the richness in blood vessels and lymphatic vessels, squamous carcinomas of the tongue are prone to metastasis, even in the early stages of the disease, which is why their prognosis is severe. Among the most important factors of aggression were recorded: the histological degree of malignancy, the size of the lesions, the depth of the invasion and the patterns of invasivity, the early presence of lymphoganglionic metastases.

Our study proposed a thorough investigation from the epidemiological, clinical-imagistic, histopathological, immunohistochemical and statistical point of view of 57 patients diagnosed with squamous carcinomas of the tongue between 2015 and 2017.

The epidemiological profile of the investigated patients shows the prevalence of oral squamous carcinomas in the 7th decade of life (49.13%), in men (the ratio of men / women is 1:1.37), most often with the origin in the mobile portion of the tongue (2/3 of the investigated cases). The clinical study frequently highlighted asymptomatic evolution of such cases. Pain (54.38%) and ulceration (43.86%) were the dominant symptoms when they became symptomatic. In addition, about 1/3 of these patients had clinically detectable metastases at the time of diagnosis.

Histopathologically, we showed the presence of several subtypes, namely: conventional, acantholytic, basaloid, sarcomatoid and verrucous, the conventional type predominating in more than half of the cases (54.4%). Particularly for this location, the histopathological study revealed an aggressive profile of these tumors, most of the cases being diagnosed in advanced stages pTNM (III-II), as moderately differentiated histological forms, with moderate Brandwein-Gensler histological risk score, but also with lymphoganglionic metastases, with perineural invasion and resection margins invaded in about 1/3 of the investigated cases.

The immunohistochemical study revealed the important potential of invasivity for particular localisations of oral squamous carcinomas, markings of the types CXCR4, MMP-9, D2-40, CD10 and F-actin, taking into account prognostic markers. At molecular level, an immunoprofile such as this reveals intrinsic potential, its own motility capacity and the coexistence of tumor cells, especially those before invasion, with stromal cells for

the locoregional extension of squamous carcinomas of the tongue. In addition, the phase between these markers can be molecular targets of a much more effective treatment than the classic one, significantly increasing the survival rate in some kind of patients with cancer.

CHAPTER I. EPIDEMIOLOGY OF THE ORAL CANCERS - presents the latest incidence data, mortality, as well as geographical, age and gender distribution and topographic localisation of these cancers.

CHAPTER II. PATHOGENESIS OF THE ORAL CANCERS - first provides general data about the process of oral carcinogenesis, the intrinsic molecules and molecular mechanisms underlying the initiation, growth and progression of such cancers. Thus, they are discussed in distinct subchapters: (1) alterations in the expression of oncogenes / oncoproteins; (2) alterations in the expression of tumor suppressor genes; (3) the role of HPV infection in oral carcinogenesis; (4) the role of immortalisation in oral carcinogenesis; (5) the role of angiogenesis in oral carcinogenesis.

CHAPTER III. TUMORAL INVASIVE PROCESS, ITS PARTICULARITIES IN THE SQUAMOS CARCINOMAS OF THE HEAD AND NECK - first presents general data on cancer growth and progression, then discusses the main types of patterns of invasive tumor growth and finally presents data of the particularities of the process of molecular invasivity in squamous carcinomas of the head and neck.

STUDY OBJECTIVES

1. The evaluation of the epidemiological, clinical-imagistic and histopathological characteristics of the squamous carcinomas of the tongue from the prognostic point of view, for a period of 3 years, by quantifying the following parameters:

- epidemiological: gender and age of the patients, location of tumors,environment of origin, risk factors;

- clinical-imagistic: symptomatology, clinical signs, latency period of the disease, imagistic aspects of locoregional invasivity using computerised tomography and nuclear magnetic resonance;

- histopathological: the histological subtype, according to the WHO classification (2005); the degree of differentiation; the appreciation of the pattern of tumor invasion; the presence / absence of perineural invasion; the quantification of inflammatory infiltrate; the assessment of the histopathological risk score; the assessment of the status of the resection margins; the assessment of the pTNM stage of the disease, according to WHO recommendations (2005);

2. Establishing the invasive immunoprofile of these tumors by investigating:

- the influence of the stromal-parenchymal component interaction in oral squamous carcinomas in the invasivity process, respectively the CD10 antibody;

- the investigation of the intrinsic local invasion potential of lingual carcinomatous cells using markers: CXCR4, MMP2, MMP9 and podoplanin;

- the establishment of the degree of involvement in the epithelio-mesenchymal transition (EMT) process of mesenchymal transcription factors using the Snail marker;

- the innvestigation of markers responsible for the intrinsic motility of cancer cells, respectively of the F-actin marker;

3. The outline of a specific statistical invasive profile of squamous carcinomas of the tongue based on the statistical analysis of the results obtained through the aforementioned studies.

CHAPTER IV. MATERIALS AND METHODS - provides data about the studied material and the methods used in research.

RESEARCHED MATERIAL - from the cases of the Laboratory of Pathological Anatomy of the Emergency Clinical County Hospital of Craiova and it was represented by the archived paraffin blocks. The study was extended over a period of 3 years, a number of 57 cases of lingual tumors being selected during the period 2015-2017, that were the object of the histopathological study.

USED METHODS

The epidemiological study aimed to collect the following data of the patients included in the study: gender, age, location of tumors, environment of origin and risk factors. The clinical-imagistic study followed the history of the disease, the results of the general and

local clinical examination (swelling, lingual asymmetry, the macroscopic appearance of the tumors, the presence of lymph-ganglionic metastases), the results of the computerised tomography and nuclear magnetic resonance, regarding the size of the tumors and their locoregional extension.

THE HISTOPATHOLOGICAL ANALYSIS followed morphological variables such as: the histopathological subtype, the degree of differentiation; the assessment of tumor invasion pattern, according to Bryne M et al. classification, the presence / absence of perineural invasion, the quantification of inflammatory infiltrate, the assessment of histopathological risk score according to Brandwein-Gensler et al., the presence / absence of vascular invasion and / or lymphatic invasion, the status of the resection margins and the assessment of the pTNM stage of the disease, according to WHO recommendations (2005).

THE IMMUNOHISTOCHEMICAL STUDY investigated tumor reactivity for a series of markers addressed to the study: (1) the influence of the stromal-parenchymal component interaction of the oral squamous carcinomas during the invasivity process, respectively the CD10 antibody; (2) the investigation of the intrinsic local invasion potential of lingual carcinomatous cells using markers: CXCR4, MMP2, MMP9 and podoplanin; (3) the establishment of the degree of involvement in the epithelio-mesenchymal transition (EMT) process of mesenchymal transcription factors using the Snail marker; (4) the investigation of the markers responsible for the intrinsic motility of cancer cells, respectively of the F-actin marker.

We resorted to simple reactions for which we used as a working method - the LSAB technique (Labelled Streptavidin-Biotin2 System) and the kit of the company Dako (Redox, Romania - K0675) and the development was done with chromogen DAB (brown color). The immunomarking of the above-specified antibody was semi-quantitatively quantified using the IRS score - Remmele & Stegner (1987), obtained by multiplying the results of the quantitative score based on the count of the marked cells (score 1 = <25% marked cells); score 2 = 26-49% marked cells; score 3 = 50-74% marked cells and score 4 => 75% marked cells) with those of marking intensity (score 1 = weak; score 2 = moderate and score 3 = strong).

In the immunohistochemical study we used concentrated antibodies, whose main characteristics are shown in the table below:

Antibody	*Clone/ Producer*	*Dilution*	*Antigenic exposure*	*Positive control*
CD10	Mouse, Monoclonal, 56C6, DakoCytomation	1:50	0.1 M citrate, pH 6	Lymph node
CXCR4	Rabbit, Policlonal, Thermo scientific	1:500	0.1 M citrate, pH 6	Squamous carcinoma
MMP2	Rabbit, Policlonal, Santa Cruz Biotechnology(sc-8835-R)	1:50	0.1 M citrate, pH 6	Granulation tissue
MMP9	Mouse, Monoclonal 7 Biotechnology (sc-13520 B)	1:50	0.1 M citrate, pH 6	Granulation tissue
Podoplanin	Mouse, Monoclonal D2-40, Dako	1:100	0.1 M citrate, pH 6	Lymph node
F-Actin	Mouse, Monoclonal, LifeSpan Biosciences	1:50	0.1 M citrate, pH 6	Prostate
Snail	Rabbit, Policlonal, Abcam	1:50	0.1 M citrate, pH 6	Breast carcinoma

Antibodies used in the study of squamous carcinomas of the tongue

For statistical analysis were used Student t tests, Anova, chi square and Pearson, using SPSS 10 software and the images were acquired using the Nikon Eclipse 55i microscope and the Image-Pro Plus software.

CHAPTER V - RESULTS

The epidemiological analysis revealed the wide distribution of the cases from the 3rd to the 8th decade. The maximum incidence was registered in the 7th decade, with a number of 28 patients, representing about half of the cases (49.13%), followed by the 6th decade with 14% and the 5th decade respectively with 12,28% of the patients investigated. The mean age for the whole group studied was 55.81 ± 14.98 and the median age was 63 years. It was observed the prevalence of cases, especially in men, 33 of the patients were men, which represented 57.89% of the total number of patients diagnosed with squamous carcinoma of the tongue. The majority of the cases had the mobile parts of the tongue affected, respectively 38 cases, which represented 66.66%. The base of the tongue was affected in only 19 cases, representing about 1/3, being in second place after the tongue

margins (35.1%). At the same time, we recorded the prevalence of the cases, especially of the patients coming from the rural area. There were 35 cases, which represented 61.40% of the cases investigated. The most frequent risk factor was alcohol, being recognized anamnestically in 17 cases, accounting for almost 30% of the investigated cases. In second place was the association of alcohol and smoking, highlighted in 13 cases, accounting for about 23% of the total patients included in the study group.

Clinical-imagistic study. The results of the clinical trial indicate pain as the most common symptom, recognized by the patients included in the study. Thus, this symptom was reported in 31 cases, representing about 54%. In the second place were the cases in which the patients reported persistent ulcerative lesions, initially asymptomatic, subsequently painful and about 50% of the cases were associated with bleeding.

From the anamnestic data collected from only 31 cases (54.38% of the investigated cases), we were able to establish the latency period of the disease. Thus, we found an average duration of about 9 to 14 months from the onset of the symptomatology and the presentation of the patients for diagnosis. In 19 of the 57 cases investigated, the patients presented with clinically detectable lymphoganglionic dissemination from the moment of diagnosis, which represented about 33.33%. In these cases, lymph nodes were larger than 1 cm, with increased consistency and adherence to the adjacent planes. The most commonly imagistic investigations was the computerised tomography (CT). Only 27 patients (47.37%) from the total of 57 could benefit from this type of investigation. In all these cases, the primary tumor was highlighted and the completion of the examination after the injection of the contrast solution allowed a better estimation of the lesion limits and the estimation of the lymphoganglionic dissemination.

The histopathological study. According to the WHO criteria (2005) for the classification of head and neck tumors [Johnson N et al., 2005], the 57 cases of squamous carcinoma studied could be classified into the following histopathological varieties: conventional squamous carcinoma, verrucous squamous carcinoma, squamous carcinoma, acantholytic squamous carcinoma, basaloid squamous carcinoma and sarcomatoid squamous carcinoma. The most frequent histopathological subtype of squamous carcinoma of the tongue, within our casuistry was the conventional subtype diagnosed in 31 cases, representing about 54.4% of the total investigated cases. In the second place, at some distance, was the acantholytic subtype, with a number of 14 cases, representing about 24.56% of the investigated casuistry.

Using the Broder criteria [Broders AC, 1941] for microscopic classification of cancers, the 57 cases of squamous carcinomas of the tongue studied, most of them belonged to the category of moderately differentiated squamous carcinomas - G2, respectively 25 of the 57 cases, which represented 44% of the casuistry. Based on Bryne et al.'s criteria (1989) for grading malignancy in relation with the tumor invasion subtype of

the host tissue, we noted that most of the cases of squamous carcinoma of the tongue were included in type-3 invasion pattern, respectively 24 cases, representing about 42.1% of the total casuistry. Based on the density of the inflammatory infiltrate from the tumor-host tissue, the pattern of neoplastic invasion and the presence or absence of perineural invasion, we established the Brandwein-Gensler score of histological risk [Brandwein-Gensler et al., 2005] for the investigated cases and recorded the Brandwein-Gensler moderate grade score in 28 cases, accounting almost half of the casuistry. Based on the WHO criteria for pTNM grading of tumors, with localisation in the oral cavity, we realised for the studied cases that the most frequently diagnosed pTNM stage was the stage III, with a number of 23 cases, which represented about 40,35%.

Immunohistochemical analysis. The evaluation of the impact of stromal-parenchymal component interaction of the invasivity with CD10 marker revealed that the tumor immunoreactivity was variable, the lowest reactivity being detected in the well differentiated forms, while the highest tumor reactivity was detected in the moderately differentiated forms (IRS = 3.34 ±1.64), the general pattern of reactions being diffuse, somewhat more intense on the periphery of the tumor proliferation and on the invasion front. The subcellular pattern of the reactions was predominantly membranous, but it was also cytoplasmic. At the tumor tissue level we recorded reactivity for CXCR4 in all investigated cases (100%), but the IRS scores ranged from 1 to 9, the mean IRS value was 4.54 ±1.90. A maximum reactivity (IRS = 9) was observed in 2 cases of CSO, with a differentiation degree G1 and also G2, developed at the mobile part of the tongue, at a woman over 60 years old and with stage III pTNM, respectively at a man over 60 years old and with stage IV pTNM. The pattern of the tumor reactivity was predominantly cytoplasmic and membranous. The tumor reactivity for MMP-2 was recorded in 46 cases (85.18%), the average IRS recorded score being 1.18 ±1.41, with values ranging from 0 to 4 (recorded in one case at a person over 60 years, female gender, at the mobile part of the tongue, with differentiation degree G2 and located in stage IV pTNM). The pattern of the tumor reactions for MMP-2 was heterogeneous, varying from cytoplasmic, present especially in the well differentiated forms, to a cytoplasmic one, visible especially in the moderately differentiated and, respectively, the nuclear one present only in the weak differentiated tumor forms. At tumor level, the reactivity for MMP-9 was higher, compared to that registered for MMP-2, the mean value of IRS for the investigated cases being 4.02 ± 2.16. The IRS scores for the investigated marker ranged from 0 to 9. Tumor reactivity was higher in the moderately and weak differentiated forms, compared to the well differentiated ones, in the first ones the reactivity being cytoplasmic and in the last ones membranous. In the tumor samples, the highest reactivity for CXCR4 was recorded in the cases of moderately differentiated squamous carcinoma (IRS = 5.96 ±2.44), the subcellular pattern of the reactions being predominantly membranous and less cytoplasmic.

In addition, in such cases, the reactivity was recorded mainly at the level of the invasion front, the reactions being diffused in the cells from the composition of the neoplastic proliferations at this level. In the tumor tissue, the reactivity for F-actin was by far the highest compared to D2-40 and CD10. Similarly, the well differentiated forms of squamous carcinoma of the tongue showed the lowest reactivity (IRS = 2.63 ±1.01), the reactions for F-actin being diffused in the neoplastic proliferation (Fig. 5.82), somewhat more intense towards the invasion front. Reactivity was present in cells from the periphery of the proliferation, missing from the keratotic pearls.In the tumor tissue, the reactivity for Snail was by far the lowest, compared to the other investigated markers. The lowest reactivity was recorded in the well differentiated forms of squamous carcinoma of the tongue (IRS = 0.27 ±0.42), the reactions for this marker being present in the keratotic pearls and in the cells from the periphery of the carcinomatous proliferations. The highest reactivity was recorded in the weak differentiated forms (IRS = 1.85 ±1.12), the predominant pattern being cytoplasmic and the reactivity being more evident, especially in the invasion front.

CHAPTER VI - DISCUSSIONS

In 2012, worldwide, the age-standardized rate of oral cancer incidence was estimated at 2.7 new cases per 100,000 persons, with notable differences related to gender, age and geographic region [Shield KD et al., 2017]. It is worrying, however, that in recent years there has been an alarming increase in the incidence of tongue cancer at young people, especially among white people [Ng JH et al., 2017; Patel SC et al., 2011; Saba NF et al., 2011]. Literature data indicate that, in the early stages, oral cancers, regardless of location, develop asymptomatically, being accidentally discovered during examinations performed in the dental offices [Scully C, Bagan J, 2009]. In advanced stages, oral cancers are most commonly diagnosed under the clinical aspect of painful ulcers [McGurk M, Scott SE, 2010; Pentenero M et al., 2011]. Strictly in their tongue localisation, the most commonly encountered clinical aspect is an extended ulceration, followed by a vegetative one. At the time of diagnosis, it appears that about 40% of the patients also have locoregional lymphoganglionic metastases.

Furthermore, if primary tumors have a diameter bigger than 4 cm, lymphoganglionic dissemination is present in 90% of the cases. CT scan is a standard method for detecting primary tumors and their bone invasion. However, it has its limitations in detecting small and well differentiated tumors, even in the case of contrast examination [Blatt S et al., 2016]. Performance can be improved by contrast CT, which allows a better evaluation of the neoplastic infiltration of adjacent tissues and especially the musculature and also allows the differentiation between local recurrence and non-specific tissue changes after radio- and chemotherapy [Blatt S et al. , 2016; Trojanowska A, 2011].

Regarding the tongue localisation, a series of studies have shown that the MRI investigation can specify with great accuracy the depth of the invasion of tongue cancers, having a great value in predicting the possible lymphoganglionic dissemination [Alsaffar HA et al., 2016; Park JO et al., 2011; Singh A et al., 2017].

Most studies indicate conventional squamous carcinoma as the most common neoplasm developed in the oral epithelium, with etiological factors including smoking and chronic ethylism [Johnson N et al., 2005; Mignogna MD et al., 2004]. According to the last classification of the oral cavity tumors [Johnson N et al., 2005] besides the conventional type, 6 other histopathological varieties of squamous carcinoma have been described: verrucous carcinoma, basaloid carcinoma, fusiform cell carcinoma (sarcomatoid carcinoma), papillary carcinoma, acantholytic carcinoma and adenoscuamos carcinoma.

The management of the patients with squamous carcinoma of the tongue continues to be based primarily on TNM staging, despite the numerous histological, immunohistochemical and molecular parameters with prognostic value reported in the literature [Bello IO et al., 2010; da Silva SD et al., 2011]. However, its major role, both prognostic and therapy planning, is significant especially for advanced stages of squamous carcinoma of the tongue, while its usefulness for early T1 / T2N0M0 forms is low [Kantola S et al., 2000; Piazza C et al., 2014a; Po Wing Yuen A et al., 2002]. Some authors, in order to increase the prognostic value of the TNM system, have also proposed the incorporation of the depth of the tumor invasion into the system [Hubert Low TH et al., 2015; Piazza C et al., 2014].

The aggression of tongue cancers is the degradation of the extracellular matrix, achieved by the competition of matrixmetaloproteinases secreted by the tumor cells and some of the associated tumor stromal cells [Yu T et al., 2011]. In regulating the secretion of these enzymes, chemokinin CXCR4 also intervenes, with numerous studies showing that tumor expression levels of CXCR4, MMP-2, MMP-9 and MMP-13 in oral squamous carcinomas have been linked [Yu T et al., 2011 ; Chu CY et al., 2007; Ishikawa T et al., 2006]. In the study undertaken by Piatelli A et al. it was shown that between the stromal expression of CD10 and the rate of lymphoganglionic metastasis, the rate of recurrence and the histological degree of differentiation, there would be statistically significant correlations [Piatelli A et al., 2006].

The degradation of the matrix is the basis of the aggressiveness of the tongue cancers. The authors suggested that CD10 would play important roles in differentiation, growth and tumor invasion, facilitating the development of metastases in oral squamous carcinomas. Studies in the literature indicate variations in CXCR4 expression in oral squamous carcinomas, in percentages ranging from 28.6% to 100% [Almofti A et al., 2004; Delilbasi CB et al., 2004; Ishikawa T et al., 2006; Muller A et al., 2006], variations that can be explained by using different expression quantification systems for this marker and /

or using different clones of CXCR4 [Lee JI et al., 2009]. A number of studies have indicated a direct correlation between the level of CXCR4 expression in primary tumors and the expression of this marker in lymphoganglionic metastases, respectively [Ishikawa T et al., 2006; Lee JI et al., 2009; Albert S et al., 2012; Al-Jokhadar et al., 2017]. Most authors did not find statistically significant correlations between MMP-2 levels and different clinical and morphological parameters, including: gender, tumor stages, nuclear grading, tumor differentiation degree, smoking exposure [Patel BP et al., 2007; Mishev G et al., 2014; Yorioka CW et al., 2002]. However, most studies indicate a positive correlation between the level of MMP-2 expression and lymphoganglionic metastasis and the prognosis of patients with squamous carcinomas of the head and neck [Patel BP et al., 2007; De Vicente JC et al., 2001; Hong SD et al., 2000; Cat K et al., 2005; Pu Y et al., 2014]. The vast majority of authors observed increased levels of MMP-9 expression in squamous carcinomas of the head and neck, including the oral level, but the prognostic significance of this expression remains a contradictory topic. It is supported by the idea that MMP-9 is not for sure the factor involved in the process of tumor invasivity at the head and neck level and that it can play fluctuating roles in this process [Vilen ST et al., 2013]. Numerous studies have shown an overexpression of D2-40 in oral squamous carcinomas and a correlation of its expression with the degree of tumor differentiation, the maximum levels of expression being recorded in cases with low tumor differentiation [A G D et al., 2017; Inoue H et al., 2012; Patil A et al., 2015; Prasad B et al., 2015]. Most studies indicate a significant correlation between the level of D2-40 expression in oral squamous carcinomas and the rate of cervical lymphoganglionic metastasis [Huber GF et al., 2011; Kreppel M et al., 2012; Chung MK et al., 2010; Yuan P et al., 2006]. A data review on F-actin expression in oral squamous carcinomas did not reveal any study that aimed at this. However, indirect data regarding the participation of the co-partners of this cytoskeletal protein in the process of invasivity and metastasis of oral squamous carcinomas have been highlighted in several studies. Thus, in the study by Yamada S et al, it was found that cortactin overexpression is correlated highly with aggressive phenotypes of oral squamous carcinomas [Yamada S et al., 2010]. Although, in oral squamous carcinomas, immunoreactivity for Snail has been reported predominantly in the invasion front, even though its expression rate in such cancers has been low [Zidar N et al., 2008]. Also, the association of Snail expression with the metastasis potential in oral squamous carcinomas was not observed [Franz M et al., 2009; Schwock J et al., 2010] although esophageal squamous carcinomas have been reported to have significant correlations between Snail expression at the invasion front and lymphoganglionic metastasis rate, as well as the tumor stage [Usami Y et al., 2008].

CHAPTER VII - CONCLUSIONS

- The epidemiological profile of the patients investigated shows the prevalence of oral squamous carcinomas in the 7th decade of life (49.13%), in men (the ratio of men / women is 1: 1.37), most often with their origin in the mobile part of the tongue (2/3 of the casuistry).

- The clinical study highlighted the generally asymptomatic evolution of such cases. Pain (54.38%) and ulceration (43.86%) were the dominant symptoms when they became symptomatic. About 1/3 of these patients had clinically detectable metastases at the time of the diagnosis. In less than half of the cases (47.37%), CT investigations were possible and in less than 20% MRI investigations.

- Histopathologically, we showed the presence of several subtypes, respectively: conventional, acantholytic, basaloid, sarcomatoid and verrucous, the conventional type predominating in more than half of the cases (54.4%). Reported at the Brandwein-Gensler histological risk score, almost half of the cases showed moderate scores, being slightly more aggressive, moderately and weak differentiated forms, with grade 2 inflammatory stroma and perineural invasion. In less than 1/3 of the cases, we noted the presence of lymphoganglionic metastases, the most frequently diagnosed stage N being N1 (53% of the cases with lymphatic designation). Most patients were diagnosed in advanced stages of the disease, 40.35% in stage III-pTNM and 33.33% in stage II-pTNM.
- Immunoreactivity for CD10 was more evident in the moderately and weak differentiated forms, particularly on the invasion front, proving its involvement in the invasive process of squamous carcinomas of the tongue, secondary facilitating interactions between tumor cells and the adjacent stroma.

- For the CXCR4 marker we recorded some of the highest immunoreactivity scores, being second after the F-actin marking. At the tumor level, the immunomarking was more evident for the moderately and weak differentiated forms, especially in the case of metastatic ones.
- Although, the tumor reactivity for MMP-2 was low, lower than the markings with CXCR4 and MMP-9, we recorded different patterns of reactivity depending on the degree of differentiation of the squamous carcinomas under study. At the tumor level, the immunoreactivity for MMP-9 was higher than MMP-2, with the moderate and weak differentiated forms having the highest reactivity.

- Statistically, we observed the existence of moderate direct correlations at tumor level between the expressions CXCR4 and MMP9 and respectively between MMP-2 and MMP-9. Thus, the tumor cells from the squamous carcinomas of the tongue would have an intrinsic bias of invasivity conferred by the expression of the 3 markers, especially on the

invasion front, more evident in the moderately and weak differentiated forms and in the metastatic cases.

- D2-40 marking was more evident in the tumor cells from the periphery of tumor proliferation and in the invasion front of the moderately differentiated forms of squamous carcinoma of the tongue, the acantholytic forms and the cases with lymphoganglionic dissemination presenting maximum reactivity. For the F-actin marker, the highest reactivity was recorded especially in the acantholytic forms and in the cases associated with the desmoplastic stroma and / or inflammatory tumor stroma, the reactions being more evident in the invasion front.

- Statistically, we noted the existence of a correlation between the tumor reactivity for each of the markers D2-40, CD10 and F-actin and, respectively, the differentiation degrees and the pTNM stages of the patients with tongue cancer. Thus, the highest reactivity was recorded at the cases with degree G2-G3 of histological differentiation and, respectively, at the patients in the stages III-IV pTNM.

- The immunoreactivity profile investigated by us highlights the increased invasivity potential of this particular localisation of oral squamous carcinomas, markers such as CXCR4, MMP-9, D2-40, CD10 and F-actin being considered as prognostic markers.

- At molecular level, an immunoprofile highlights the intrinsic invasive potential, the capacity of motility and the co-operation between the tumor cells, especially those of the invasion front, with stromal cells for the locoregional extension of the squamous carcinomas of the tongue.

- In addition, each of these markers can be molecular targets of a much more efficient treatment than the classic one, significantly increasing the survival rate of these pacients.

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