ORIGINAL PAPER

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

# Dendritic cells: friends or foes of laryngeal cancer?

C. DOROŞ[1)], N. BALICA[1)], ANCA MARIA CÎMPEAN[2)]

[1)]*ENT Department*
[2)]*Department of Microscopic Morphology, Histology, Angiogenesis Research Center*
*"Victor Babeş" University of Medicine and Pharmacy, Timisoara*

## Abstract

*Background*: Dendritic cells are important keyplayers of various malignant tumors but less studied in laryngeal malignancies. We assessed the immunohistochemical expression and distribution of dendritic cells in different types of laryngeal carcinomas and tried to find if they could influence evolution and prognosis of such malignancies. *Materials and Methods*: Immunohistochemistry was applied on 49 laryngeal tumors. Dendritic cells were identified by using S100 protein staining. The specimens were then evaluated for dendritic cells presence, number and distribution. *Results*: S100 positive cells were identified in all cases of squamous cell carcinoma, being absent in the case of adenoid cell carcinoma. Squamous cells carcinomas had the highest numbers of S100 positive cells. For them, we encountered an inverse correlation between peri- and intra-tumor S100 positive cells density. Intraepithelial dendritic cells density was lower for undifferentiated squamous cell carcinoma, also as for stroma of well-differentiated squamous cell carcinoma. Poorly differentiated carcinoma had a higher density of stromal S100 positive cells. S100 positive cells were identified in tumor area with squamous differentiation in all cases, and in peritumor area in 41 cases (83.67%). S100 positive cells density was correlated with tumor grade but not with invasion. *Conclusions*: Taking together, our results suggest that migration of stromal dendritic cells inside tumor areas could be an important component of the antitumor immune response induction and thus, S100 positive dendritic cells may be considered as a favorable prognostic factor in laryngeal carcinomas.

***Keywords***: laryngeal cancer, dendritic cells, S100 protein, prognosis.

## Introduction

Immunologic therapies have shown promise results in the management of several tumor types on experimental animal models and clinical trials [1–4]. Molecular mechanisms concerning the dendritic cells involvement in antitumor immune response remain controversial, despite of a large number of studies published in the field [5–9]. It seems that dendritic cells are involved not only in the antitumor immune response, but also in other pathogenic mechanisms of malignancies as tumor angiogenesis [10], invasion [11] and metastasis [12, 13].

Laryngeal cancer is still a tumor condition with unpredictable evolution and prognosis. In the last years, histopathology [14], grading and tumor angiogenesis [15] have been investigated as a potential prognostic and therapeutic factors. The data found in the literature is far to be convincible and thus, a more accurate and fine approach of laryngeal malignancies is needed. Because of these reasons, new directions in the laryngeal cancer research have been followed. The immunologic concept of laryngeal tumor development, invasion and metastasis is relatively new and less studied. Dendritic cells are cellular components of the normal laryngeal stratified squamous epithelium and also can be found in metaplastic epithelium. Thus, their presence in the tumor epithelium from laryngeal carcinoma is not a surprise as other authors already stated [16, 17]. Despite of these evidences, few studies suggested a potential involvement of dendritic cells in the laryngeal cancer development and progression and highlighted their potential prognostic and therapeutic role for this type of malignancy [18, 19].

For laryngeal cancer, dynamic feature of dendritic cells, their ability to migrate and to change in number and molecular phenotype included them as a potential prognostic factor for patients with and without metastasis [20, 21].

Except few studies, which detected molecular features of laryngeal dendritic cells, many researchers prefer immunohistochemistry for their assessment because of a proper correlation between morphology, dendritic cells distribution, density and variability. Also, few studies about dendritic cells were done by their assessment on paraffin embedding human specimens compared with *in vitro* models and animal tumor model.

Based on previous evidences, our study assessed peritumor and intratumor S100 positive dendritic cells and correlate with invasion, grade, and histopathology and we tried to highlight the dendritic cells prognostic impact.

## Materials and Methods

We selected for present study 49 retrospective cases of laryngeal tumors previously obtained by laryngectomy (total or fronto-lateral) or removed by endoscopic

technique. Paraffin-embedded specimens were sliced by Thermo Shandon automated Microtome and we obtained 5-μm serial sections for each case. Modified Broders system grading was applied on Hematoxylin and Eosin stained slides. Additional sections were immunostained for S100 protein (polyclonal, prediluted, DakoCytomation, Carpinteria, USA). Incubation with primary antibodies for 30 minutes at room temperature was followed by labeled Streptavidin–Biotin system (LSAB+) working system. The final reaction product was identified with 3,3'-diaminobenzidine. Counterstain was performed by using modified Lillie' Hematoxylin. The nuclei were stained with modified Lillie's Hematoxylin. To avoid overestimation of S100 positive cells, we performed CD68 macrophages specific immunostain for the each specimens by applying the same method. Nerve was used as positive control and the nuclear and cytoplasmic positive distribution was assessed. All steps of the immunohistochemical procedure were performed in an automated manner with DakoAutostainer System.

Evaluation of S100 positive cell density has been performed in tumor and peritumor area using "hot-spot" method, at ×400 magnification. Only cells with S100 positive identifiable branched bodies were quantified; isolated branches were excluded. Positive signals were counted in an automated manner by using Lucia G software (Nikon), choosing for each case three fields of tumor and area with the highest cell density.

Images were captured with Nikon Eclipse E600 microscope camera, recorded in JPEG format and processed by microscopic image analyzer. The presence of dendritic cells was correlated with inflammatory infiltrate. Inflammatory infiltrate was quantified absent, rare, moderate or high.

## Results

On Hematoxylin and Eosin stained specimens, we found leukoplasia in three (6.12%) cases, *in situ* carcinoma for one (2.04%) case, one (2.04%) case of adenoid cell carcinoma, and squamous cell carcinoma in 44 (89.79%) cases. The malignant lesions were graded as G1 – 12 (24.48%) cases, G2 – 24 (48.97%) cases, and G3 – eight (16.32%) cases.

In the normal stratified squamous non-cornified epithelium adjacent to the tumor, S100 positive dendritic cells bodies were distributed in the suprabasal layer, sending branches to the upper basal epithelial layers. The mean value of dendritic cells density inside the normal epithelial area was 12.66/field ×400. In cases of leukoplasia the number and morphology of S100 positive dendritic cells were similar with those from normal squamous stratified epithelium (Figure 1a). A significant decrease of intraepithelial dendritic cells number was noticed in case of *in situ* carcinoma (mean 9/field ×400). Also, peritumor stroma of *in situ* carcinoma lacked dendritic cells (Figure 1b).

S100 protein positive cells were identified in all cases of squamous cell carcinoma, being absent in the case of adenoid cell carcinoma. S100 positive dendritic cells varied in number and size, most of them being of medium size with intensely branched cytoplasmic processes (Figure 1c). For squamous cell carcinoma, the highest numbers of S100 positive dendritic cells was noticed inside malignant epithelial areas (Figure 1d), and also, mixed with keratosic pearls of well-differentiated type. Small dendritic cells with short but well-branched processes were found in less number (mean 5.6/field ×400) inside tumor areas of undifferentiated squamous cell. Stromal-positive dendritic cells showed a particular distribution and density according with histopathologic type of laryngeal cancer.

An inverse correlation has been found between peritumor and intratumor dendritic cells density in squamous cell carcinoma type. S100 stromal positive dendritic cells were rare in well differentiate carcinoma, and numerous in poor differentiate carcinoma (4.75 *vs.* 8.55). These cells had less evident dendritic morphology, being observed as isolated or grouped cells surrounding malignant epithelial areas (Figure 2, a and b).

Inflammatory infiltrate represented a constant finding when we assessed peritumor S100 positive cells, especially in cases with a high-density of stromal dendritic cells. Stromal tissue surrounding necrotic tumor areas was free of S100 positive dendritic cells in all cases. Immunostaining for CD68 was used in the quantification of stromal dendritic cells to differentiate between oval or polygonal in shape non-branched macrophages and peritumor dendritic cells. This method certified us the predominance of dendritic cells in the stromal component around the tumor areas.

Variations have been found concerning the dendritic cells density for malignant epithelial areas with a different tumor grade. High dendritic cells density was significantly correlated with G2 tumor areas, while, low density or absence of them was observed in G3 malignant laryngeal tumor. By this finding, an indirect relationship between dendritic cells and tumor grade has been suggested for laryngeal carcinomas.

## Discussion

Forty-three out of 27 000 articles found in the literature about laryngeal cancer, reported the role of dendritic cells in its progression, invasion and metastasis and, of them, few described a correlation between dendritic cells and prognosis.

The neglected quantification of number, distribution and morphology of dendritic cells in normal stratified squamous epithelium of the larynx delayed the appearance of evidences concerning dendritic cells role in the pathogenesis of laryngeal premalignant lesions or early stages of its malignant progression. Between 1995 and 2008, three articles suggested the role of dendritic cells in premalignant lesions of the larynx [22–24], but, until now, their role has not been certified. The lack of dendritic cells from stroma surrounding *in situ* carcinoma from our study, together with the decrease of intraepithelial dendritic cells number, partially correlate with previous studies and strongly support the hypothesis that premalignant and *in situ* lesions of laryngeal epithelium are ineffective monitoring by the immune system and, this, could be, a clue in the progression from premalignant stage to malignant transformation of the larynx epithelium [23].

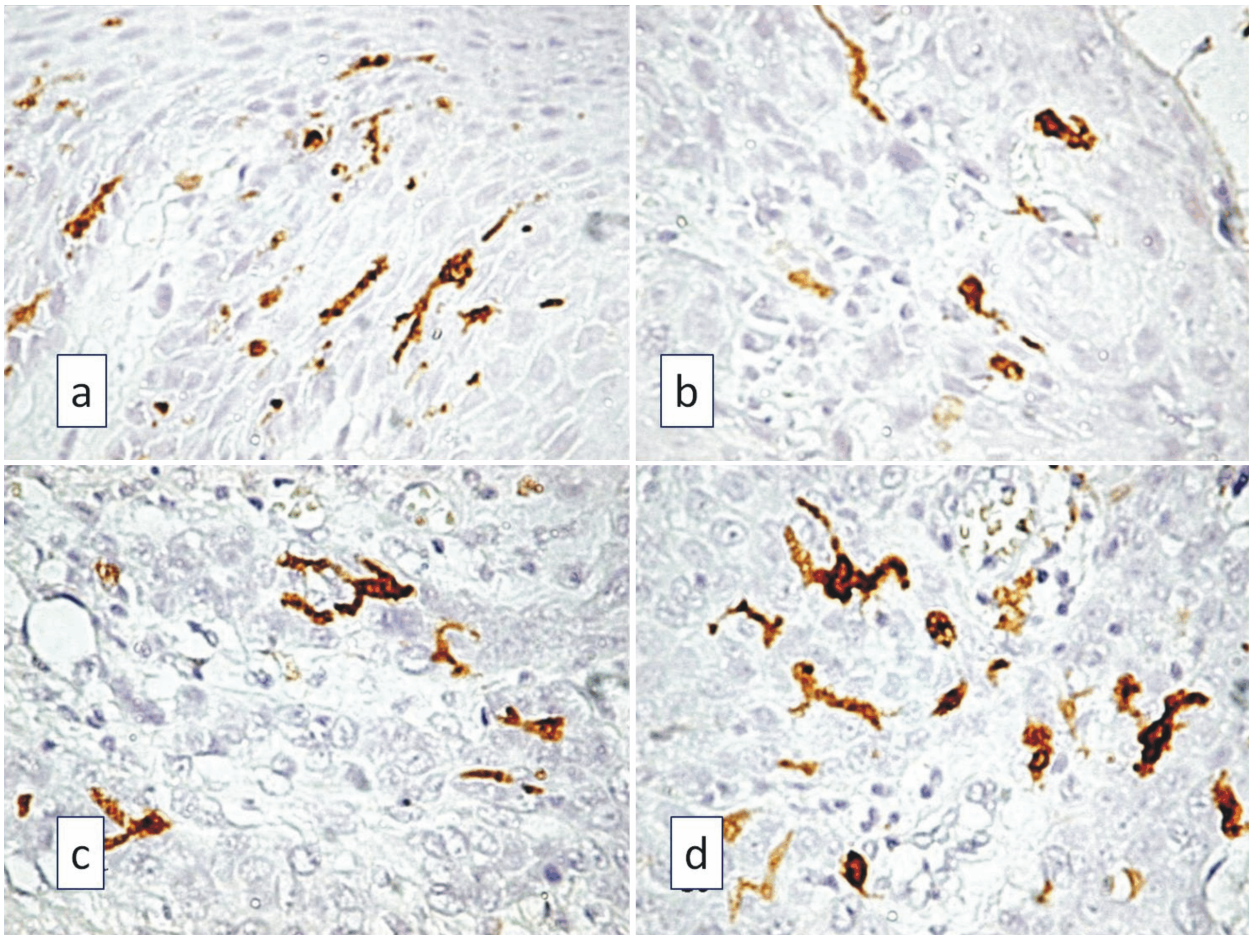


**Figure 1** – ***Variable distribution of S100 positive dendritic cells in laryngeal lesions. Leukoplasia, S100 protein positive cell suprabasal distribution (a, ×400). In situ carcinoma – small number of positive dendritic cells inside epithelial area (b, ×400). Branched, medium size dendritic cells in malignant epithelial areas and stroma of a squamous cell carcinoma (c, ×400). High number of S100 positive cells in intratumor area of squamous cell carcinoma (d, ×400).***

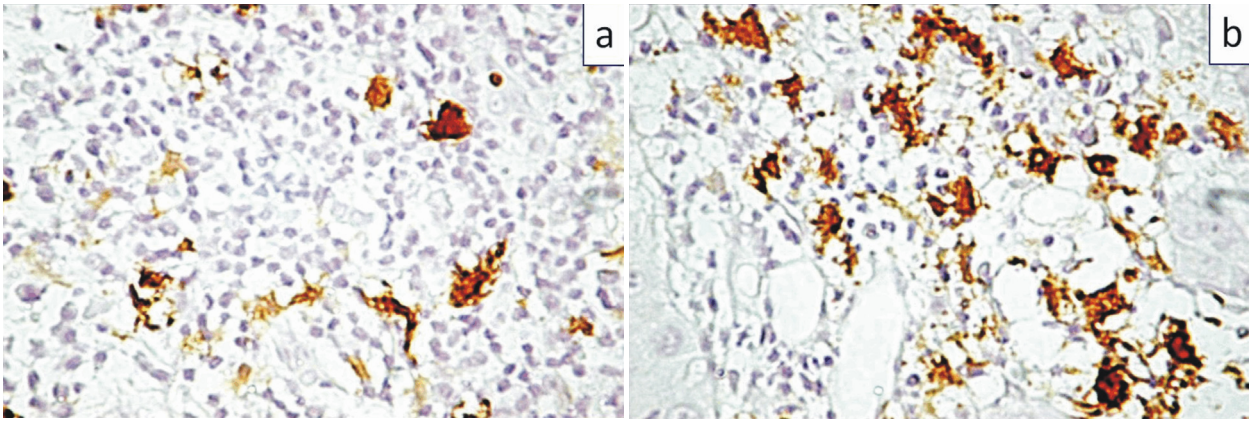


**Figure 2** – ***Isolated (a, ×400) and grouped (b, ×400) S100 positive dendritic cells cells inside the inflammatory infiltrate surrounding tumor epithelial areas.***

We found here differences concerning the presence, distribution and number of dendritic cells between different histopathologic types of laryngeal cancer. This finding was also reported by several papers [14, 18–20] but, none of them explained the mechanism by which these differences appear. The prevalence of dendritic cells in squamous cell carcinoma types both inside tumor areas and peritumor stromal component suggests a particular molecular profile of squamous cell carcinoma compared with other histopathologic entities. Few molecular signals of dendritic cells variability have been studied. Among them, matrix metalloproteinase-9, survivin, TIMPs and p63 were recently highlighted [25–27]. Together with therapeutic fails and poor prognosis, variability of dendritic cells among different types of laryngeal cancer strongly suggest the need for a molecular classification of laryngeal cancer in addition to conventional one, as a more efficient support for therapeutic approach and for improving long-term prognosis and survival. Migration of dendritic cells around tumor epithelial areas observed in poorly differentiated carcinoma may suggest a change of tumor

cells phenotype by acquisition of new tumor antigens able to activate new immunogenic cascade as a defense against tumor condition.

Inflammation is one of the most prominent component of the tumor microenvironment. In the early stages of laryngeal cancer, increased stromal chronic inflammation correlates with improved disease outcome [28]. Our data about the presence of inflammatory infiltrate in almost all cases with high number of peritumor S100 positive dendritic cells together with their absence in the stroma around necrotic tumor areas, sustain the interplay between tumor cells and dendritic cells in the development and progression of the laryngeal cancer by an unknown mechanisms at present time. Another important field less studied for laryngeal tumors is represented by the involvement of dendritic cells in the progression of viral induced malignancies of the larynx [29].

## Conclusions

Present paper reported particular aspects of dendritic cells distribution and density according with histo-pathologic types of laryngeal cancer. Assessment of both intratumor and peritumor dendritic cells morphology and density is mandatory for a better understanding of dendritic cells role in laryngeal cancer and suggested the presence of a dynamic population of dendritic cells specific for each type of laryngeal carcinoma. There is no correlation between S100 positive dendritic cell density and invasion level, but an important correlation was observed with G2 and G3 tumor grade. The presence of S100 positive dendritic cells may be considered as favorable prognostic factor in certain subtypes of laryngeal cancer, being still controversial for others. A potential ineffective immune defense suggested by low number of dendritic cells found in preinvasive stages of laryngeal cancer in the present study could be an important step in the development of new DCs based targeted therapies to improve the prognosis and survival of the patients with laryngeal cancer.

**Acknowledgments**

We are grateful to Diana Tătucu for her contribution to the primary processing of the specimens and Amalia Raluca Ceauşu for great technical support of immuno-histochemical stainings.

### References

[1] Marsh JC, Goldfarb J, Shafman TD, Diaz AZ, *Current status of immunotherapy and gene therapy for high-grade gliomas*, Cancer Control, 2013, 20(1):43–48.

[2] Son CH, Shin DY, Kim SD, Park HS, Jung MH, Bae JH, Kang CD, Yang K, Park YS, *Improvement of antitumor effect of intratumoral injection of immature dendritic cells into irradiated tumor by cyclophosphamide in mouse colon cancer model*, J Immunother, 2012, 35(8):607–614.

[3] Michielsen AJ, Noonan S, Martin P, Tosetto M, Marry J, Biniecka M, Maguire AA, Hyland JM, Sheahan KD, O'Donoghue DP, Mulcahy HE, Fennelly D, Ryan EJ, O'Sullivan JN, *Inhibition of dendritic cell maturation by the tumor microenvironment correlates with the survival of colorectal cancer patients following bevacizumab treatment*, Mol Cancer Ther, 2012, 11(8):1829–1837.

[4] Indelicato DJ, Finkelstein SE, *Dendritic cell immunotherapy in soft tissue sarcoma*, Immunotherapy, 2012, 4(10):1023–1029.

[5] Patel J, Bozeman EN, Selvaraj P, *Taming dendritic cells with TIM-3: another immunosuppressive strategy used by tumors*, Immunotherapy, 2012, 4(12):1795–1798.

[6] Strioga M, Schijns V, Powell DJ Jr, Pasukoniene V, Dobrovolskiene N, Michalek J, *Dendritic cells and their role in tumor immunosurveillance*, Innate Immun, 2013, 19(1):98–111.

[7] de Gruijl TD, van de Ven R, *Chapter six – Adenovirus-based immunotherapy of cancer: promises to keep*, Adv Cancer Res, 2012, 115:147–220.

[8] Becker Y, *Molecular immunological approaches to biotherapy of human cancers – a review, hypothesis and implications*, Anticancer Res, 2006, 26(2A):1113–1134.

[9] Astarita JL, Acton SE, Turley SJ, *Podoplanin: emerging functions in development, the immune system, and cancer*, Front Immunol, 2012, 3:283.

[10] Fainaru O, Almog N, Yung CW, Nakai K, Montoya-Zavala M, Abdollahi A, D'Amato R, Ingber DE, *Tumor growth and angiogenesis are dependent on the presence of immature dendritic cells*, FASEB J, 2010, 24(5):1411–1418.

[11] Sato J, Fujiwara M, Kawakami T, Sumiishi A, Sakata S, Sakamoto A, Kurata A, *Fascin expression in dendritic cells and tumor epithelium in thymoma and thymic carcinoma*, Oncol Lett, 2011, 2(6):1025–1032.

[12] Gerlini G, Sestini S, Di Gennaro P, Urso C, Pimpinelli N, Borgognoni L, *Dendritic cells recruitment in melanoma metastasis treated by electrochemotherapy*, Clin Exp Metastasis, 2013, 30(1):37–45.

[13] Sawant A, Hensel JA, Chanda D, Harris BA, Siegal GP, Maheshwari A, Ponnazhagan S, *Depletion of plasmacytoid dendritic cells inhibits tumor growth and prevents bone metastasis of breast cancer cells*, J Immunol, 2012, 189(9): 4258–4265.

[14] Ferlito A, Thompson LDR, Cardesa A, Gnepp DR, Devaney KO, Rodrigo JP, Hunt JL, Rinaldo A, Takes RP, *The importance of histological types for treatment and prognosis in laryngeal cancer*, Eur Arch Otorhinolaryngol, January 2013, *in press*.

[15] Lionello M, Staffieri A, Marioni G, *Potential prognostic and therapeutic role for angiogenesis markers in laryngeal carcinoma*, Acta Otolaryngol, 2012, 132(6):574–582.

[16] Todryk S, *A sense of tumour for the immune system*, Immunology, 2002, 107(1):1–4.

[17] Feng Y, Zhang B, Tan H, Su H, He W, *Relationship among lymphatic metastasis, pericancerous lymphocytic reaction and dendritic cell infiltration in laryngeal carcinoma cells*, Zhonghua Er Bi Yan Hou Ke Za Zhi, 2001, 36(4):264–266.

[18] Yilmaz T, Gedikoglu G, Celik A, Onerci M, Turan E, *Prognostic significance of Langerhans cell infiltration in cancer of the larynx*, Otolaryngol Head Neck Surg, 2005, 132(2):309–316.

[19] Esteban F, Concha A, Huelin C, Pérez-Ayala M, Pedrinaci S, Ruiz-Cabello F, Garrido F, *Histocompatibility antigens in primary and metastatic squamous cell carcinoma of the larynx*, Int J Cancer, 1989, 43(3):436–442.

[20] Klatka J, Grywalska E, Tabarkiewicz J, Polak A, Rolinski J, *Dendritic cell subsets in neoplastic tissue and peripheral blood of laryngeal cancer patients: relation with grade and stage of the disease*, Oncol Rep, 2012, 28(1):207–217.

[21] Li X, Takahashi Y, Sakamoto K, Nakashima T, *Expression of dendritic cell phenotypic antigens in cervical lymph nodes of patients with hypopharyngeal and laryngeal carcinoma*, J Laryngol Otol, 2009, 123(Suppl S31):5–10.

[22] Thompson AC, Griffin NR, *Langerhans cells in normal and pathological vocal cord mucosa*, Acta Otolaryngol, 1995, 115(6):830–832.

[23] Sprinzl GM, Hussl B, Obrist P, Yoneda K, Thumfart WF, Romani N, Schrott-Fischer A, *Dendritic cells in precancerous lesions of the larynx*, Laryngoscope, 2000, 110(1):13–18.

[24] Sadri M, McMahon J, Parker A, *Laryngeal dysplasia: aetiology and molecular biology*, J Laryngol Otol, 2006, 120(3):170–177.

[25] Wang BQ, Zhang CM, Gao W, Wang XF, Zhang HL, Yang PC, *Cancer-derived matrix metalloproteinase-9 contributes to tumor tolerance*, J Cancer Res Clin Oncol, 2011, 137(10):1525–1533.

[26] Dong P, Li X, Zhu Z, Yu Z, Lu G, Sun Z, Wang S, *Expression of S-100 positive dendritic cell, TIMP-1 and p63 and its significance in the primary laryngeal carcinoma*, Lin Chuang Er Bi Yan Hou Ke Za Zhi, 2005, 19(3):127–129.
[27] Szczepański M, Stelmachowska M, Stryczyński L, Golusiński W, Samara H, Mozer-Lisewska I, Zeromski J, *Assessment of expression of toll-like receptors 2, 3 and 4 in laryngeal carcinoma*, Eur Arch Otorhinolaryngol, 2007, 264(5):525–530.
[28] Chang J, Fang TJ, Yung K, van Zante A, Miller T, Al-Jurf S, Schneider S, Courey M, *Clinical and histologic predictors of voice and disease outcome in patients with early glottic cancer*, Laryngoscope, 2012, 122(10):2240–2247.
[29] Tezal M, Scannapieco FA, Wactawski-Wende J, Hyland A, Marshall JR, Rigual NR, Stoler DL, *Local inflammation and human papillomavirus status of head and neck cancers*, Arch Otolaryngol Head Neck Surg, 2012, 138(7):669–675.

***Corresponding author***
Anca Maria Cîmpean, Associate Professor, MD, PhD, Department of Microscopic Morphology / Histology, Angiogenesis Research Center, "Victor Babeş" University of Medicine and Pharmacy, 2 Eftimie Murgu Square, 300041 Timişoara, Romania; Phone +40256–204 476, e-mail: ancacimpean1972@yahoo.com

*Received: January 10th, 2013*

*Accepted: March 5th, 2013*