*Rom J Morphol Embryol* 2020, 61(4):1259–1278 ISSN (print) 1220–0522, ISSN (online) 2066–8279 doi: 10.47162/RJME.61.4.27

ORIGINAL PAPER

RJME Romanian Journal of Morphology & Embryology http://www.rjme.ro/

# Analysis of the distribution and expression of some tumor invasiveness markers in palate squamous cell carcinomas

ADRIAN PĂTRU[1,2], VALERIU ŞURLIN[1,3], CLAUDIU MĂRGĂRITESCU[4], EDUARD MIHAI CIUCĂ[2], MARIUS MATEI[5], MIRCEA-SEBASTIAN ŞERBĂNESCU[6], ADRIAN CAMEN[2]

[1]*Doctoral School, University of Medicine and Pharmacy of Craiova, Romania*

[2]*Department of Oral and Maxillofacial Surgery, University of Medicine and Pharmacy of Craiova, Romania*

[3]*Department of Surgery, University of Medicine and Pharmacy of Craiova, Romania*

[4]*Department of Pathology, University of Medicine and Pharmacy of Craiova, Romania*

[5]*Department of Histology, University of Medicine and Pharmacy of Craiova, Romania*

[6]*Department of Medical Informatics and Biostatistics, University of Medicine and Pharmacy of Craiova, Romania*

## Abstract

Oral cancer remains an important global health issue and despite recent diagnostic and therapeutic advances, it continues to have an unfavorable prognostic and decreased survival. Although palatal tumors represent one of the rarest locations of oral squamous cell carcinomas (SCCs), they are among the most aggressive local tumors, leaving behind important morpho-functional disabilities. In order to explain such local aggressiveness, the present study aims to investigate the immunohistochemical expression in palate SCCs of some markers known to be involved in the process of tumor invasiveness, such as Wiskott–Aldrich syndrome like (WASL), Claudin-1 (CLDN1), Integrin beta-6 (ITGB6) and c-Mesenchymal to epithelial transition protein (c-Met). We have found here a higher tumor WASL and CLDN1 reactivity in well-differentiated (G1) palate SCCs, and regardless the histological type, degree of differentiation or tumor topography, an overexpression at the invasion front, and in those palate' SCC cases with muscular invasiveness and with lymph node (LN) dissemination. ITGB6 and c-Met had a higher reactivity in moderately differentiated (G2) palate SCCs, especially at the periphery of tumor proliferations, at the invasion front and in those high invasive cases and as well as in those that associated LN dissemination. All four investigated markers were also positive at the level of LN metastatic proliferations. None of the markers could statistically stratify on age group and pain, and on bone and perineural invasion while all of them statistically stratified on survival and grading. We concluded that these markers have a prognostic role allowing the identification of those cases with an unfavorable clinical evolution and decreased survival.

***Keywords***: immunohistochemistry, oral cavity, palate, squamous cell carcinomas, tumor invasiveness markers.

## Introduction

Oral cancer is an important global public health issue, being the 16th most common neoplasm worldwide, with an estimated incidence in 2018 of about 355 000 new diagnoses from which more than 177 000 were expected to die [1]. Worldwide, there are differences in incidence and mortality, with the highest rates in developing countries, especially in India and other South/Eastern Asia regions [2, 3]. Despite the progress in research and therapy, survival has not improved significantly in the last years, continuing to be late clinically detected with poor prognosis and important effects on the quality of life of these patients [4, 5]. The most important prognostic factor in oral squamous cell carcinomas (SCCs) is the presence of lymph node (LN) metastasis, which decreases the survival rate with about 50% [6]. This aspect suggests an aggressive behavior of these tumors characterized by a propensity for rapid local invasion and spread. Invasion of cancer cells into the underlying stroma is the first step during cancer progression and metastasis. In oral SCCs, the pattern of invasion at the tumor invasive front has been shown to have predictive value for such patients [7]. Thus, in the cases with individual tumor cells in the invasive front, an increased rate of lymph LN metastases and a worsening of the prognosis were recorded [8, 9]. Although palatal tumors represent one of the rarest locations of oral SCCs, they are among the most aggressive local tumors, leaving behind important morpho-functional disabilities [10, 11].

### Aim

In order to explain such local aggressiveness further thorough studies are needed, this even more as in tumor processes of invasiveness and metastasis are involved a large number of factors and signaling pathways. In this context, the present study aims to investigate the immunohistochemical (IHC) expression in palate SCCs of some markers known to be involved in the process of tumor invasiveness, such as Wiskott–Aldrich syndrome like (WASL), Claudin-1 (CLDN1), Integrin beta-6 (ITGB6) and c-Mesenchymal to epithelial transition protein (c-Met).

## Materials and Methods

Palate SCC archival cases from the Laboratory of Pathology, Emergency County Hospital of Craiova, Romania, between 2010 and 2019, were retrieved. In our study were enrolled 45 cases of palate SCC, that had sufficient paraffin-embedded tissue specimens. All microscopic slides were reviewed by two oral pathologists, both to confirm the diagnosis and grading of tumor according to the diagnostic criteria established by *World Health Organization* (WHO) Classification (2005) [12]. After confirmation of the respective histopathology, 4 μm-thick seriate sections were cut and processed for immunohistochemistry. Briefly, the tissue sections were further deparaffinized, and hydrated in decreasing ethanol series. Antigen retrieval was performed by incubating the slides with 0.1 M pH 6 citrate buffer, and microwaving at 650 W for 20 minutes, and then the slides were allowed to cool to room temperature (RT) for further 30 minutes. The nonspecific binding was prevented by incubation for one hour, at RT, in 2% bovine serum albumin (BSA) solution. Then, we incubate the slides, overnight, at 4ºC, with the primary antibodies, as detailed in Table 1.

**Table 1 –** ***The antibodies and immunostaining protocol***

| Antibody | Clone / Manufacturer, Catalog No. | Dilution | External positive control |
|---|---|---|---|
| WASL | Rabbit, polyclonal / Sigma-Aldrich, HPA005750 | 1:100 | Colon |
| CLDN1 | Rabbit, polyclonal / Thermo Scientific, RB-9209-P | 1:100 | Breast carcinoma |
| ITGB6 | Rabbit, polyclonal / Sigma-Aldrich, HPA023626 | 1:200 | Colon |
| c-Met | Rabbit, monoclonal / BioSB, EP1454Y | 1:20 | Breast carcinoma |

c-Met: c-Mesenchymal to epithelial transition protein; CLDN1: Claudin-1; ITGB6: Integrin beta-6; WASL: Wiskott–Aldrich syndrome like.

A three-step indirect immunoperoxidase technique was performed using the Labeled Streptavidin–Biotin 2 (LSAB2) enzyme detection system and the correspondent Dako kit (Redox, Romania – K0675). The reactions were visualized using the 3,3'-Diaminobenzidine (DAB, Dako, K3468) and for counterstaining, the Mayer's Hematoxylin kit (Tunic, Bio-Optica, Romania – M06002) was used. Negative control staining was carried out by substituting the primary antibodies with phosphate-buffered saline (PBS) solution. The IHC assessment was performed and agreed upon by two pathologists who were blinded to the patient data and using the semiquantitative IHC score (immunoreactive score – IRS), as described by Remmele & Stegner [13].

Clinical and histopathological (HP) data were stored in an Excel 2016 (Microsoft, USA) file. Each patient was described by several fields: gender, age group, pain, survival, tumor localization, grading, bone invasion, muscular invasion, perineural invasion, stage, tumor T score (TNM), nodule N score (TNM), and IRS for WASL, CLDN1, ITGB6, and c-Met. The IRS were computed by multiplying the qualitative score (0, 1, 2, 3 – quantifies the intensity of the reactions) with the semi-quantitative one (0, 1, 2, 3, 4 – quantifies the number of positive cells, the thresholds being 25%, 50% and 75%), thus obtaining a final score ranging from 0 to 12. Each score was independently given, then agreed on by two pathologists. For better visualization and for inferential statistics, due to a small dataset, the IRS were resampled, values ranging from 1 to 6 were considered Class A, and values higher than 6 were considered Class B. In this manner, seen as categorical data, the IRS could be stratified and viewed as contingency tables, thus, $\chi^2$ (*chi*-squared) test or Fisher's exact tests were used to assess the differences, a $p$-value $<0.05$ was considered statistically significant.

Seen as continuous numerical data, different stratification of the IRS permitted the computation of average scores. Thus, continuous statistics could be applied. Normal data distribution was assessed through the Shapiro–Wilk test, and since all the analyzed subgroups had a normal distribution, in order to compare the means of two or more groups Student's $t$-test and analysis of variance (ANOVA), parametric tests were used; a $p$-value $<0.05$ was considered statistically significant. Correlation between numerical data was assessed through Pearson's $r$ correlation coefficient. The resulted $r$ value of the test is the covariance of two variables, divided by the product of their standard deviations, a number between -1 and 1, where 1 means positive correlation, -1 negative correlation and zero means no correlation. Empirically set, the thresholds for $r$ are: [0; 0.2] – very weak, lack of correlation, [0.2; 0.4] – weak correlation, [0.4; 0.6] – reasonable correlation, [0.6; 0.8] – high correlation, [0.8; 1] – very high correlation. Descriptive and inference statistical assessment were done in part in Excel, and in part in GraphPad Prism 9 (GraphPad, USA).

The study protocol was approved by the Ethics Committee of the University of Medicine and Pharmacy of Craiova, Romania.

## Results

The clinico-morphological data of our casuistry were published in a previous article [14]. Briefly, the average age was of 60.17±10.04 years, minimum 46 years, maximum 76 years, most patients were men (5.43:1) and most of the tumors developed from hard palate (24 cases). Histopathologically, at the hard palate, SCC prevailed the conventional well-differentiated tumor, and the papillary and verrucous SCC variant, while for the soft palate SCC, we noticed the predominance of moderately and poorly differentiated conventional SCC and the basaloid and acantholytic SCC forms. For hard palate lesions, 64% of the cases were diagnosed in stages II and III pTNM, while 90.47% of soft palate SCC presented in stages III and IV pTNM. Overall, about 69% of the patients did not survive more than 12 months after surgery, the number of deaths being almost double for those with tumors of the soft palate compared with those with hard palate tumors.

### WASL immunoexpression

Positive immunostaining for WASL was observed in the cytoplasm of basal and suprabasal keratinocytes of normal palatine epithelium adjacent to the tumors (Figure 1A). However, in the hyperplastic and dysplastic epithelium, the reaction was much more extended towards the upper layers but decreasing progressively in intensity towards them (Figure 1B). In addition to cytoplasmic reactivity, a nuclear reactivity was also observed, which was more evident in hyperplastic and dysplastic epithelia. WASL cytoplasmic reactivity was also observed in the minor salivary glandular tissue (reactions were much more intense in the excretory ducts compared with glandular

acini, and among the latter the reactivity was higher in serous acini and serous part of acini mixed compared to mucous acini) (Figure 1C), in the in adipose tissue (Figure 1D), smooth muscle tissue, striated muscle tissue (Figure 1E), lymphoid tissue (more evident in the germinal centers) (Figure 1D), in vascular endothelial cells (Figure 1F), fibroblasts and macrophages.

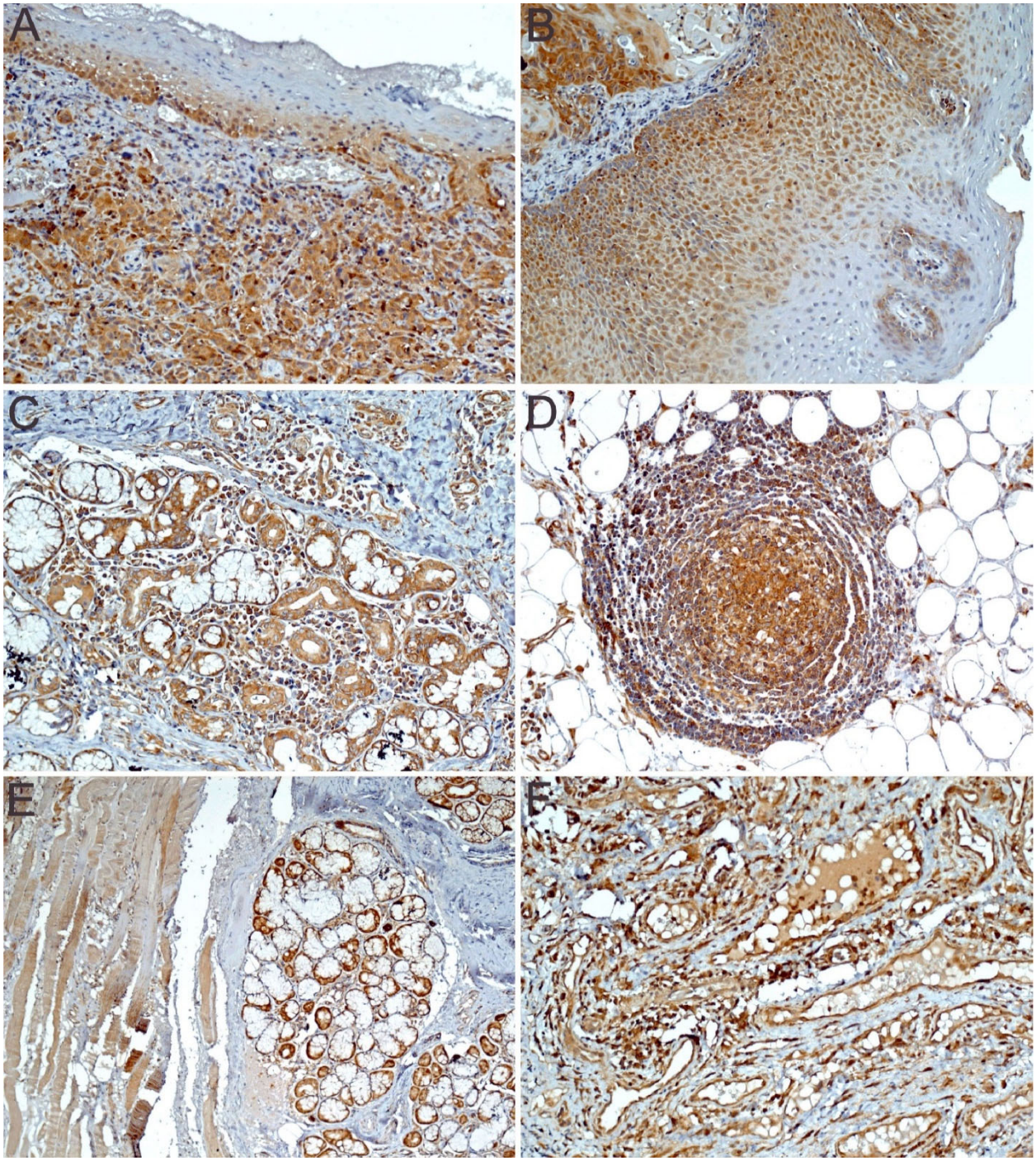


**Figure 1 –** ***WASL reactivity: (A) In cytoplasm of basal and suprabasal keratinocytes of normal palatine epithelium adjacent to the tumors; (B) Cytoplasmic and even nuclear reactivity extended towards the upper layers in the hyperplastic and dysplastic epithelium; (C) Cytoplasmic reactivity in the minor salivary glands with higher intensity in the excretory ducts compared with glandular acini; (D) Reactivity at the level of adipose tissue and in the lymphoid tissue especially in the germinal centers; (E) Reactivity in the striated muscle tissue; (F) Reactivity at the level of vascular endothelial cells, fibroblasts and macrophages. Anti-WASL antibody immunolabeling: (A–C and F) ×200; (D and E) ×100. WASL: Wiskott–Aldrich syndrome like.***

The WASL reactivity was more obvious in the tumor samples, but this reactivity was heterogeneous and varying depending on the histological subtype and the status of the loco-regional tumor progression. The highest IRS were recorded in the verrucous palate SCC (IRS=10) (Figure 2A), WASL staining being more obvious at the invasive front compared with superficial part of the tumor (Figure 2B). Also, we noticed a high reactivity in the neoplastic cells from the periphery of proliferations compared to those inside the tumor, this reactivity progressively decreasing

with the degree of neoplastic cells keratinization. This aspect was recorded in all investigated cases regardless of histological subtype. On the second place as reactivity were the well-differentiated (G1) palate SCCs (with the median IRS=8), with WASL reactivity more obvious at the periphery of tumor proliferations (Figure 2C). A slightly lower reactivity was observed in the papillary palate SCC cases (the IRS scores were 6 and 8) but with similar pattern (Figure 2D). Then, there were the moderately differentiated (G2) palate SCCs, with the median IRS=4. The WASL reactivity was more obvious at the invasive front compared with superficial tumor part (Figure 2, E and F).

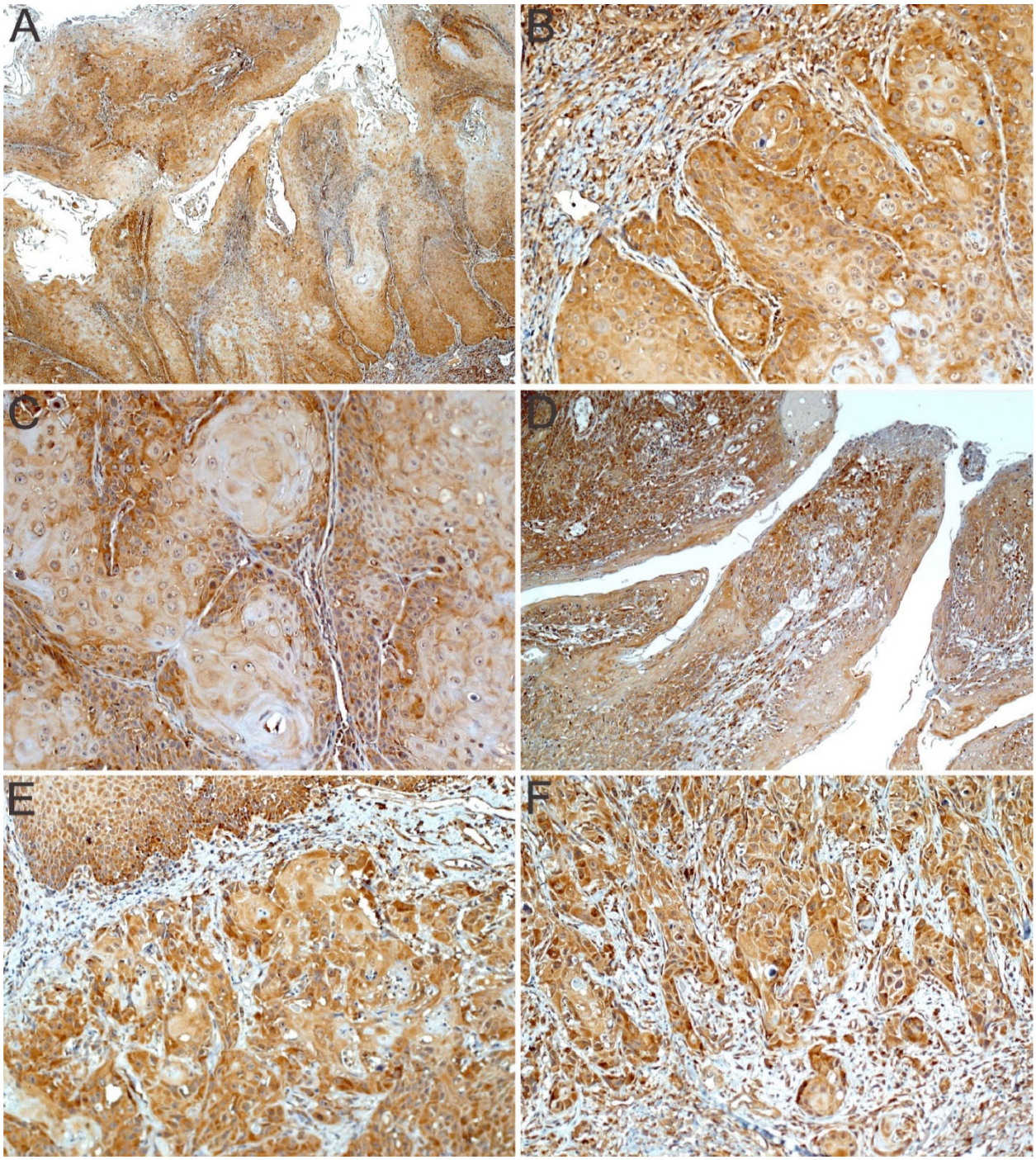


**Figure 2 –** ***WASL reactivity in tumor samples of palate SCC: (A) The most reactive specimens were those from the patients with verrucous palate SCC subtype; (B) The reactivity was more obvious at the invasive front compared with superficial part of the tumor; (C) A slightly lower reactivity was recorded in well-differentiated (G1) palate SCC, especially at the periphery of tumor proliferations; (D) These cases were followed as reactivity by the papillary palate SCC subtype; (E and F) In these cases, reactivity was more obvious at the invasive front compared with superficial tumor part. Anti-WASL antibody immunolabeling: (A) ×50; (B, C, E and F) ×200; (D) ×100. SCC: Squamous cell carcinoma; WASL: Wiskott–Aldrich syndrome like.***

A slightly lower reactivity was recorded in those two cases of acantholytic SCC, respective IRS=4 and IRS=6. The WASL reactivity was more obvious at the level of the tumor acantholysis zones (Figure 3A), the discohesive tumor cells being both cytoplasmic and nuclear positive (Figure 3B). For the basaloid palate SCC cases, we noticed

for the IRS scores values of 3 and 4 and a peculiar finding, we noticed a nuclear WASL reactivity (Figure 3C). The least reactive proved to be the poorly differentiated palate SCC cases, in which we recorded an IRS=2. In these cases, the WASL reactivity was obvious in those areas with moderately differentiated SCC morphology, while for the undifferentiated tumor zones, we noticed either weak cytoplasmic and nuclear reactivity or no reactivity (Figure 3, D and E). Also, regardless histological variant, the palate SCC cases with LN dissemination proved to be more reactive to WASL. Moreover, this reactivity was also present at the level of tumor proliferations disseminated in the LNs, but with a lower intensity (Figure 3F). In addition, in all tumor specimens that associated inflammatory stroma, more obvious in the acantholytic forms, we also noticed a WASL reactivity at the level of endothelial cells, fibroblasts, lymphocytes and macrophages.

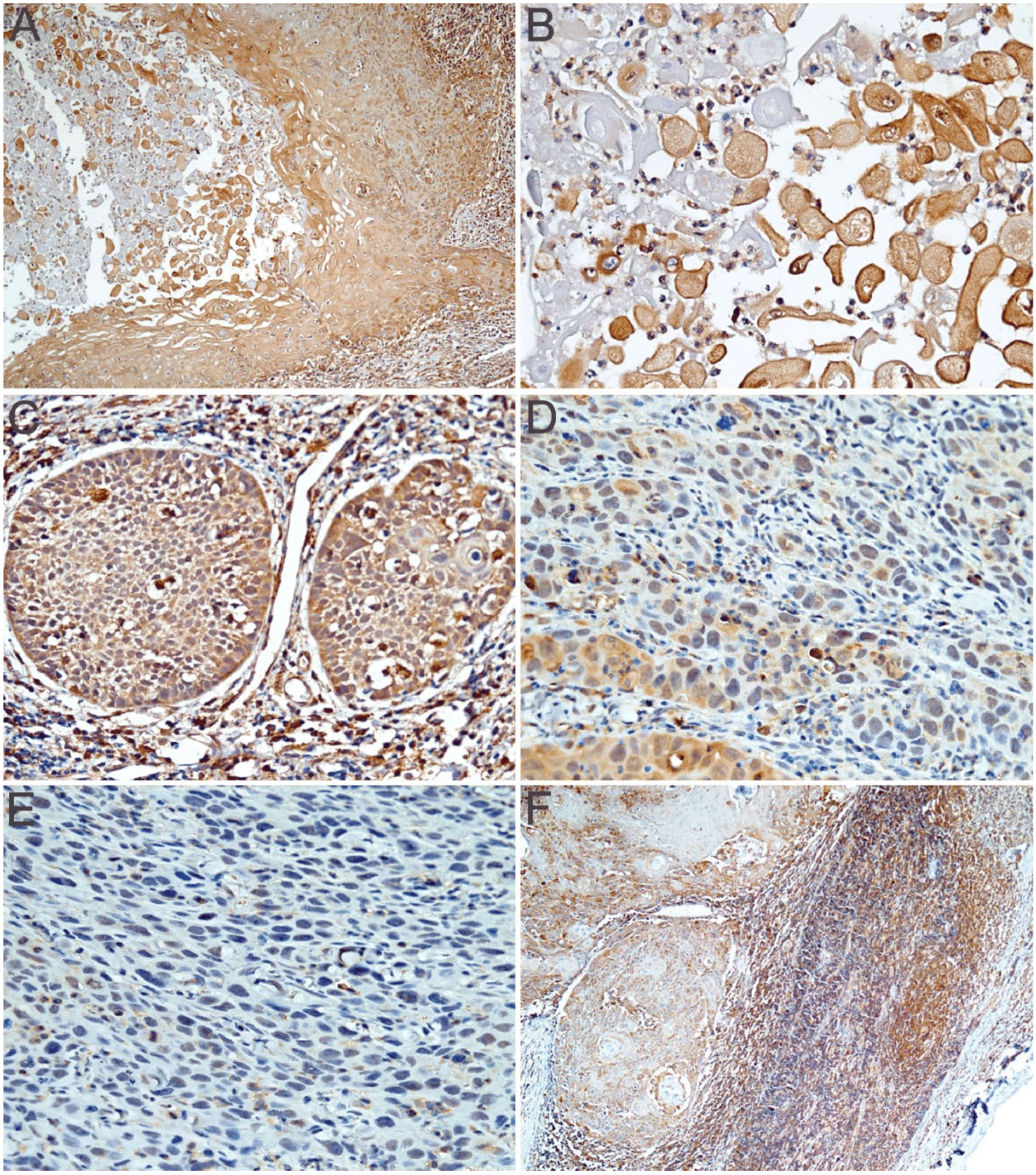


**Figure 3 –** ***WASL reactivity in tumor samples of palate SCC: (A) A slightly lower reactivity was recorded in the acantholytic SCC cases and it was more obvious at the level of the tumor acantholysis zones; (B) Both cytoplasmic and nuclear reactivity in the discohesive tumor cells; (C) A nuclear reactivity present in the basaloid palate SCC cases; (D and E) Weak cytoplasmic and nuclear reactivity or no reactivity in the undifferentiated areas of palate G3 SCC cases; (F) Weak reactivity at the level of tumor proliferations disseminated in the lymph nodes. Anti-WASL antibody immunolabeling: (A and F) ×100; (B–E) ×400. G3: Poorly differentiated tumor; SCC: Squamous cell carcinoma; WASL: Wiskott–Aldrich syndrome like.***

From the contingency tables, WASL showed significantly different average IRS for tumor localization (the Fisher's exact test statistical value was 0.0172; the result was significant at $p<0.05$.), hard and soft palate average scores being 6.29±2.22 and 4.43±2.24, respectively, Student's *t*-test $p=0.009$, significant at $p<0.05$. A graphical representation could be seen in Figure 4. Other significant findings from the resulted contingency tables showed WASL stratification with muscle invasion (the Fisher's exact test statistical value was 0.0005; the result was significant at $p<0.05$.), survival (the Fisher's exact test statistical value was 0.0001; the result was significant at $p<0.05$), and nodule N score (the *chi*-squared statistical value was 7.2009; the *p*-value was 0.027311; the result was significant at $p<0.05$).

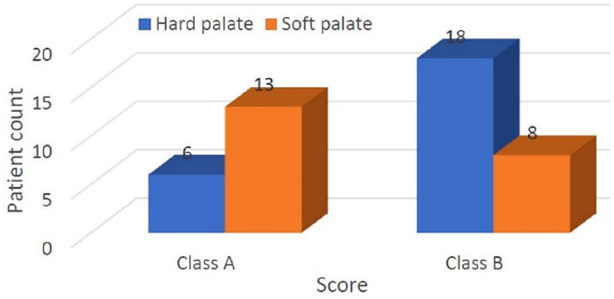


**Figure 4 –** ***WASL distribution on localization: Class A groups the IRS scores ranging from 1 to 6; Class B groups the IRS scores higher than 6. IRS: Immunoreactive score; WASL: Wiskott–Aldrich syndrome like.***

Findings from the continuous data analysis showed grading stratification of WASL, average G1 score was 7.61±1.29, while G2 was 5.06±1.25 and G3 was 2.10±0.99, ANOVA $p<0.001$, with individual Student's *t*-test between classes $p<0.001$. WASL partly stratified on gender, stage, tumor T score, with no consistent location differences. WASL did not show any significant stratification with age, pain, bone and perineural invasion.

### CLDN1 immunoexpression

In the normal palate mucosa adjacent to neoplastic proliferation, we found CLDN1 expression more obvious in the keratinocytes from the lower half of the epithelium with predominantly membranous pattern (Figure 5A). The basal and parabasal layer were not positive to this marker. In the hyperplastic and dysplastic epithelium from the vicinity of neoplastic proliferations, we recorded an increase in CLDN1 reactivity, being observed a cytoplasmic positive reaction in the lower half of the epithelium and even a nuclear pattern in the basal and parabasal epithelial cells (Figure 5B). A strong reaction was noticed in the excretory ducts from minor salivary glands and also a weak staining in their serous acini or serous counterpart of mixed acini (Figure 5C). In addition, weak CLDN1 staining was noticed in endothelial blood vessel cells, the smooth muscle fibers and in the germinal centers of lymphoid follicles.

In tumor tissue, the reactivity was more obvious in the moderate G2 tumors (IRS=9) and acantholytic variant (IRS=9 and IRS=10), where the highest IRS were recorded. In moderate G2 tumors the CLDN1 expression was more obvious inside rather than at the periphery of tumor proliferations, and the dominant pattern was membranous with a peculiar honeycomb appearance (Figure 5D). Also, inside the tumor proliferations we noticed a cytoplasmic reactivity but with a variable intensity from one cell to another. At the invasive tumor front, the CLDN1 reactivity varied depending on the invasion pattern. Thus, in the forms with 1 and 2 invasive pattern, a reduction of the marking intensity was observed especially towards the periphery of proliferations (Figure 5E), while in the tumors with 3 and 4 invasive pattern, an intensification of reactivity was observed in all invasive cells and focally even a faint nuclear staining was noticed (Figure 5F).

In the acantholytic variant, the CLDN1 reactivity decreased progressively until it disappeared towards the lumen proliferations (Figure 6A). Inside these lumens, tumor acantholytic cells temporarily retain a granular cytoplasmic reactivity (Figure 6B). In G1 palate SCCs, the CLDN1 reactivity was a little lower (IRS=8), with more intense staining inside tumor islands than the peripheral basaloid neoplastic cells. At the level of keratin pearls, the reactivity was either absent or had a faint thin concentric ring pattern (Figure 6C). A somewhat similar reactivity was also present in both cases of papillary palate SCCs, where we record an IRS of 8. The prevalent pattern was membranous, more obvious at the level of the neoplastic keratinocytes from inside proliferations than the basaloid neoplastic cells from periphery and in the superficial tumor part then invasive front (Figure 6D). A slightly weaker reactivity was observed in the case of palate verrucous SCC (IRS=6) but with very similar pattern as it was noticed in papillary cases (Figure 6E). In the basaloid palate SCC cases we recorded a weak reactivity (IRS=1 and IRS=2) restricted to center of the tumor islands with a cytoplasmic prevalent pattern (Figure 6F).

The lowest CLDN1 reactivity was noticed in G3 palate SCCs (IRS=1), with a peculiar weak nuclear pattern in the poorly differentiated cells and a higher cytoplasmic reaction in those more differentiated neoplastic cells (Figure 7A). In addition, we observed a tendency to have a higher CLDN1 reactivity in cases with advanced stages and LN dissemination. At the level of LN metastases, the CLDN1 reactivity was present but with a lower intensity then in the primary tumors (Figure 7B).

From the contingency tables, CLDN1 showed statistical stratification with muscle invasion (the Fisher's exact test statistical value was 0.0272; the result was significant at $p<0.05$), survival (the Fisher's exact test statistical value was 0.0192; the result was significant at $p<0.05$), and nodule N score (the *chi*-squared statistical value was 13.0721; the *p*-value was 0.00145; the result was significant at $p<0.05$). Findings from the continuous data analysis showed grading stratification of CLDN1, average G1 score was 7.00±1.03, while G2 was 8.75±0.68 and G3 was 2.18±2.64, ANOVA $p<0.001$, with individual Student's *t*-test between classes $p<0.001$. Other findings from the continuous data analysis showed gender stratification of CLDN1, average female score was 8.14±1.07, while average male score was 6.13±3.09, Student's *t*-test $p=0.004$, significant at $p<0.05$. CLDN1 partly stratified on stage with no consistent location differences. CLDN1 did not show any significant stratification with age, pain, localization, bone and perineural invasion, and tumor T score.

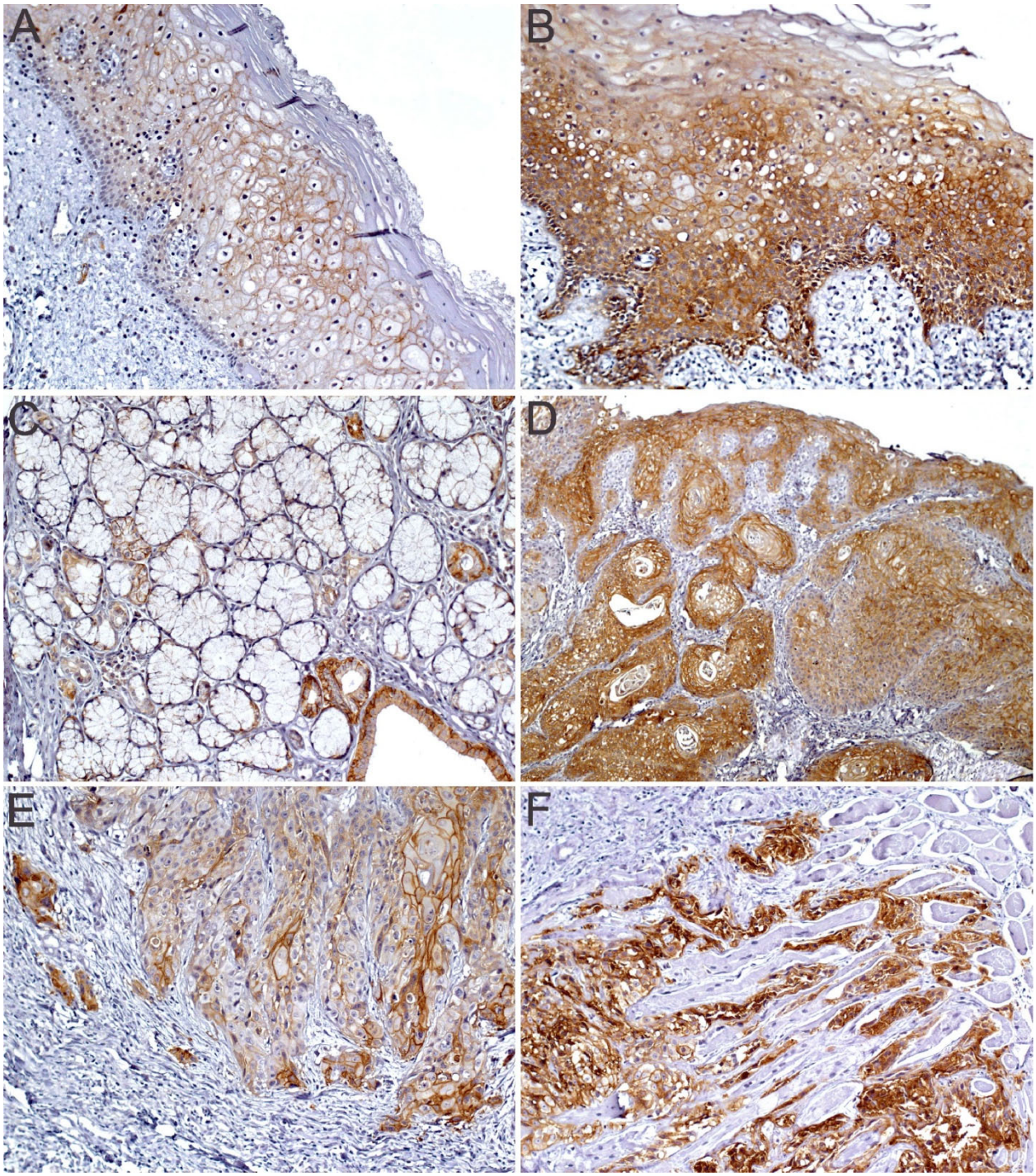


**Figure 5 –** ***CLDN1 reactivity: (A) Keratinocytes reactivity from the lower half of the normal adjacent epithelium with predominantly membranous pattern; (B) A cytoplasmic positive reaction in the lower half of the hyperplastic and dysplastic epithelium and even a nuclear pattern in the basal and parabasal epithelial cells; (C) A strong reaction in the excretory ducts from minor salivary glands; (D) A strong reaction with membranous prevalent pattern in the palate G2 SCC cases; (E) A reduction in tumor reactivity was noticed in the periphery of neoplastic proliferations, especially in those cases with 1 and 2 invasive pattern; (F) A strong reaction was noticed in all invasive cells from those palate SCC with 3 and 4 invasive pattern. Anti-CLDN1 antibody immunolabeling: (A–C, E and F) ×200; (D) ×100. CLDN1: Claudin-1; G2: Moderately differentiated tumor; SCC: Squamous cell carcinoma.***

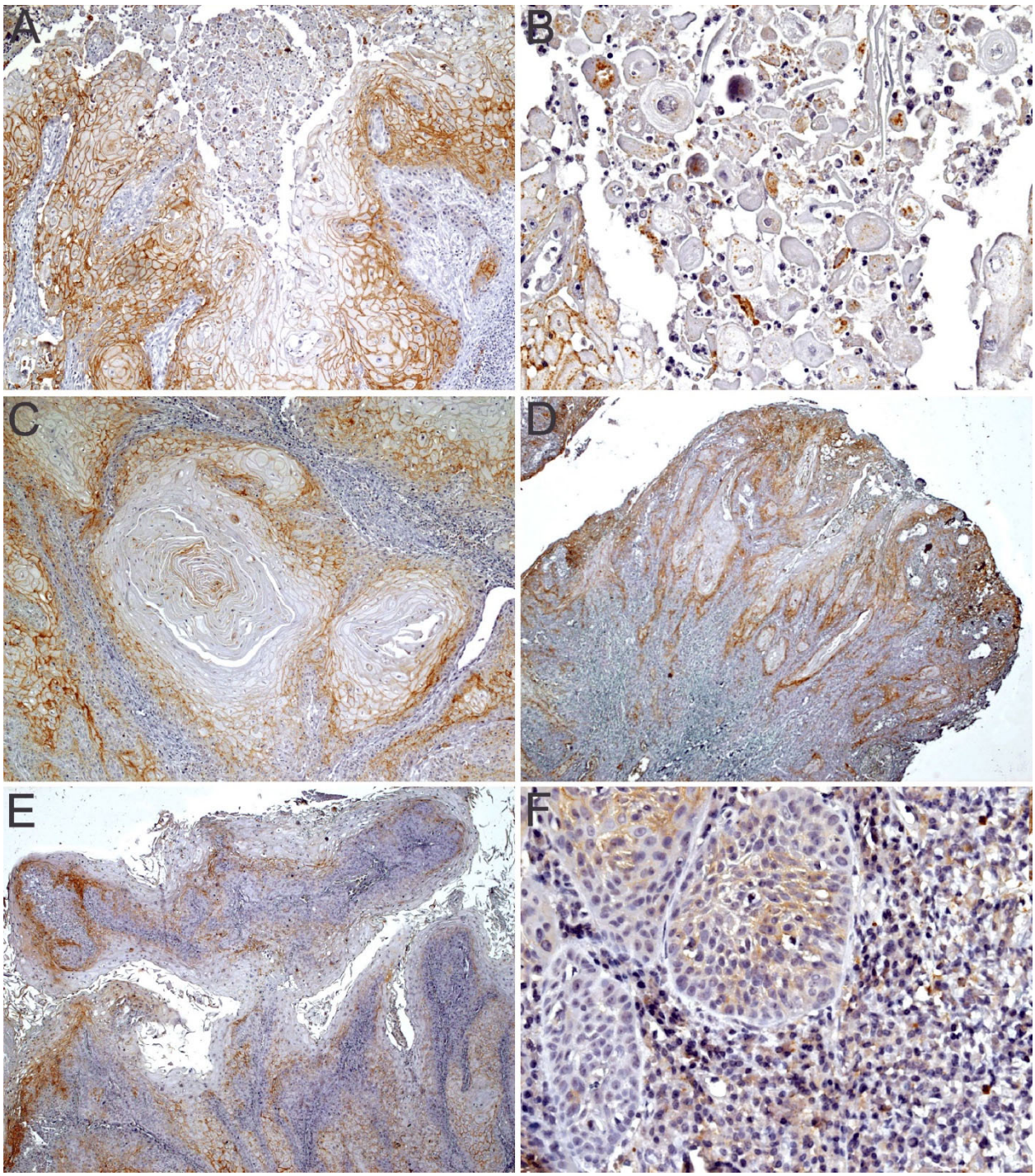


**Figure 6 –** ***CLDN1 reactivity: (A) A progressive reactivity decreasing in the acantholytic variant with no reactivity towards the lumen proliferations; (B) Inside these lumens, tumor acantholytic cells temporarily retain a granular cytoplasmic reactivity; (C) A lower reactivity was noticed in the G1 SCC cases with either absent or a faint thin concentric ring pattern at the level of keratin pearls; (D) In the papillary palate SCC cases was observed a membranous reactivity pattern more obvious at the level of the neoplastic keratinocytes from inside proliferations than the basaloid neoplastic cells from periphery; (E) A slightly weaker reactivity was observed in the case of palate verrucous SCC; (F) In the basaloid palate SCC cases, we recorded a weak reactivity restricted to center of the tumor islands, with a cytoplasmic prevalent pattern. Anti-CLDN1 antibody immunolabeling: (A and C) ×100; (B and F) ×400; (D and E) ×50. CLDN1: Claudin-1; G1: Well-differentiated tumor; SCC: Squamous cell carcinoma.***

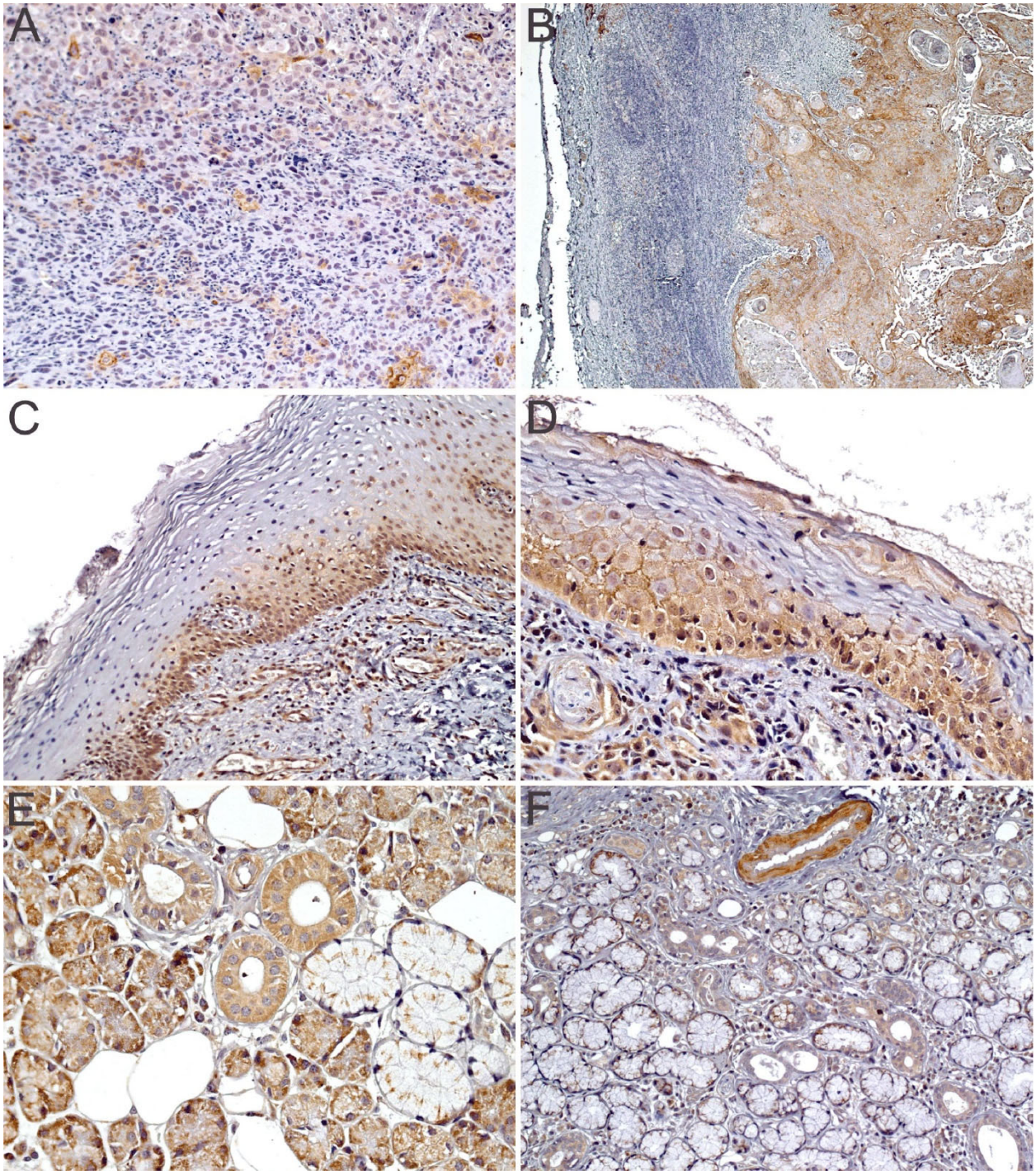


**Figure 7** – ***CLDN1 reactivity: (A) In G3 palate SCCs, we noticed a peculiar weak nuclear pattern in the poorly differentiated cells and a higher cytoplasmic reaction in those more differentiated neoplastic cells; (B) The reactivity was also present at the level of lymph node metastases but with a lower intensity then in the primary tumors. Anti-CLDN1 antibody immunolabeling: (A) ×200; (B) ×50. CLDN1: Claudin-1; G3: Poorly differentiated tumor; SCC: Squamous cell carcinoma. ITGB6 reactivity: (C) Reactivity was present in the lower part of the tumor adjacent epithelium, with membranous and cytoplasmic pattern; (D) A nuclear pattern was observed in the atypical cells from hyperplastic and dysplastic epithelium; (E) Reactivity at the level of minor salivary glands, with serous acinar cells as the most reactive, followed by the ductal epithelial cells and as the least reactive were the mucous acinar cells; (F) An intense positive reaction was observed at the level of smooth muscle fibers and at the level of endothelial cells from intra- and peritumoral blood vessels. Anti-ITGB6 antibody immunolabeling: (C and F) ×200; (D and E) ×400. ITGB6: Integrin beta-6.***

### ITGB6 immunoexpression

In the resection margins, the normal palate epithelium far away from the tumor tissue was not positive to ITGB6, but the epithelium adjacent to tumor tissue and the hyperplastic and dysplastic epithelium presented ITGB6 reactivity. This reactivity was present in the lower part of the epithelium, with membranous and cytoplasmic pattern (Figure 7C) and even with a nuclear pattern in atypical cells (Figure 7D). We also noticed an ITGB6 reactivity at the level of minor salivary glands, with serous acinar cells as the most reactive, followed by the ductal epithelial cells and the least reactive were the mucous acinar cells (Figure 7E). An intense positive ITGB6 reaction was observed at the level of smooth (Figure 7F) and striated muscle fibers and at the level of endothelial cells from intra- and peritumoral blood vessels (Figure 7C).

The highest ITGB6 reactivity was recorded in the G2 palate SCCs (IRS=9), with a cytoplasmic and nuclear dominant pattern (Figure 8A). This reactivity was more obvious at the periphery than inside of neoplastic proliferation and also at the invasive front compared with tumor superficial part (Figure 8B). A slightly lower reactivity was noticed in those two cases of palate papillary SCCs, in both being recorded an IRS score of 8. The reaction was more intense in the neoplastic epithelium that bordered the papillae cores and at the invasion front with a cytoplasmic prevalent pattern (Figure 8C). In the single case of verrucous palate SCC, the IRS score was about 4 and the reactivity was most cytoplasmic and more intense in the basal and parabasal neoplastic cells (Figure 8D). A decrease in reactivity has also been recorded in G3 palate SCCs (the median recorded IRS=3) where the dominant pattern was the nuclear one (Figure 8E). A similar reactivity was observed in those two basaloid palate SCCs (both cases had IRS=3), with the nuclear ITGB6 staining more prevalent at the invasive front and at the periphery of neoplastic proliferations (Figure 8F).

The ITGB6 does not seem to statistically stratify with any of the described parameters as resulted from the contingency tables. However, findings from the continuous data analysis showed survival stratification of ITGB6, survival group a having an average score of 5.35±3.10, while group B of 3.57±2.38, Student's *t*-test $p=0.043$, significant at $p<0.05$. Another significant stratification of the ITGB6 was on grading, average G2 score being 8.06 ±1.85, while for G1 and G3 2.83±1.65, and 2.80±0.42, respectively. Student's *t*-test showed significant differences between G2 and G1 (Student's *t*-test $p<0.001$, not significant at $p<0.05$), between G2 and G3 (Student's *t*-test $p<0.001$, not significant at $p<0.05$), and no differences between G1 and G2 (Student's *t*-test $p=0.936$, not significant at $p<0.05$). ITGB6 did not show any significant stratification with location, gender, age, pain, bone, muscle and perineural invasion, stage, tumor T score, nodule N score.

With intermediate reactivity between G2 and G3 palate SCCs were those two cases of acantholytic carcinomas (in both cases was recorded IRS=6) in which the prevalent ITGB6 reactivity was at the membrane level, especially in those neoplastic cells that rim the pseudoglandular lumens (Figure 9A). A membrane and cytoplasmic reactivity were also noticed in some acantholytic tumor cells. The lowest reactivity was observed in G1 palate SCCs (IRS=2) with membranous and cytoplasmic pattern (Figure 9B). This reactivity was more obvious at the periphery of neoplastic proliferation and was absent at the level of keratin pearls. Regardless histological variant, the highest IRS scores were recorded in those cases associated with LN dissemination. The neoplastic proliferation from the LN metastasis kept their ITGB6 reactivity (Figure 9C).

### c-Met immunoexpression

In the normal epithelium, the c-Met reactivity was either absent or weak cytoplasmic positive in the basal and parabasal layers (Figure 9D). A slightly higher reactivity was observed in the hyperplastic and dysplastic epithelium next to the neoplastic proliferations with a prevalent cytoplasmic pattern extended to the intermediate layer. A slightly more intense reactivity was noticed in the minor salivary glands and especially in the epithelium of the excretory ducts with a dual cytoplasmic and membranous pattern (Figure 9E). We also observed a weak c-Met reactivity, with a predominantly cytoplasmic pattern in smooth and striated muscle fibers, nerve fibers, fibroblasts, cells from the stromal inflammatory infiltrate and endothelial cells form intra- and peritumoral blood vessels (Figure 9F).

In tumor specimens, the reactivity to c-Met was higher than in the normal mucosa or in areas with hyperplastic and dysplastic changes. The highest reactivity was noticed in the G2 palate SCCs (IRS=9). In the superficial part of tumors, the c-Met was with a prevalent membranous pattern, while at the invasion front the prevalent pattern was a cytoplasmic one (Figure 10, A and B). Regardless tumor topography, the c-Met reactivity was higher at neoplastic cells from the periphery with a dual cytoplasmic and membranous pattern, while those inside the proliferations had lower reactivity and predominantly with membranous pattern (Figure 10C). Then followed the papillary palate SCCs cases, both cases presented IRS=6 (Figure 10D). The reactivity was more obvious at the surface of papillary projections, with both cytoplasmic and membranous pattern (Figure 10E) than at the invasive front were the c-Met reactivity was weak and with cytoplasmic pattern (Figure 10F).

A slightly lower reactivity was presented by the only case of verrucous palate SCC, with the IRS=4 (Figure 11A). The reactivity was the same as in the papillary palate SCCs cases, with the tumor superficial part as more reactive than the invasion front (Figure 11, A and B). In the G3 palate SCCs was recorded an IRS=3, the reactivity been weak and with cytoplasmic prevalent pattern (Figure 11C). A higher reactivity was recorded in those areas where were present carcinomatous islands with squamous differentiation. In both cases of basaloid palate SCCs, the c-Met reactivity was also weak, the average IRS score values were about 3 and 4. The prevalent subcellular pattern was the cytoplasmic one and it was more obvious at the neoplastic cells from the periphery of basaloid proliferations (Figure 11D). For the G1 palate SCCs, we recorded an IRS=2 the reactivity being present at the periphery of tumor islands with cytoplasmic prevalent pattern, the keratin pearls being devoid of reactivity (Figure 11E). The least reactive were the two

acantholytic carcinomas, where were recorded IRS values of 1 and 2. Positive reaction with cytoplasmic pattern was noticed in the outermost layers of tumor proliferations while in the acantholytic tumor cells no reactivity was recorded (Figure 11F). The reactivity was higher in those cases with LN metastases, the intensity of the reactions being even higher at the level of metastases than compared to the primary tumor. Also, we noticed moderate reactivity in those cases that associated an inflammatory stroma, and also some reactivity was present in the stromal fibroblasts and endothelial cells from intra- and peritumoral vessels.

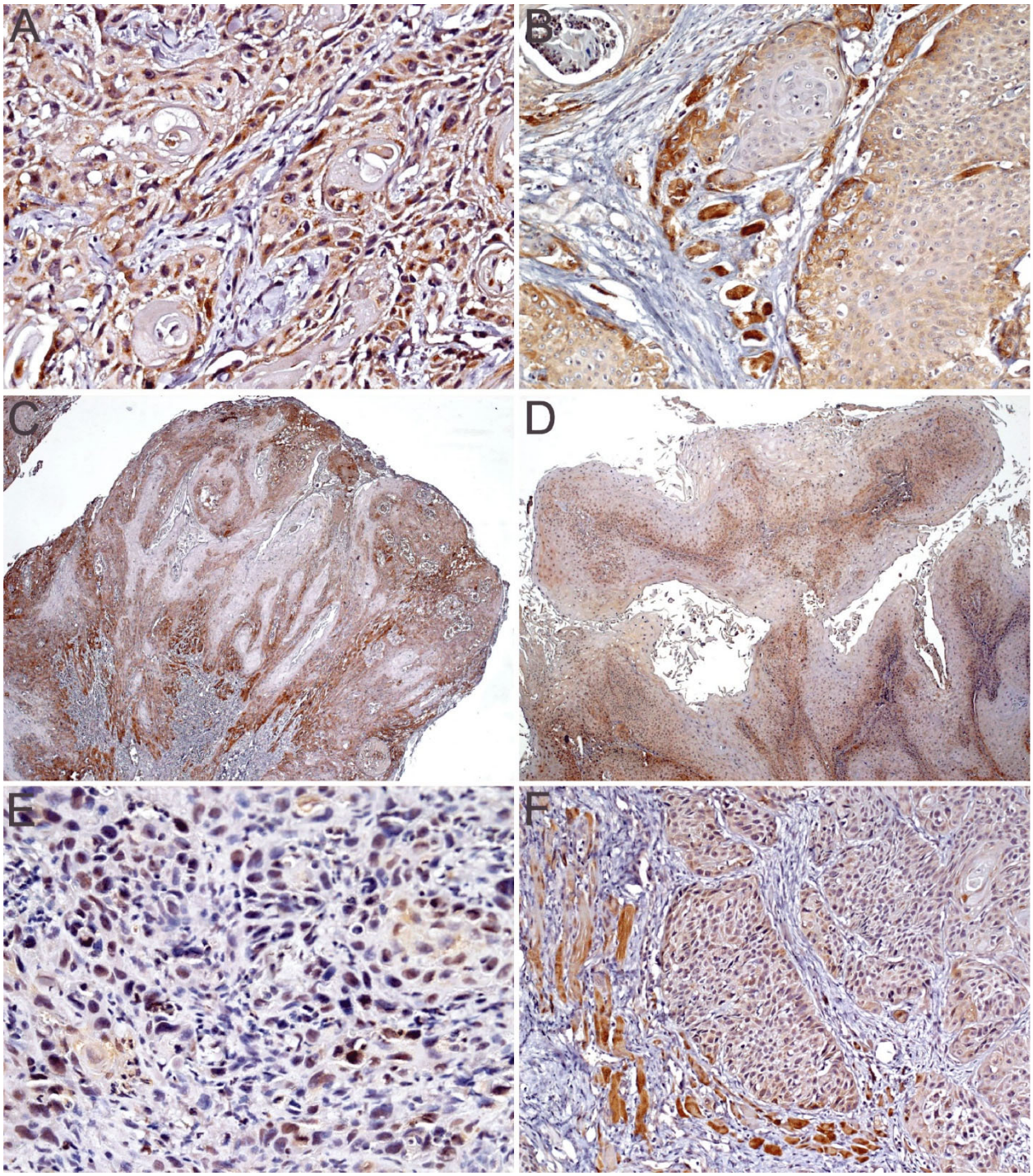


**Figure 8 –** ***ITGB6 reactivity: (A) The highest ITGB6 reactivity was recorded in the G2 palate SCCs, with a cytoplasmic and nuclear dominant pattern; (B) The reactivity was more obvious at the periphery than inside of neoplastic proliferation and also at the invasive front compared with tumor superficial part; (C) In the palate papillary SCCs, the reaction was more intense in the neoplastic epithelium that rim the papillae cores and at the invasion front, with a cytoplasmic prevalent pattern; (D) In the verrucous palate SCC, the reactivity was most cytoplasmic and more intense in the basal and parabasal neoplastic cells; (E) A decrease in reactivity was recorded in G3 palate SCCs, where the dominant pattern was the nuclear one; (F) A similar reactivity was observed in the basaloid palate SCCs, with the nuclear staining more prevalent at the invasive front and at the periphery of neoplastic proliferations. Anti-ITGB6 antibody immunolabeling: (A and E) ×400; (B and F) ×200; (C and D) ×50. G2: Moderately differentiated tumor; G3: Poorly differentiated tumor; ITGB6: Integrin beta-6; SCC: Squamous cell carcinoma.***

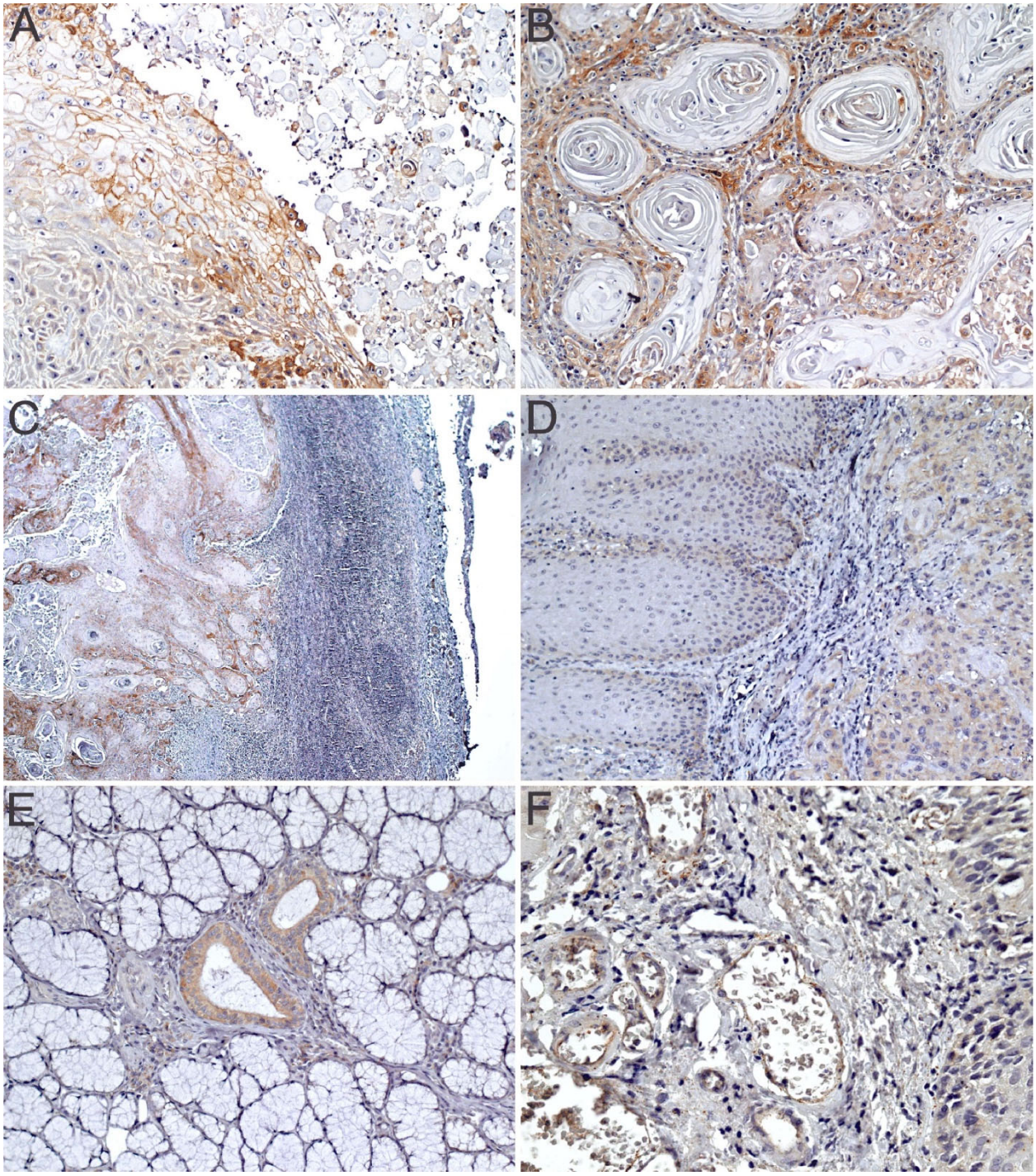


**Figure 9 –** ***ITGB6 reactivity: (A) In the acantholytic carcinomas, the prevalent reactivity was at the membrane level, especially in those neoplastic cells that rim the pseudoglandular lumens; (B) The slightest reactivity was observed in G1 palate SCCs, with membranous and cytoplasmic pattern; (C) The neoplastic proliferation from the lymph node metastasis kept their reactivity. Anti-ITGB6 antibody immunolabeling: (A) ×200; (B) ×100; (C) ×50. G1: Well-differentiated tumor; ITGB6: Integrin beta-6; SCC: Squamous cell carcinoma. c-Met reactivity: (D) Either absent or weak cytoplasmic positive reaction in the basal and parabasal layers of normal adjacent epithelium; (E) A slightly more intense reactivity was noticed in the minor salivary glands and especially in the epithelium of the excretory ducts, with a dual cytoplasmic and membranous pattern; (F) A weak c-Met reactivity, with a predominantly cytoplasmic pattern was also noticed in fibroblasts, cells from the stromal inflammatory infiltrate and endothelial cells form intra- and peritumoral blood vessels. Anti-c-Met antibody immunolabeling: (D) ×100; (E) ×200; (F) ×400. c-Met: c-Mesenchymal to epithelial transition protein.***

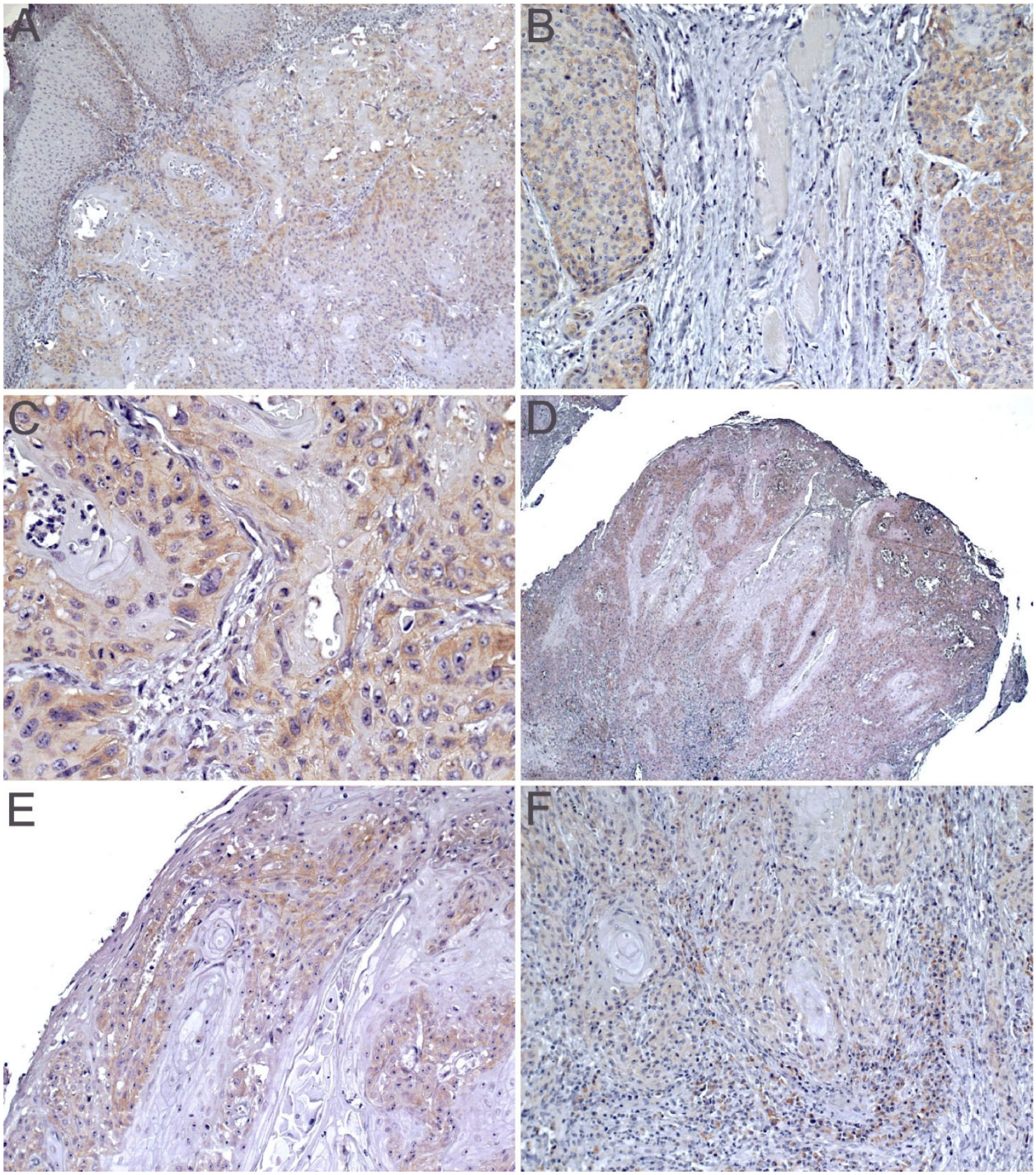


**Figure 10 –** ***c-Met reactivity: (A and B) The highest reactivity was noticed in the G2 palate SCCs, with a prevalent membranous pattern in the superficial part of tumors, while at the invasion front, the prevalent pattern was a cytoplasmic one; (C) Regardless tumor topography, the reactivity was higher at neoplastic cells from the periphery, with a dual cytoplasmic and membranous pattern, while those inside the proliferations had lower reactivity and predominantly with membranous pattern; (D) A slighter reactivity was noticed in the papillary palate SCCs cases; (E) The reactivity was more obvious at the surface of papillary projections, with both cytoplasmic and membranous pattern; (F) On the contrary, at the invasive front, the reactivity was weak and with cytoplasmic pattern. Anti-c-Met antibody immuno-labeling: (A) ×100; (B, E and F) ×200; (C) ×400; (D) ×50. c-Met: c-Mesenchymal to epithelial transition protein; G2: Moderately differentiated tumor; SCC: Squamous cell carcinoma.***

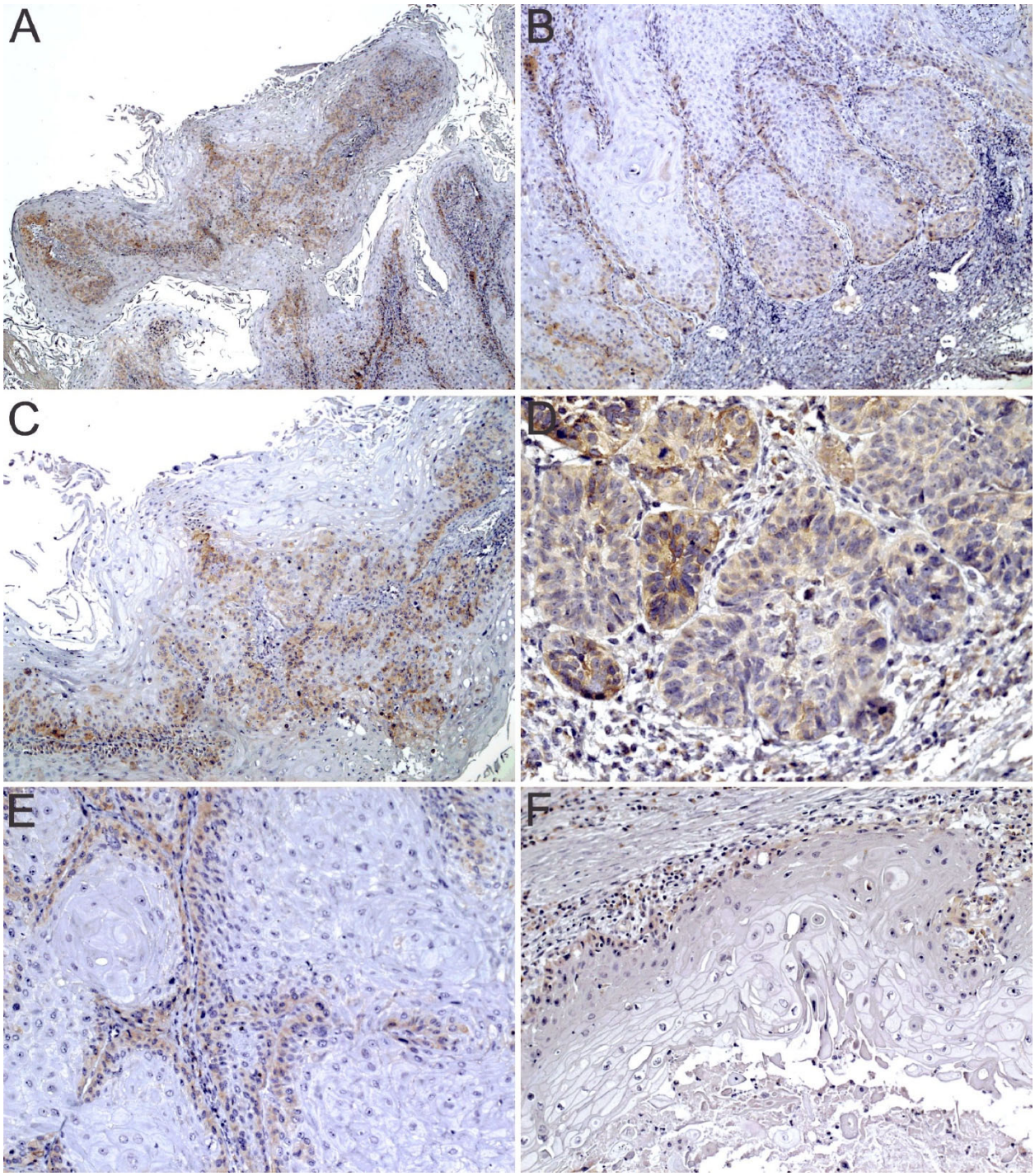


**Figure 11 –** ***c-Met reactivity: (A) A slightly lower reactivity was presented in the verrucous palate SCC case; (B) The reactivity was the same as in the papillary palate SCCs cases, with the tumor superficial part as more reactive than the invasion front; (C) In the G3 palate SCC cases, the reactivity was weak and with cytoplasmic prevalent pattern; (D) In the basaloid palate SCC cases, the reactivity was also weak, with a prevalent cytoplasmic pattern that was more obvious at the neoplastic cells from the periphery of basaloid proliferations; (E) In the G1 palate SCC cases, the reactivity was present at the periphery of tumor islands, with cytoplasmic prevalent pattern, and the keratin pearls being devoid of reactivity; (F) The least reactive were acantholytic carcinomas, where we noticed a cytoplasmic pattern in the outermost layers of tumor proliferations, while in the acantholytic tumor cells, we did not observe any reactivity. Anti-c-Met antibody immunolabeling: (A) ×50; (B and C) ×100; (D) ×400; (E and F) ×20. c-Met: c-Mesenchymal to epithelial transition protein; G1: Well-differentiated tumor; G3: Poorly differentiated tumor; SCC: Squamous cell carcinoma.***

As seen in the ITGB6, c-Met does not seem to statistically stratify with any of the described parameters as resulted from the contingency tables. Findings from the continuous data analysis showed survival stratification of c-Met, survival group a having an average score of 5.13±3.17, while group B of 2.36±1.74, Student's *t*-test $p$=0.001, significant at $p$<0.05. Another significant stratification of the c-Met was on grading, average G2 score being 7.53±2.48, while for G1 and G3 1.78±0.73, and 3.20±0.36, respectively. Student's *t*-test showed significant differences between G2 and G1 (Student's *t*-test $p$<0.001, not significant at $p$<0.05), between G2 and G3 (Student's *t*-test $p$<0.001, not significant at $p$<0.05), and between G1 and G2 (Student's *t*-test $p$<0.001, significant at $p$<0.05). Similar to ITGB6, the highest reactivity was at G2, only that, this time, the G1 and G2 averages scores were also different. c-Met did not show any significant stratification with gender, age, pain, location, bone, muscle and perineural invasion, stage, tumor T score, nodule N score.

Regardless of the localization or any other parameters, we have computed the correlation between the four IHC markers. The results can be seen in Table 2.

**Table 2 –** ***Immunohistochemistry–IRS correlation***

| | WASL | CLDN1 | ITGB6 | c-Met |
|---|---|---|---|---|
| **WASL** | 1 | 0.47 | -0.20 | -0.30 |
| **CLDN1** | | 1 | 0.58 | 0.40 |
| **ITGB6** | | | 1 | 0.83 |
| **c-Met** | | | | 1 |

c-Met: c-Mesenchymal to epithelial transition protein; CLDN1: Claudin-1; IRS: Immunoreactive score; ITGB6: Integrin beta-6; WASL: Wiskott–Aldrich syndrome like.

WASL has a reasonable correlation with CLDN1, having weak inverse correlations with ITGB6 and c-Met. CLDN1 has a reasonable correlation with ITGB6 and c-Met, while very high correlation is present between c-Met and ITGB6, as we expected from the similar stratification on grading. All four markers statistically stratified on survival and grading. WASL and CLDN1 stratified on muscle invasion, while only WASL stratified on location, and only CLDN1 on gender. None of the described markers stratified on age group, pain, bone and perineural invasion.

## Discussions

In a Report of the *International Agency for Research on Cancer* (IARC), who used the resources of GLOBOCAN database, for 2020, it was estimated an incidence of 377 713 new cases of lip and oral cavity cancers (about 2% of all sites cancer) from which 177 757 would die (1.8% of all sites) [15]. Oral SCC is by far the most common SCC of the head and neck, but lately a decrease in its incidence was recorded. Therefore, if in 2012 the oral SCC was considered the 11$^{th}$ most common cancer in the world, in 2018 it became the 18$^{th}$ most common cancer worldwide [16]. This incidence decrease would be due to the lesser chewing habits and geographic heterogeneity, although this type of cancer continues to be the most common cancer in South Asia, South Central Asia, as well as the Pacific Islands. Among oral SCC sites, tumors developed on the tongue and buccal mucosa appear to be the most common, being followed by tumors of the lips and palate [17]. Despite earlier detection and therapeutic progress, the survival rate of patients with oral SCCs has not improved more than 3% to 4% over the last decades [18, 19]. In addition, there were notable differences in the five-year relative survival rate depending on the oral topography of these SCCs. Thus, according to the *Surveillance, Epidemiology, and End Result* (SEER) program database, during 2010–2016 period, it was about 92% for lip, 67% for tongue, 51% for the floor of mouth and 49% for oropharynx SCCs [20]. In fact, it is well known that that SCC of the anterior two-third of the tongue, the floor of the mouth, and the lower alveolar ridge have a high risk of metastasis to loco-regional LNs and showed relatively poor prognosis [21].

In general, palate cancers are rare, the hard palate lesions represent only 5% of all oral cavity cancers [11], while soft palate SCCs accounts for about 5–12% of oropharyngeal cancers [10] and approximately 15% of head and neck SCCs [22]. Hard palate SCC has a better prognosis than other cancer localizations and is less likely to develop regional and distant metastasis. The T tumor stage has the highest impact on disease-free and overall survival in such cases. Thus, patients with ≥T2 stages, with distant metastasis and positive resection margins were reported to have poor prognosis [23]. The overall five-year survival rates for hard palate SCC were quoted around 62% to 66.3% [24, 25]. The soft palate SCCs are aggressive tumors, with rapid onset and a major negative impact regardless of tumor size at diagnosis [26]. For the T1 and T2 soft palate SCCs was reported a five-year overall survival rate about 91% to 100% and respectively 70% to 75% [27], while for the T3 and T4 lesions despite multimodal treatment the rate values dropped to 33% to 47% [28, 29]. The incidence of LN metastases for soft palate SCCs was estimated to be around 20% for T1–T2 tumors and about 60% to 70% for T3–T4 tumors [30]. In order to a better prognostic and therapeutically stratification of patients with palate SCC, we aimed to investigate the expression in such tumors of a series of biomarkers known to be involved in cancerous loco-regional invasiveness, such as WASP, CLDN1, ITGB6 and c-Met.

### Wiskott–Aldrich syndrome protein

Wiskott–Aldrich syndrome protein (WASP) belongs to a family of five proteins involved in the remodeling of actin cytoskeleton by which they regulate cell movement, cell signaling, and cell division. More recently, the WASP family members were identified in the nucleus with direct impact on nuclear shape and chromatin organization, but they also are involved in regulating gene expression [31, 32]. Participating in the initiation of invadopodium formation, WASP play a key role in the initiation of invasion, being linked to cancer progression and metastases in some human cancers [33–35].

Our study revealed that the WASL was expressed with variable intensity in normal palate mucosa at the level of basal and suprabasal keratinocytes, in minor salivary glands, smooth and striated muscle tissue, adipocytes, endothelial cells, fibroblasts, lymphocytes and macrophages. A higher reactivity was noticed in hyperplastic and dysplastic areas of palate mucosa adjacent to the tumor

proliferations. The highest reactivity was highlighted in tumor tissue and was more obvious in the tumor cells from the periphery of palate SCCs regardless histological type, degree of differentiation or tumor topography. The prevalent subcellular pattern reactivity was the cytoplasmic one, but a nuclear pattern was noticed in the acantholytic, basaloid and poorly differentiated palate SCCs. The most reactive tumors were the verrucous variant and the well-differentiated forms with a higher intensity in the invasion front. At the opposite pole were the basaloid variant and the poorly differentiated forms of palate SCCs. Regardless of the histological variant or degree of differentiation high IRS scores were recorded in those palate SCC cases with muscular invasiveness and with LN dissemination.

Reviewing the literature data, we did not find any information on the expression of this marker in the oral mucosa or in the SCCs with this location. However, in terms of the WASP protein family expression in SCCs with other locations, Wang *et al.* (2010), proves N-WASP cytoplasmic reactivity in esophageal SCC, and their absence in normal squamous mucosa [36]. Authors reveled significant correlation of N-WASP tumor expression and LN metastasis and pathological staging suggesting that this biomarker may play an important role in the tumorigenesis of such tumors, and it could be served as prognostic and diagnostic markers of esophageal SCC. Kimura *et al.* (2010), showed that enhancement of N-WASP–actin-related proteins 2/3 (Arp2/3) complex signal in primary SCC of the breast, fact that promotes the lamellipodia formation and subsequently the invasive behavior of the neoplastic cells [37]. The authors suggest a breast cancer classification based on the mechanisms of cell motility in three groups: (*i*) group with an N-WASP–Arp2/3 complex signal, (*ii*) group with WASP-family verprolin-homologous protein 2 (WAVE2)–Arp2/3 complex signal, and (*iii*) group without Arp2/3 complex. The authors concluded that this classification could have prognostic and therapeutic impact for patients with such tumors.

### Claudins

Claudins, a group of membrane proteins are the major component of the tight junctions which are mainly found in endothelial or epithelial cells. So far, 24 members of this family of proteins have been described, and CLDN1 is the most studied [38]. Its role as either a tumor promoter or suppressor is controversial, both an increase and a decrease in CLDN1 expression have been shown to be associated with tumorigenesis [39]. Thus, in prostate cancer [40], breast cancer [41] and melanocytic neoplasms [42], loss of CLDN1 expression has been associated with cancer progression and invasion while in papillary thyroid carcinoma [43], oral SCC [44], ovarian cancers [45] and other human malignancies [38], CLDN1 overexpression has been associated with aggressiveness and the acquisition of high malignant phenotype.

Our investigation proved a higher reactivity in the G1 and G2 palate SCCs with a membranous prevalent pattern achieving the particular appearance of honeycomb. Also, we noticed a tendency to shift the reactivity pattern from the membranous to a cytoplasmic and even nuclear one as the degree of tumor differentiation decreases. The lowest reactivity being recorded in the basaloid and G3 palate SCCs. In addition, we noticed that the CLDN1 reactivity was more obvious inside the tumor proliferations compared to the periphery as well at the invasion front than in the upper tumor part. Beside G3 cases, the nuclear CLDN1 reactivity was also observed in the basal and parabasal layers from the hyperplastic adjacent epithelia and more extended in atypical cells from the overlying layers from the dysplastic adjacent epithelia and also at the invasion front especially in neoplastic proliferations that have invaded the underlying striated muscle tissue. A higher reactivity was also recorded in cases with advanced stages and LN dissemination, the positive CLDN1 staining being also observed at the level of metastatic neoplastic proliferations.

Regarding the reactivity CLDN1 in the normal oral mucosa, the literature data is controversial. Thus, while some authors did not detect the expression of this marker [46], other found its expression in normal squamous mucosa, especially in its intermediate layer [47–49]. Also, some authors revealed a strong CLDN1 expression in the dysplastic basal cell layer of the in-situ SCCs [44, 47, 49]. It was suggested that in the early stages of oral carcinogenesis, there is an increase in CLDN1 expression and then during progression to invasive disease there is a loss of its expression [49]. Most of authors indicated an enhanced CLDN1 expression in oral SCCs samples with membranous and/or cytoplasmic and nuclear pattern in well-differentiated areas and no reactivity in poorly differentiated regions [46–49]. Ouban & Ahmed reported differential pattern of CLDN1 expression among tumors of different sites within the oral cavity [49]. Thus, most SCCs developed in lip, cheek, and gingiva showed weak or loss CLDN1 expression while most tongue cases presented moderate to strong expression. However, Babkair *et al.* do not found such different CLDN1 expressions depending on the oral tumor topography [46]. The same authors showed that inhibiting CLDN1 expression will increase the cells dissociation and consequently decreases the invasiveness of SCC cells. In fact, in a previous study, Oku *et al.* demonstrated that increased expression of CLDN1 was associated with matrix metalloproteinase-2 (MMP-2) activation that led to an increase in invasiveness of oral SCCs [44]. In another study, Sappayatosok & Phattarataratip found that high CLDN1 expression correlated with perineural and vascular invasion suggesting the involvement of such biomarker in the progression of oral SCCs [50]. At the same time, it was shown that the presence of CLDN1 expression in the invasive front was associated with high risk of recurrences and neck LN dissemination [51]. Given that CLDN1 can be recognized by cytotoxic T-lymphocytes [52], this molecule may serve as novel target for the oral SCCs therapy.

### Integrins

Integrins are a family of transmembrane-type receptor proteins comprising 24 different integrin receptors, mediating signaling between the intracellular and extracellular environments and playing a major role in the cell–cell and cell–extracellular matrix adhesion achievement [53]. In addition, it was proved that they are involved in cell motility, invasion, proliferation, and migration [54]

and thus they are also involved in tumor growth and metastasis [55]. ITGB6 turned out to be a receptor for fibronectin and cytotactin, and thus this integrin can regulate cancer cell proliferation, apoptosis, migration, epithelial to mesenchymal transition, invasion, metastasis, chemoresistance and cancer cell survival [53, 56–58].

Our investigation does not find ITGB6 expression in the normal palate epithelium, but this was present in hyperplastic and dysplastic epithelium adjacent to tumor proliferations. Some reactivity was also noticed in the acini and ductal epithelium of minor salivary gland, smooth and striated muscle fibers, and endothelial cells from the intra- and peritumoral blood vessels. In tumor tissue, the ITGB6 expression was far higher than in normal or dysplastic adjacent epithelium. The highest ITGB6 reactivity was recorded in the G2 palate SCCs, with a cytoplasmic and nuclear dominant pattern. At the opposite pole were the cases of G1 palate SCCs with cytoplasmic and membranous prevalent pattern. With an intermediate reactivity were the cases of the acantholytic, basaloid and G3 palate SCCs. Regardless histological variant the reactivity was more obvious at the invasion front and in the neoplastic cells from the periphery of tumor proliferations. Also, we noticed a higher reactivity in those high invasive cases and as well as in those that associated LN dissemination. In addition, we recorded an ITGB6 reactivity at the level of intra- and peritumoral vessels and even in the tumor proliferations from the LN metastasis.

Literature date showed that ITGB6 was not detectable in normal oral epithelium but was highly expressed in both dysplastic stratified squamous epithelium and SCC [59, 60]. Many studies had proved that ITGB6 was overexpressed in oral epithelial dysplasia, leukoplakia, *in situ* carcinomas, verrucous carcinomas and oral SCCs [58, 59, 61]. Most authors were agreeing that the highest tumor reactivity was present at the invasion front [59, 62]. The involvement of this biomarker in the growth and invasiveness of oral SCCs has been demonstrated by a series of *in vivo* and *in vitro* transfection studies [63, 64]. Thus, it was found that overexpression of ITGB6 in malignant keratinocytes up-regulates MMP-9 and MMP-2 expression and promotes invasion. Moreover, Dang *et al.* found that ITGB6 overexpression led to activation of urokinase plasminogen activator, which in turn up-regulates MMP-9 and MMP-2 expression and promotes invasion of h6-transfected oral SCC cells [65]. Li *et al.*, proved that neoplastic cells form oral SCCs overexpressed ITGB6 and MMP3, especially those from the periphery of invasive tumor islands simultaneously with a high rate of collagen fibers degradation in their adjacent stroma [66]. The authors concluded that such reactivity justify the invasiveness behavior of such tumors and the overexpression of these markers correlate with an unfavorable clinical prognostic and decreased survival. More recently, Yu *et al.* recorded a high ITGB6 reactivity in oral SCCs with LN metastasis compared with those without lymphatic dissemination, therefore, the authors proposed this marker as a predictor of LN metastasis [67].

### c-Met

c-Met is a receptor tyrosine kinase for the hepatocyte growth factor (HGF) implicated in a series of cellular processes, such as embryogenesis, cell growth, cell differentiation, and angiogenesis [68]. However, is abnormal activation turned out to be involved in development and progression of multiple human cancers, including the head and neck SCCs [69, 70]. It was proved that its activation suppresses E-cadherin expression and increase expression of MMPs, which leads to the acquisition of an invasive phenotype by tumor cells [71, 72]. Also, it seems that c-Met positive cancer cells from head and neck SCCs have the properties of cancer stem cells, which would be responsible for the chemo- and radiotherapy resistance of these tumors [73]. Moreover, meta-analysis studies showed that c-Met expression represent a significant adverse prognostic marker in patients with head and neck SCCs [69, 72].

We noticed a weak reactivity in normal samples with a slightly higher reactivity in the hyperplastic and dysplastic epithelium next to the neoplastic proliferations, with a prevalent cytoplasmic pattern extended to the intermediate layer. Also, some reactivity was recorded in tumor stroma especially in those cases that associated an inflammatory infiltrate in their stroma and in the endothelium of the tumor vessels. In tumor specimens, the highest reactivity was observed in G2 palate SCCs, followed by papillary and verrucous palate HP forms. At the opposite pole, the least reactive were the acantholytic and G1 palate SCCs. An intermediate reactivity was recorded in the basaloid and G3 palate SCCs. Regardless HP forms, the tumor reactivity was higher at the cells from the periphery of tumor proliferations, and the dominant pattern was the cytoplasmic one. Also, we noticed that those cases that developed LN metastases were also the most intensely reactive, the c-Met-positive reaction being observed even with higher intensity at the level of metastatic proliferations.

In one of the first studies regarding the expression of this marker in oral SCCs, Lo Muzio *et al.*, showed its expression in the suprabasal layers of normal oral mucosa and in the cells of minor salivary glands [74]. In the tumor specimens, they recorded a 78% positivity and 100% reactivity in the LN metastases. Also, the authors found a statistically significant positive correlation between c-Met expression and patient survival, suggesting that this marker could be used as a prognostic factor in such patients. Some later, Freudlsperger *et al.*, found that in 82.9% of oral SCCs, c-Met was positive in more than 50% of the tumor cells and the only significant correlation recorded was a negative one between its expression and clinical tumor stage [75]. Then, Zhao *et al.* found a significant correlation between c-Met overexpression and advanced clinical stage, positive LN status, and recurrences [76]. Also, the authors reported that this marker play an important role in oral SCC LN metastasis as a regulator of lymphangiogenesis through an indirect pathway involving vascular endothelial growth factor-C (VEGF-C).

## Conclusions

We showed a higher tumor WASL reactivity in G1 palate SCCs, and regardless histological type, degree of differentiation or tumor topography an overexpression at the invasion front, and in those palate' SCC cases with muscular invasiveness and with LN dissemination.

Also, we noticed a tendency to shift the reactivity pattern from the membranous to a cytoplasmic and even nuclear one as the degree of tumor differentiation decreases. For CLDN1, ITGB6, and c-Met, we observed a higher reactivity in G2 palate SCCs, especially at the periphery of tumor proliferations, at the invasion front and in those high invasive cases and as well as in those that associated LN dissemination. All four investigated markers were also positive at the level of LN metastatic proliferations. From the clinical point of view none of the markers proved to statistically stratify on age group and pain. From the pathologist's view, none of them statistically stratified on bone and perineural invasion. The only marker with statistical stratification on tumor localization was WASL. Taken together, our study confirms the prognostic role of these markers allowing the identification of those cases with an unfavorable clinical evolution and decreased survival.

**Conflict of interests**

The authors declare that there is no conflict of interests regarding the publication of this paper. All authors read and approved the final manuscript.

## References


[1] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin, 2018, 68(6):394–424. https://doi.org/10.3322/caac.21492 PMID: 30207593

[2] Gupta N, Gupta R, Acharya AK, Patthi B, Goud V, Reddy S, Garg A, Singla A. Changing trends in oral cancer – a global scenario. Nepal J Epidemiol, 2016, 6(4):613–619. https://doi.org/10.3126/nje.v6i4.17255 PMID: 28804673 PMCID: PMC5506386

[3] Rivera C. Essentials of oral cancer. Int J Clin Exp Pathol, 2015, 8(9):11884–11894. PMID: 26617944 PMCID: PMC4637760

[4] Omura K. Current status of oral cancer treatment strategies: surgical treatments for oral squamous cell carcinoma. Int J Clin Oncol, 2014, 19(3):423–430. https://doi.org/10.1007/s10147-014-0689-z PMID: 24682763

[5] Oskam IM, Verdonck-de Leeuw IM, Aaronson NK, Witte BI, de Bree R, Doornaert P, Langendijk JA, Leemans CR. Prospective evaluation of health-related quality of life in long-term oral and oropharyngeal cancer survivors and the perceived need for supportive care. Oral Oncol, 2013, 49(5):443–448. https://doi.org/10.1016/j.oraloncology.2012.12.005 PMID: 23318122

[6] Woolgar JA, Triantafyllou A, Lewis JS Jr, Hunt J, Williams MD, Takes RP, Thompson LD, Slootweg PJ, Devaney KO, Ferlito A. Prognostic biological features in neck dissection specimens. Eur Arch Otorhinolaryngol, 2013, 270(5):1581–1592. https://doi.org/10.1007/s00405-012-2170-9 PMID: 22983222

[7] Dissanayaka WL, Pitiyage G, Kumarasiri PV, Liyanage RL, Dias KD, Tilakaratne WM. Clinical and histopathologic parameters in survival of oral squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol, 2012, 113(4):518–525. https://doi.org/10.1016/j.oooo.2011.11.001 PMID: 22668430

[8] Sandu K, Nisa L, Monnier P, Simon C, Andrejevic-Blant S, Bron L. Clinicobiological progression and prognosis of oral squamous cell carcinoma in relation to the tumor invasive front: impact on prognosis. Acta Otolaryngol, 2014, 134(4):416–424. https://doi.org/10.3109/00016489.2013.849818 PMID: 24628337

[9] Siriwardena SBSM, Tsunematsu T, Qi G, Ishimaru N, Kudo Y. Invasion-related factors as potential diagnostic and therapeutic targets in oral squamous cell carcinoma – a review. Int J Mol Sci, 2018, 19(5):1462. https://doi.org/10.3390/ijms19051462 PMID: 29758011 PMCID: PMC5983574

[10] Iyer NG, Nixon IJ, Palmer F, Kim L, Whitcher M, Katabi N, Ghossein R, Shah JP, Patel SG, Ganly I. Surgical management of squamous cell carcinoma of the soft palate: factors predictive of outcome. Head Neck, 2012, 34(8):1071–1080. https://doi.org/10.1002/hed.21878 PMID: 22109978

[11] Truitt TO, Gleich LL, Huntress GP, Gluckman JL. Surgical management of hard palate malignancies. Otolaryngol Head Neck Surg, 1999, 121(5):548–552. https://doi.org/10.1016/S0194-5998(99)70084-7 PMID: 10547468

[12] Johnson N, Franceschi S, Ferlay J, Ramadas K, Schmid S, MacDonald DG, Bouquot JE, Slootweg PJ. Squamous cell carcinoma. In: Barnes L, Eveson JW, Reichart P, Sidransky D (eds). Pathology and genetics of head and neck tumours. World Health Organization (WHO) Classification of Tumours, International Agency for Research on Cancer (IARC) Press, Lyon, France, 2005, 168–175. https://screening.iarc.fr/doc/BB9.pdf

[13] Remmele W, Stegner HE. Vorschlag zur einheitlichen Definition eines Immunreaktiven Score (IRS) für den immunhistochemischen Östrogenrezeptor-Nachweis (ER-ICA) im Mammakarzinomgewebe [Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue]. Pathologe, 1987, 8(3):138–140. PMID: 3303008

[14] Pătru A, Şurlin V, Mărgăritescu C, Ciucă E, Mărgăritescu OC, Camen A. Palate squamous cell carcinomas: a ten-year single institute experience. Curr Health Sci J, 2020, 46(4):358–370. https://doi.org/10.12865/CHSJ.46.04.06 PMID: 33717510 PMCID: PMC7948021

[15] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin, 2021, 71(3):209–249. https://doi.org/10.3322/caac.21660 PMID: 33538338

[16] Anwar N, Pervez S, Chundriger Q, Awan S, Moatter T, Ali TS. Oral cancer: clinicopathological features and associated risk factors in a high risk population presenting to a major tertiary care center in Pakistan. PLoS One, 2020, 15(8):e0236359. https://doi.org/10.1371/journal.pone.0236359 PMID: 32760151 PMCID: PMC7410283

[17] García-Martín JM, Varela-Centelles P, González M, Seoane-Romero JM, Seoane J, García-Pola MJ. Epidemiology of oral cancer. In: Panta P (ed). Oral cancer detection. Springer, Cham, 2019, 81–93. https://doi.org/10.1007/978-3-319-61255-3_3

[18] Bjerkli IH, Jetlund O, Karevold G, Karlsdóttir Á, Jaatun E, Uhlin-Hansen L, Rikardsen OG, Hadler-Olsen E, Steigen SE. Characteristics and prognosis of primary treatment-naïve oral cavity squamous cell carcinoma in Norway, a descriptive retrospective study. PLoS One, 2020, 15(1):e0227738. https://doi.org/10.1371/journal.pone.0227738 PMID: 31945122 PMCID: PMC6964975

[19] Gatta G, Botta L, Sánchez MJ, Anderson LA, Pierannunzio D, Licitra L; EUROCARE Working Group. Prognoses and improvement for head and neck cancers diagnosed in Europe in early 2000s: The EUROCARE-5 population-based study. Eur J Cancer, 2015, 51(15):2130–2143. https://doi.org/10.1016/j.ejca.2015.07.043 PMID: 26421817

[20] American Cancer Society (ACS). Survival rates for oral cavity and oropharyngeal cancer. Last revised: February 2, 2021; accessed: March 23, 2021. https://www.cancer.org/cancer/oral-cavity-and-oropharyngeal-cancer/detection-diagnosis-staging/survival-rates.html https://www.cancer.org/content/dam/CRC/PDF/Public/8765.00.pdf

[21] Suresh GM, Koppad R, Prakash BV, Sabitha KS, Dhara PS. Prognostic indicators of oral squamous cell carcinoma. Ann Maxillofac Surg, 2019, 9(2):364–370. https://doi.org/10.4103/ams.ams_253_18 PMID: 31909017 PMCID: PMC6933976

[22] Keus RB, Pontvert D, Brunin F, Jaulerry C, Bataini JP. Results of irradiation in squamous cell carcinoma of the soft palate and uvula. Radiother Oncol, 1988, 11(4):311–317. https://doi.org/10.1016/0167-8140(88)90202-2 PMID: 3131842

[23] Hakim SG, Steller D, Sieg P, Rades D, Alsharif U. Clinical course and survival in patients with squamous cell carcinoma of the maxillary alveolus and hard palate: results from a single-center prospective cohort. J Craniomaxillofac Surg, 2020, 48(1):111–116. https://doi.org/10.1016/j.jcms.2019.12.008 PMID: 31884030

[24] Givi B, Eskander A, Awad MI, Kong Q, Montero PH, Palmer FL, Xu W, De Almeida JR, Lee N, O'Sullivan B, Irish JC, Gilbert R, Ganly I, Patel SG, Goldstein DP, Morris LGT. Impact of

elective neck dissection on the outcome of oral squamous cell carcinomas arising in the maxillary alveolus and hard palate. Head Neck, 2016, 38(Suppl 1):E1688–E1694. https://doi.org/10.1002/hed.24302 PMID: 26614119 PMCID: PMC4927080

[25] Yang X, Song X, Chu W, Li L, Ma L, Wu Y. Clinicopathological characteristics and outcome predictors in squamous cell carcinoma of the maxillary gingiva and hard palate. J Oral Maxillofac Surg, 2015, 73(7):1429–1436. https://doi.org/10.1016/j.joms.2014.12.034 PMID: 25869748

[26] Espinosa Restrepo F, Martínez Capoccioni G, Martín Martín C. T1–T2 squamous cell carcinoma of the uvula: a little big enemy. Otolaryngol Head Neck Surg, 2012, 146(1):81–87. https://doi.org/10.1177/0194599811421110 PMID: 21900539

[27] Douglas WG, Rigual NR, Giese W, Bauer J, Wiseman SM, Loree TR, Schwarz J, Alrawi S, Hicks WL Jr. Advanced soft palate cancer: the clinical importance of the parapharyngeal space. Otolaryngol Head Neck Surg, 2005, 133(1):66–69. https://doi.org/10.1016/j.otohns.2005.03.007 PMID: 16025055

[28] Hansen E, Panwala K, Holland J. Post-operative radiation therapy for advanced-stage oropharyngeal cancer. J Laryngol Otol, 2002, 116(11):920–924. https://doi.org/10.1258/00222150260369462 PMID: 12487672

[29] Osborne RF, Brown JJ. Carcinoma of the oral pharynx: an analysis of subsite treatment heterogeneity. Surg Oncol Clin N Am, 2004, 13(1):71–80. https://doi.org/10.1016/S1055-3207(03)00117-0 PMID: 15062362

[30] Cohan DM, Popat S, Kaplan SE, Rigual N, Loree T, Hicks WL Jr. Oropharyngeal cancer: current understanding and management. Curr Opin Otolaryngol Head Neck Surg, 2009, 17(2):88–94. https://doi.org/10.1097/moo.0b013e32832984c0 PMID: 19373958

[31] Alekhina O, Burstein E, Billadeau DD. Cellular functions of WASP family proteins at a glance. J Cell Sci, 2017, 130(14):2235–2241. https://doi.org/10.1242/jcs.199570 PMID: 28646090 PMCID: PMC5536917

[32] Verboon JM, Sugumar B, Parkhurst SM. Wiskott–Aldrich syndrome proteins in the nucleus: aWASH with possibilities. Nucleus, 2015, 6(5):349–359. https://doi.org/10.1080/19491034.2015.1086051 PMID: 26305109 PMCID: PMC4915506

[33] Gligorijevic B, Wyckoff J, Yamaguchi H, Wang Y, Roussos ET, Condeelis J. N-WASP-mediated invadopodium formation is involved in intravasation and lung metastasis of mammary tumors. J Cell Sci, 2012, 125(Pt 3):724–734. https://doi.org/10.1242/jcs.092726 PMID: 22389406 PMCID: PMC3367832

[34] Pichot CS, Arvanitis C, Hartig SM, Jensen SA, Bechill J, Marzouk S, Yu J, Frost JA, Corey SJ. Cdc42-interacting protein 4 promotes breast cancer cell invasion and formation of invadopodia through activation of N-WASp. Cancer Res, 2010, 70(21):8347–8356. https://doi.org/10.1158/0008-5472.CAN-09-4149 PMID: 20940394 PMCID: PMC2970640

[35] Sălan AI, Mărăşescu PC, Camen A, Ciucă EM, Matei M, Florescu AM, Pădureanu V, Mărgăritescu C. The prognostic value of CXCR4, *α*-SMA and WASL in upper lip basal cell carcinomas. Rom J Morphol Embryol, 2018, 59(3):839–849. PMID: 30534824

[36] Wang WS, Zhong HJ, Xiao DW, Huang X, Liao LD, Xie ZF, Xu XE, Shen ZY, Xu LY, Li EM. The expression of CFL1 and N-WASP in esophageal squamous cell carcinoma and its correlation with clinicopathological features. Dis Esophagus, 2010, 23(6):512–521. https://doi.org/10.1111/j.1442-2050.2009.01035.x PMID: 20095995

[37] Kimura F, Iwaya K, Kawaguchi T, Kaise H, Yamada K, Mukai K, Matsubara O, Ikeda N, Kohno N. Epidermal growth factor-dependent enhancement of invasiveness of squamous cell carcinoma of the breast. Cancer Sci, 2010, 101(5):1133–1140. https://doi.org/10.1111/j.1349-7006.2010.01527.x PMID: 20219074

[38] Tsukita S, Furuse M, Itoh M. Multifunctional strands in tight junctions. Nat Rev Mol Cell Biol, 2001, 2(4):285–293. https://doi.org/10.1038/35067088 PMID: 11283726

[39] Myal Y, Leygue E, Blanchard AA. Claudin 1 in breast tumorigenesis: revelation of a possible novel “claudin high” subset of breast cancers. J Biomed Biotechnol, 2010, 2010:956897. https://doi.org/10.1155/2010/956897 PMID: 20490282 PMCID: PMC2871677

[40] Sheehan GM, Kallakury BV, Sheehan CE, Fisher HA, Kaufman RP Jr, Ross JS. Loss of claudins-1 and -7 and expression of claudins-3 and -4 correlate with prognostic variables in prostatic adenocarcinomas. Hum Pathol, 2007, 38(4):564–569. https://doi.org/10.1016/j.humpath.2006.11.007 PMID: 17306334

[41] Tokés AM, Kulka J, Paku S, Szik A, Páska C, Novák PK, Szilák L, Kiss A, Bögi K, Schaff Z. Claudin-1, -3 and -4 proteins and mRNA expression in benign and malignant breast lesions: a research study. Breast Cancer Res, 2005, 7(2):R296–R305. https://doi.org/10.1186/bcr983 PMID: 15743508 PMCID: PMC1064136

[42] Cohn ML, Goncharuk VN, Diwan AH, Zhang PS, Shen SS, Prieto VG. Loss of claudin-1 expression in tumor-associated vessels correlates with acquisition of metastatic phenotype in melanocytic neoplasms. J Cutan Pathol, 2005, 32(8):533–536. https://doi.org/10.1111/j.0303-6987.2005.00324.x PMID: 16115050

[43] Németh J, Németh Z, Tátrai P, Péter I, Somorácz A, Szász AM, Kiss A, Schaff Z. High expression of claudin-1 protein in papillary thyroid tumor and its regional lymph node metastasis. Pathol Oncol Res, 2010, 16(1):19–27. https://doi.org/10.1007/s12253-009-9182-9 PMID: 19578981

[44] Oku N, Sasabe E, Ueta E, Yamamoto T, Osaki T. Tight junction protein claudin-1 enhances the invasive activity of oral squamous cell carcinoma cells by promoting cleavage of laminin-5 gamma2 chain *via* matrix metalloproteinase (MMP)-2 and membrane-type MMP-1. Cancer Res, 2006, 66(10):5251–5257. https://doi.org/10.1158/0008-5472.CAN-05-4478 PMID: 16707450

[45] Kleinberg L, Holth A, Trope CG, Reich R, Davidson B. Claudin upregulation in ovarian carcinoma effusions is associated with poor survival. Hum Pathol, 2008, 39(5):747–757. https://doi.org/10.1016/j.humpath.2007.10.002 PMID: 18439941

[46] Babkair H, Yamazaki M, Uddin MS, Maruyama S, Abé T, Essa A, Sumita Y, Ahsan MS, Swelam W, Cheng J, Saku T. Aberrant expression of the tight junction molecules claudin-1 and *zonula occludens*-1 mediates cell growth and invasion in oral squamous cell carcinoma. Hum Pathol, 2016, 57:51–60. https://doi.org/10.1016/j.humpath.2016.07.001 PMID: 27436828

[47] Dos Reis PP, Bharadwaj RR, Machado J, Macmillan C, Pintilie M, Sukhai MA, Perez-Ordonez B, Gullane P, Irish J, Kamel-Reid S. Claudin 1 overexpression increases invasion and is associated with aggressive histological features in oral squamous cell carcinoma. Cancer, 2008, 113(11):3169–3180. https://doi.org/10.1002/cncr.23934 PMID: 18991282

[48] Lourenço SV, Coutinho-Camillo CM, Buim ME, Pereira CM, Carvalho AL, Kowalski LP, Soares FA. Oral squamous cell carcinoma: status of tight junction claudins in the different histopathological patterns and relationship with clinical parameters. A tissue-microarray-based study of 136 cases. J Clin Pathol, 2010, 63(7):609–614. https://doi.org/10.1136/jcp.2009.070409 PMID: 20591911

[49] Ouban A, Ahmed A. Analysis of the distribution and expression of claudin-1 tight junction protein in the oral cavity. Appl Immunohistochem Mol Morphol, 2015, 23(6):444–448. https://doi.org/10.1097/PAI.0000000000000104 PMID: 25517868

[50] Sappayatosok K, Phattarataratip E. Overexpression of claudin-1 is associated with advanced clinical stage and invasive pathologic characteristics of oral squamous cell carcinoma. Head Neck Pathol, 2015, 9(2):173–180. https://doi.org/10.1007/s12105-014-0559-z PMID: 25078758 PMCID: PMC4424206

[51] De Vicente JC, Fernández-Valle Á, Vivanco-Allende B, Santamarta TR, Lequerica-Fernández P, Hernández-Vallejo G, Allonca-Campa E. The prognostic role of claudins -1 and -4 in oral squamous cell carcinoma. Anticancer Res, 2015, 35(5):2949–2959. PMID: 25964581

[52] Kondo S, Demachi-Okamura A, Hirosawa T, Maki H, Fujita M, Uemura Y, Akatsuka Y, Yamamoto E, Shibata K, Ino K, Kikkawa F, Kuzushima K. An HLA-modified ovarian cancer cell line induced CTL responses specific to an epitope derived from claudin-1 presented by HLA-A*24:02 molecules. Hum Immunol, 2013, 74(9):1103–1110. https://doi.org/10.1016/j.humimm.2013.06.030 PMID: 23806269

[53] Barczyk M, Carracedo S, Gullberg D. Integrins. Cell Tissue Res, 2010, 339(1):269–280. https://doi.org/10.1007/s00441-009-0834-6 PMID: 19693543 PMCID: PMC2784866

[54] Desgrosellier JS, Cheresh DA. Integrins in cancer: biological implications and therapeutic opportunities. Nat Rev Cancer. 2010, 10(1):9–22. https://doi.org/10.1038/nrc2748 PMID: 20029421 PMCID: PMC4383089
[55] Mizejewski GJ. Role of integrins in cancer: survey of expression patterns. Proc Soc Exp Biol Med, 1999, 222(2):124–138. https://doi.org/10.1046/j.1525-1373.1999.d01-122.x PMID: 10564536
[56] Bandyopadhyay A, Raghavan S. Defining the role of integrin alphavbeta6 in cancer. Curr Drug Targets, 2009, 10(7):645–652. https://doi.org/10.2174/138945009788680374 PMID: 19601768 PMCID: PMC2888263
[57] Ramos DM, Dang D, Sadler S. The role of the integrin alpha v beta6 in regulating the epithelial to mesenchymal transition in oral cancer. Anticancer Res, 2009, 29(1):125–130. PMID: 19331141
[58] Thomas GJ, Nyström ML, Marshall JF. Alphavbeta6 integrin in wound healing and cancer of the oral cavity. J Oral Pathol Med, 2006, 35(1):1–10. https://doi.org/10.1111/j.1600-0714.2005.00374.x PMID: 16393247
[59] Regezi JA, Ramos DM, Pytela R, Dekker NP, Jordan RCK. Tenascin and beta 6 integrin are overexpressed in floor of mouth *in situ* carcinomas and invasive squamous cell carcinomas. Oral Oncol, 2002, 38(4):332–336. https://doi.org/10.1016/s1368-8375(01)00062-8 PMID: 12076695
[60] Ylipalosaari M, Thomas GJ, Nystrom M, Salhimi S, Marshall JF, Huotari V, Tervahartiala T, Sorsa T, Salo T. Alpha v beta 6 integrin down-regulates the MMP-13 expression in oral squamous cell carcinoma cells. Exp Cell Res, 2005, 309(2):273–283. https://doi.org/10.1016/j.yexcr.2005.06.008 PMID: 16024014
[61] Hamidi S, Salo T, Kainulainen T, Epstein J, Lerner K, Larjava H. Expression of alpha(v)beta6 integrin in oral leukoplakia. Br J Cancer, 2000, 82(8):1433–1440. https://doi.org/10.1054/bjoc.1999.1130 PMID: 10780523 PMCID: PMC2363375
[62] Jones J, Watt FM, Speight PM. Changes in the expression of alpha v integrins in oral squamous cell carcinomas. J Oral Pathol Med, 1997, 26(2):63–68. https://doi.org/10.1111/j.1600-0714.1997.tb00023.x PMID: 9049904
[63] Ramos DM, But M, Regezi J, Schmidt BL, Atakilit A, Dang D, Ellis D, Jordan R, Li X. Expression of integrin beta 6 enhances invasive behavior in oral squamous cell carcinoma. Matrix Biol, 2002, 21(3):297–307. https://doi.org/10.1016/s0945-053x(02)00002-1 PMID: 12009335
[64] Thomas GJ, Lewis MP, Hart IR, Marshall JF, Speight PM. AlphaVbeta6 integrin promotes invasion of squamous carcinoma cells through up-regulation of matrix metalloproteinase-9. Int J Cancer, 2001, 92(5):641–650. https://doi.org/10.1002/1097-0215(20010601)92:5<641::aid-ijc1243>3.0.co;2-p PMID: 11340566
[65] Dang D, Yang Y, Li X, Atakilit A, Regezi J, Eisele D, Ellis D, Ramos DM. Matrix metalloproteinases and TGFbeta1 modulate oral tumor cell matrix. Biochem Biophys Res Commun, 2004, 316(3):937–942. https://doi.org/10.1016/j.bbrc.2004.02.143 PMID: 15033492
[66] Li HX, Zheng JH, Fan HX, Li HP, Gao ZX, Chen D. Expression of *αvβ6* integrin and collagen fibre in oral squamous cell carcinoma: association with clinical outcomes and prognostic implications. J Oral Pathol Med, 2013, 42(7):547–556. https://doi.org/10.1111/jop.12044 PMID: 23331428
[67] Yu B, Cao W, Zhang C, Xia R, Liu J, Yan M, Chen W. Prediction of lymph node metastasis in oral squamous cell carcinoma based on protein profile. Expert Rev Proteomics, 2019, 16(4):363–373. https://doi.org/10.1080/14789450.2019.1584039 PMID: 30779878
[68] Maina F, Panté G, Helmbacher F, Andres R, Porthin A, Davies AM, Ponzetto C, Klein R. Coupling Met to specific pathways results in distinct developmental outcomes. Mol Cell, 2001, 7(6):1293–1306. https://doi.org/10.1016/s1097-2765(01)00261-1 PMID: 11430831
[69] Kim JH, Kim BJ, Kim HS. Clinicopathological impacts of high c-Met expression in head and neck squamous cell carcinoma: a meta-analysis and review. Oncotarget, 2017, 8(68):113120–113128. https://doi.org/10.18632/oncotarget.21303 PMID: 29348891 PMCID: PMC5762576
[70] Zhang Y, Xia M, Jin K, Wang S, Wei H, Fan C, Wu Y, Li X, Li X, Li G, Zeng Z, Xiong W. Function of the c-Met receptor tyrosine kinase in carcinogenesis and associated therapeutic opportunities. Mol Cancer, 2018, 17(1):45. https://doi.org/10.1186/s12943-018-0796-y PMID: 29455668 PMCID: PMC5817860
[71] Koontongkaew S, Amornphimoltham P, Yapong B. Tumor–stroma interactions influence cytokine expression and matrix metalloproteinase activities in paired primary and metastatic head and neck cancer cells. Cell Biol Int, 2009, 33(2):165–173. https://doi.org/10.1016/j.cellbi.2008.10.009 PMID: 18996211
[72] Vsiansky V, Gumulec J, Raudenska M, Masarik M. Prognostic role of c-Met in head and neck squamous cell cancer tissues: a meta-analysis. Sci Rep, 2018, 8(1):10370. https://doi.org/10.1038/s41598-018-28672-8 PMID: 29991692 PMCID: PMC6039483
[73] Sun S, Wang Z. Head neck squamous cell carcinoma c-Met$^+$ cells display cancer stem cell properties and are responsible for cisplatin-resistance and metastasis. Int J Cancer, 2011, 129(10):2337–2348. https://doi.org/10.1002/ijc.25927 PMID: 21225626
[74] Lo Muzio L, Leonardi R, Mignogna MD, Pannone G, Rubini C, Pieramici T, Trevisiol L, Ferrari F, Serpico R, Testa N, De Rosa G, Staibano S. Scatter factor receptor (c-Met) as possible prognostic factor in patients with oral squamous cell carcinoma. Anticancer Res, 2004, 24(2C):1063–1069. PMID: 15154624
[75] FreudIsperger C, Alexander D, Reinert S, Hoffmann J. Prognostic value of c-Met expression in oral squamous cell carcinoma. Exp Ther Med, 2010, 1(1):69–72. https://doi.org/10.3892/etm_00000012 PMID: 23136595 PMCID: PMC3490346
[76] Zhao D, Wang SH, Feng Y, Hua CG, Zhao J, Tang XF. Intratumoral c-Met expression is associated with vascular endothelial growth factor C expression, lymphangiogenesis, and lymph node metastasis in oral squamous cell carcinoma: implications for use as a prognostic marker. Hum Pathol, 2011, 42(10):1514–1523. https://doi.org/10.1016/j.humpath.2010.03.012 PMID: 21531000

***Corresponding authors***

Claudiu Mărgăritescu, Professor, MD, PhD, Department of Pathology, Faculty of Dentistry, University of Medicine and Pharmacy of Craiova, 66 1 May Avenue, 200628 Craiova, Romania; Phone +40740–152 550, e-mail: c_margaritescu2000@yahoo.com

Mircea-Sebastian Şerbănescu, Lecturer, MD, PhD, Department of Medical Informatics and Biostatistics, University of Medicine and Pharmacy of Craiova, 2 Petru Rareş Street, 200349 Craiova, Romania; Phone +40745–766 610, e-mail: mircea_serbanescu@yahoo.com

*Received: January 23, 2021* *Accepted: June 22, 2021*