

## ORIGINAL PAPER

**ASSOCIATION OF INFILTRATING CELLS WITH MICROVESSEL DENSITY IN ORAL SQUAMOUS CELL CARCINOMA**OLGA STASIKOWSKA-KANICKA<sup>1</sup>, MAŁGORZATA WĄGROWSKA-DANILEWICZ<sup>1</sup>, MARIAN DANILEWICZ<sup>2</sup><sup>1</sup>Department of Nephropathology, Medical University of Lodz<sup>2</sup>Department of Pathomorphology, Medical University of Lodz

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Several lines of evidence indicate that immune cells in the tumor microenvironment play an important role in regulating tumor progression. An immunohistochemical method was used to examine the abundance of natural killer (NK) cells, mucosal dendritic cells (DCs), macrophages, mast cells, and microvessel density in 78 cases of oral squamous cell carcinoma (OSCC): with better prognosis – OSCCBP (n = 37), and with poorer prognosis – OSCCPP (n = 41), and 18 controls. The mean numbers of macrophages and microvessels were significantly higher in the OSCCPP group in comparison to both OSCCBP and control groups. The mean number of NK cells, mast cells and DCs was lower in the OSCCPP group in comparison to the OSCCBP group, but there were no statistically significant differences between mean numbers of NK cells in tested groups. Statistically significant correlations between the number of DCs and NK cells and mast cells, as well as between microvessel density and numbers of macrophages, DCs and mast cells were revealed in both OSCCPP and OSCCBP groups. In conclusion, our findings revealed an association between the number of infiltrating cells and oral cancer prognosis. Moreover, our results suggest that the infiltrating cells (macrophages, Langerhans and mast cells) may be involved in the process of angiogenesis.

**Key words:** oral cancer, tumor microenvironment, dendritic cells, macrophages, natural killers, mast cells.

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**Introduction**

Oral cavity squamous cell carcinoma (OSCC) is a malignant neoplasia arising from the oral mucosal epithelium [1]. Oral cancer is the sixth most common cancer worldwide and remains a serious problem of public health in the developing countries.

Several lines of evidence indicate that immune cells in the tumor microenvironment play an important role in regulating tumor progression, which may determine the clinical parameters and prognosis [2, 3, 4, 5, 6]. Macrophages have been implicated as key contributors to the tumor-microenvironment dynamics [7]. Nearly all tissues contain resident macrophages which

undergo tissue-specific adaptations that facilitate their contributions towards maintaining tissue homeostasis and/or tissue-specific functions [8]. Tumor-associated macrophages (TAMs) can derive from blood monocytes or can be differentiated from resident tissue macrophages. Tumor-associated macrophages are closely involved in tumorigenesis by inducing angiogenesis, immunosuppression, and invasion. They also play an important role in tumor cell migration and metastasis [9]. Several reports have suggested that TAMs are associated with tumor growth, disease progression, and poor prognosis in some human cancers [2, 10].

Mast cells derive from hematopoietic progenitor cells and migrate into vascularized tissues, in which

they differentiate into mature cells. The wide range of biological function, ubiquitous distribution and strategic location near blood vessels, nerves, inflamed tissues and neoplastic foci enable mast cells to play a central role in a multitude of physiologic, immunologic and pathologic processes [11]. Mast cells are involved in pain, tissue damage as well as repair and allergic inflammatory reactions and contribute to the pathogenesis of a number of disorders (e.g. asthma, rhinitis, tissue remodeling, arthritis, anaphylaxis) [12, 13]. Recently, apart from their roles in the maintenance of homeostasis and in inflammation, the association of mast cells with various tumors has been described [14]. In several malignancies, mast cell density has been found to correlate with increased risk of metastasis and poor prognosis [3, 4]. Mast cell activity facilitating tumorigenesis includes production of factors that support the process of forming a new vascular system within the site of neoplastic involvement.

Dendritic cells (DCs) are a heterogeneous population of highly motile cells that originate from the precursors in the bone marrow. DCs control immune responses and are pivotal in the development of immune memory and tolerance. Malfunction of DCs contributes to diseases such as autoimmunity, allergy, and cancer. DCs may induce and maintain the antitumor immunity. DCs infiltrating primary tumors (TIDCs) play an important role in antitumor immune surveillance, as TIDCs migrating to the regional lymph nodes are capable of presenting tumor antigens to naïve tumor-specific T cells. On the other hand, in malignant tumors, DCs display different phenotype and activity with pro-tumorigenic functions as well. DCs may lose antigen-presenting activity in the tumor environment or polarize into immunosuppressive regulatory DCs, which suppress effector T cells and support tumor growth [15]. Dendritic Langerhans cells (LCs) are a subset of DCs present in skin and mucosal linings, providing immunosurveillance to these tissue compartments [16]. In the oral mucosa, an important role of LCs in evoking the antitumoral response may be expected, although no definite proofs have been provided.

Natural killer (NK) cells are a heterogeneous population of lymphocytes originating from a distinct developmental lineage [17], and are functionally characterized by their cytotoxicity and cytokine production. NK cells belong to the innate immune system, and they can react to rapid changes in host cells without prior sensitization. As part of the first line of defense, they recognize and lyse virally infected cells and tumor cells. NK cells are not only killer cells, but they also have the capacity to shape adaptive immunity by regulating T cell responses [18, 19]. NK cells can be beneficial for mounting a T cell response by modulating DC function. NK cells act by different

mechanisms depending on the DC subsets and the prevailing cytokine environment. Natural killer T (NKT) cells can modulate immune responses in autoimmunity, infections and malignancies [20]. Several studies have demonstrated important anti-tumor effects of NKT cells and have reported reduced numbers of NKT cells in patients with malignant diseases, including malignant melanoma, head and neck, breast, colon and renal cancer [5].

The tumor microenvironment is emerging as a crucial aspect in the progression of solid and hematological malignancies. In this context, macrophages and mast cells have been demonstrated to have a role in enhancing angiogenesis in cancer through the release of pro-angiogenic factors and through a complex cross-talk within the tumor microenvironment [6].

Therefore, the objectives of this study were to evaluate the abundance of NKT cells (CD56 positive), mucosal dendritic cells (langerin positive), macrophages (CD68 positive), mast cells (tryptase positive), and microvessel density (CD31 positive areas) in relation to patient outcomes. Another purpose was to find a possible association between investigated cellular populations and microvessel density.

## Material and methods

### Patients

Seventy-eight formalin-fixed, paraffin-embedded tissue specimens of oral squamous cell carcinoma (OSCC), and eighteen control cases (non-cancer mucosa originating from the Plastic and Reconstructive Surgery Department) were retrieved from archival material (Chair of Pathomorphology, Medical University of Lodz, Poland). Paraffin-embedded tissue sections taken from postoperative material were diagnosed using standard hematoxylin and eosin staining, and the histological diagnoses were established according to the current standards [21]. The main criteria for patient selection were histopathological similarities within the group (G2), and the same anatomical localization of lesions (the floor of the mouth). To find the possible relationship between the studied markers and clinical prognosis, patients with OSCC were additionally divided into two groups: with better prognosis – OSCCBP (oral squamous cell carcinoma – better prognosis) (OSCC without metastatic disease,  $n = 37$ ), and with poorer prognosis – OSCCPP (oral squamous cell carcinoma – poorer prognosis) (OSCC with metastases to regional lymph nodes and/or with distant metastases,  $n = 41$ ). The age range for the OSCCBP group was from 28 to 75 years (mean  $\pm$  SD =  $59.24 \pm 10.89$ ), for the OSCCPP group was from 40 to 84 (mean  $\pm$  SD =  $59.39 \pm 11.16$ ) and for control cases 15 to 74 (mean  $\pm$  SD =  $47.05 \pm 18.71$ ).

## Immunohistochemistry

Formalin-fixed paraffin-embedded, 3- $\mu$ m tissue sections were mounted onto SuperFrost slides (SuperFrost Plus, Gerhord Menzel GmbH, Braunschweig, Germany), and deparaffinized in xylene and ethanol of graded concentrations. For antigen retrieval, the slides were treated in a microwave oven in a solution of TRS (Target Retrieval Solution, High pH, Dako, Denmark) for 30 minutes ( $2 \times 6$  minutes 360 W,  $2 \times 5$  180 W,  $2 \times 4$  minutes 90 W). After cooling down at room temperature, they were transferred to 0.3% hydrogen peroxide in methanol, for 30 minutes, to block endogenous peroxidase activities. Sections were rinsed with Tris-buffered saline (TBS, Dako, Denmark) and incubated for 30 minutes with monoclonal mouse primary antibodies against: CD68 (Dako; clone: PG-M1, dilution 1 : 100), mast cell tryptase (Dako; clone: AA1, dilution 1 : 100), CD31 (Dako; clone: JC70A, dilution 1 : 40), CD56 (Dako; clone: 123C3, dilution 1 : 50) and 60 minutes with rabbit polyclonal to langerin (Abcam; clone: EPR15863, dilution 1 : 1000). Immunoreactive proteins were visualized using the appropriate EnVision-HRP kit (Dako, Carpinteria, CA, USA) according to the instructions of the manufacturer. Visualization was performed by incubating the sections in a solution of 3,3'-diaminobenzidine (Dako, Denmark). After washing, the sections were counterstained with Mayer's hematoxylin and mounted. For each antibody and for each sample, a negative control was processed. Negative controls were carried out by incubation in the absence of the primary antibody and always yielded negative results.

## Morphometry

### *Morphometric analysis of CD68, CD56, langerin and mast cell tryptase positive cells*

CD68, CD56, langerin and mast cell tryptase positive cells were evaluated using a computer image analysis system consisting of a PC equipped with

a Pentagram graphic tablet, Indeo Fast card (frame grabber, true-color, real-time), produced by Indeo (Taiwan), and a color TV camera Panasonic (Japan) coupled with a Carl Zeiss microscope (Germany). This system was programmed (MultiScan 18.03 software, produced by Computer Scanning Systems, Poland) to calculate the number of objects (semiautomatic function).

The number of CD68, CD56, langerin and mast cell tryptase positive cells was estimated by counting all positive cells in 7-10 high power monitor fields (HPF) (0.029 mm<sup>2</sup> each), marking immunopositive cells (semiautomatic function).

### *Morphometric analysis of microvessel density*

Microvessel density (CD31 positive areas) was evaluated using the same computer image analysis system as described above. CD31 immunostains were evaluated in the vessels only (not in the individual cells), in the most vascular areas. The results were presented as the mean number of CD31 positive vessels with visible lumina per HPF (0.029 mm<sup>2</sup>).

## Statistical methods

Differences between groups were tested using unpaired Student's t-test preceded by evaluation of normality and Levene's test. The Mann-Whitney U test was used where appropriate. Correlation coefficients were calculated using Spearman's method. Results were considered statistically significant if  $p < 0.05$ .

## Results

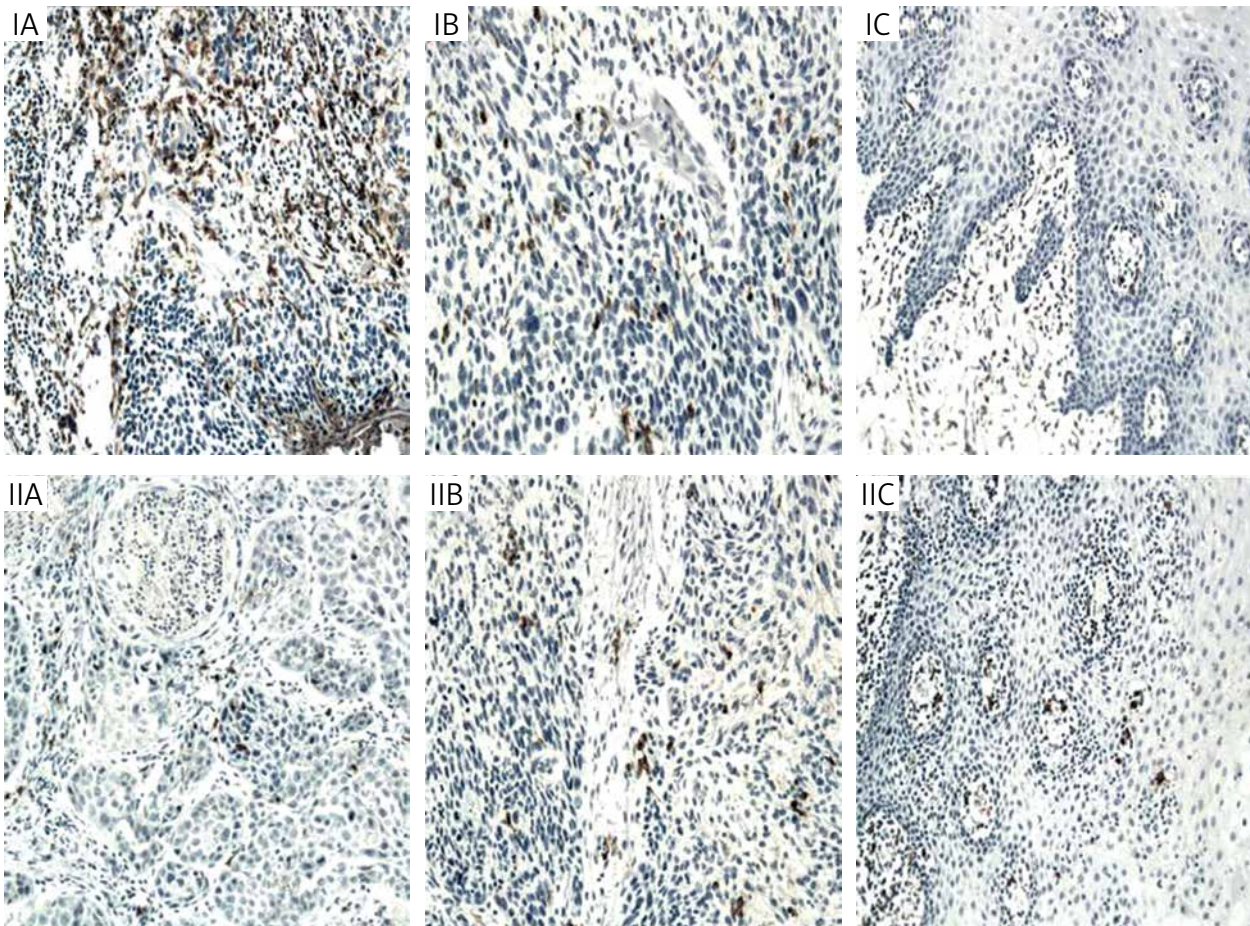
The quantitative data of the immunorexpression of tryptase, langerin, CD68, CD56 positive cells and microvessel density are presented in Table I.

The mean number of CD68 positive cells was significantly higher in the OSCCPP group (Fig. 1.IA) in comparison to both OSCCBP (Fig. 1.IB) and control groups (Fig. 1.IC). We also found a significantly lower

**Table I.** Morphometric data of langerin+ , CD56+ , tryptase+ , CD68+ cells, and CD31+ cells (vessels) in oral squamous cell carcinomas with: poorer prognosis (OSCCPP), better prognosis (OSCCBP) and controls.

GROUPS	LANGERIN+ CELLS CELLS/HPF	CD56+ CELLS CELLS/HPF	TRYPTASE+ CELLS CELLS/HPF	CD68+ CELLS CELLS/HPF	CD31+ VESSELS VESSELS/HPF
OSCCPP (n = 41)	3.8 $\pm$ 1.7	1.3 $\pm$ 0.6	8.2 $\pm$ 3.7	24.4 $\pm$ 14.6	68.4 $\pm$ 31.7
OSCCBP (n = 37)	5.2 $\pm$ 3.1	1.6 $\pm$ 0.9	12.7 $\pm$ 5.3	16.2 $\pm$ 9.6	42.7 $\pm$ 18.5
Controls (n = 18)	5.8 $\pm$ 2.7	1.5 $\pm$ 0.5	10.4 $\pm$ 4.7	12.3 $\pm$ 5.2	27.3 $\pm$ 15.2
OSCCPP vs. OSCCBP	p < 0.02	p = 0.08 (NS)	p < 0.001	p < 0.005	p < 0.001
OSCCPP vs. control	p < 0.002	p = 0.22 (NS)	p = 0.05 (NS)	p < 0.002	p < 0.001
OSCCBP vs. control	p = 0.4 (NS)	p = 0.66 (NS)	p = 0.12 (NS)	p = 0.11 (NS)	p < 0.004

NS – not significant



**Fig. 1.** I) Immunopositivity of CD68 in oral squamous cell carcinomas: A) in OSCCPP group, B) in OSCCBP group, C) in control group. Immunohistochemistry. Total magnification 100 $\times$ . II) Immunopositivity of tryptase positive mast cells in oral squamous cell carcinomas: A) in OSCCPP group, B) in OSCCBP group, C) in control group. Immunohistochemistry. Total magnification 100 $\times$

mean number of tryptase positive mast cells in OSCCPP (Fig. 1.IIA) compared to OSCCBP (Fig. 1.IIB). The mean number of langerin positive dendritic cells was significantly decreased in the OSCCPP group (Fig. 2.IA) in comparison to both OSCCBP (Fig. 2.IB), and control groups (Fig. 2.IC). The mean number of CD56 positive cells was lower in the OSCCPP group (Fig. 2.IIA) in comparison to both OSCCBP (Fig. 2.IIB) and control groups (Fig. 2.IIC), but there were no statistically significant differences between the mean numbers of CD56 positive cells in tested groups. The mean number of the vessels was significantly higher in the OSCCPP group (Fig. 3A) in comparison to OSCCBP (Fig. 3B) and control groups (Fig. 3C). We also found a significantly higher mean number of vessels in OSCCBP compared to the control group.

In both OSCCPP and OSCCBP groups there were positive significant correlations between the number of Langerhans dendritic cells and CD56 positive NK cells as well as tryptase positive mast cells, whereas the correlations between the number of Langerhans dendritic cells and CD68 positive cells were not statistically significant (Table II).

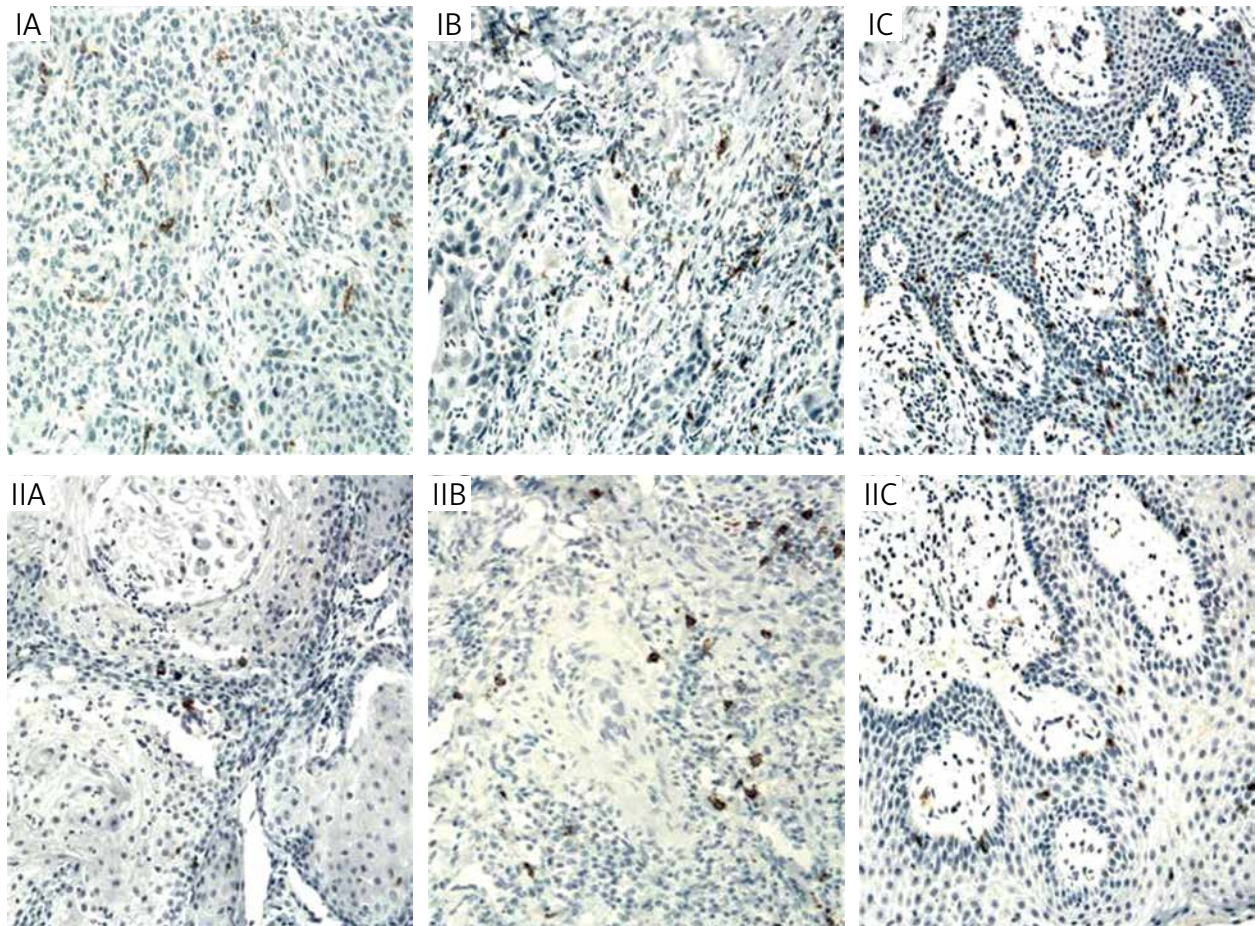
In the OSCCBP group there were positive significant correlations between the microvessel density and the number of CD68 positive macrophages and tryptase positive mast cells. In the OSCCPP group there were positive significant correlations between the microvessel density and the number of CD68 positive macrophages. We also found a negative correlation between the microvessel density and the number of tryptase positive mast cells and Langerhans dendritic cells in the OSCCPP group (Table III).

In the control group all these correlations were weak and not significant (data not shown).

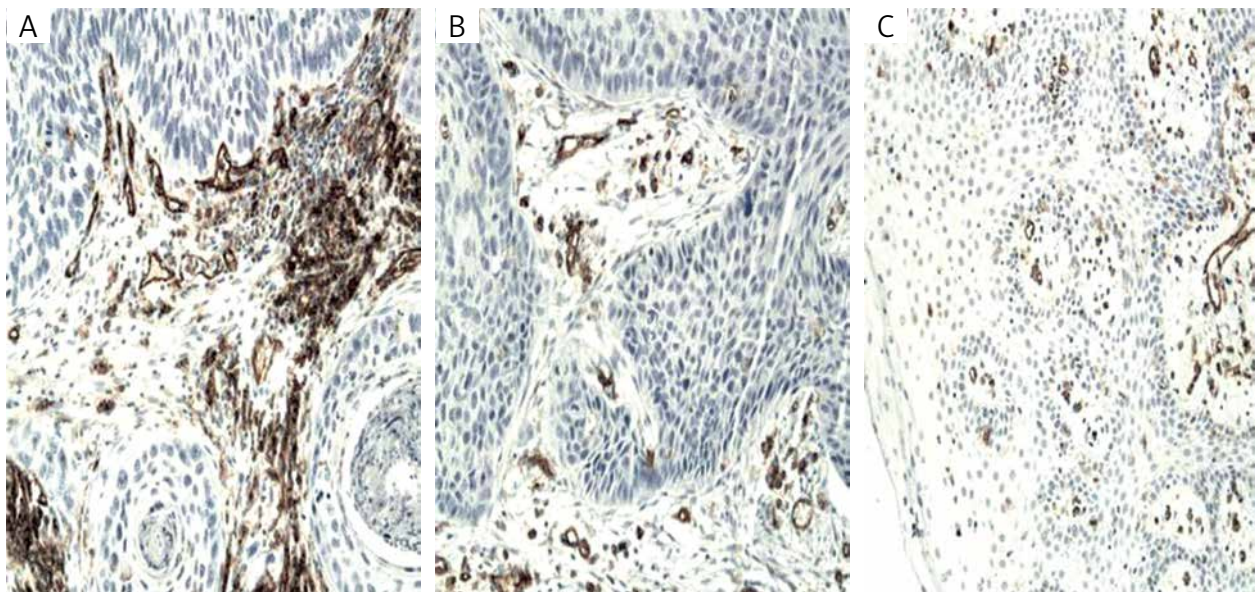
## Discussion

Tumors are not made up of a single cell type but are comprised of a mixture of cells of different lineages, including malignant cells but also innate and adaptive immune cells, fibroblasts, endothelial cells, and others. These cells have a double-edged sword function, being involved both in tumor suppression and in tumor progression and metastasis [22, 23]. Therefore, a better understanding of their role may





**Fig. 2.** I) Immunoexpression of langerin positive dendritic cells (Langerhans cells) in oral squamous cell carcinomas: A) in OSCCPP group, B) in OSCCBP group, C) in control group. Immunohistochemistry. Total magnification 100 $\times$ . II) Immunoexpression of CD56 in oral squamous cell carcinomas: A) in OSCCPP group, B) in OSCCBP group, C) in control group. Immunohistochemistry. Total magnification 100 $\times$



**Fig. 3.** CD31 positive cells (vessels) in oral squamous cell carcinomas: A) in OSCCPP group, B) in OSCCBP group, C) in control group. Immunohistochemistry. Total magnification 100 $\times$

**Table II.** Correlations between counts of langerin+ cells and CD56+ cells, tryptase+ cells as well as macrophages (CD68+ cells) in patients with oral squamous cell carcinomas with poorer prognosis (OSCCPP), and with better prognosis (OSCCBP)

CORRELATION BETWEEN	OSCCPP (N = 41)	OSCCBP (N = 37)
Langerin+ cells vs. CD56+ cells	r = 0.37, p < 0.02	r = 0.45, p < 0.006
Langerin+ cells vs. tryptase+ cells	r = 0.41, p < 0.008	r = 0.51, p < 0.002
Langerin+ cells vs. CD68+ cells	r = -0.22, p = 0.16 (NS)	r = -0.18, p = 0.28 (NS)

NS – not significant

**Table III.** Correlations between CD31+ microvessel density and langerin+ cell count, CD56+ cells, tryptase+ cells as well as macrophages (CD68+ cells) in patients with oral squamous cell carcinomas with poorer prognosis (OSCCPP), and with better prognosis (OSCCBP)

CORRELATION BETWEEN	OSCCPP (N = 41)	OSCCBP (N = 37)
Microvessel density vs. langerin+ cells	r = -0.31, p < 0.05	r = 0.32, p = 0.05(NS)
Microvessel density vs. CD56+ cells	r = 0.17, p = 0.28 (NS)	r = 0.16, p = 0.34 (NS)
Microvessel density vs. tryptase+ cells	r = -0.55, p < 0.001	r = 0.47, p < 0.004
Microvessel density vs. CD68+ cells	r = 0.49, p < 0.002	r = 0.53, p < 0.001

NS – not significant

aid further research in order to develop novel targeted therapies.

Tumor-associated macrophages are the predominant inflammatory components of immune cell infiltration in cancer stroma, which is discovered in the tumor microenvironment of many cancers [24]. Several reports have suggested that TAMs are associated with tumor initiation and development, immunosuppression, stroma formation, angiogenesis, invasion, and metastasis [6, 25, 26]. Studies in various cancers have shown that TAMs can be associated with both positive and negative prognosis [27]. In our study the mean number of CD68 positive cells was significantly higher in the OSCCPP group in comparison to both OSCCBP and control groups. Literature data show that patients with high TAM count tumors have a significantly more aggressive phenotype than those with low TAM count tumors [28, 29, 30]. In this context, our results seem to be in agreement with other findings. For instance, Ishigami *et al.* reported that patients with a high TAM count had poorer overall survival than those with a low TAM count [31].

Numerous experiments have demonstrated negative prognostic effect for mast cells, concluding that high mast cell density is related to increased risk of metastasis, poor prognosis and lower overall survival [32]. In contrast to these findings, we found a significantly lower mean number of tryptase positive mast cells in OSCCPP compared to OSCCBP. Similarly to our study, Jiang *et al.* found that a low mast cell count was associated with worse prognosis in gastric cancer [33]. Liu *et al.* reported that higher infiltration of mast cells is inversely associated with depth of invasion and lymph node status [34].

In some tumors such as breast cancer, mast cells seem to exert anti-tumor effects and are associated with favorable prognosis, whereas in some tumors such as non-small cell lung carcinoma the role of mast cells is still controversial [35]. We postulate that mechanisms underlying the mast cells' anti- and pro-tumoral activity still remain to be fully understood. In accordance with previous studies we also speculate that mast cells anti-tumor function may reflect their ability to mediate direct tumor killing, whereas their pro-tumoral effect is related to promotion of tissue remodeling, immunomodulation and production of pro-angiogenic factors [36, 37].

Infiltration of DCs into primary tumor lesions is associated with significantly prolonged patient survival and reduced incidence of metastatic disease in patients with oral, head and neck, nasopharyngeal, lung, bladder, esophageal, and gastric carcinomas [38]. In our study, high density of mucosal dendritic cells expressing the langerin marker was associated with significantly better prognosis. In agreement with our study, Ishigami *et al.* reported that the survival of patients with a high dendritic cell count was significantly better than in those with a low dendritic cell count [39]. Moreover, Tsujitani *et al.* [40] showed in gastric cancer that in cases with higher dendritic cell infiltration, survival time was significantly longer and peritoneal recurrences were rarer than in cases with only a slight infiltration. We also observed a significant correlation between number of Langerhans cells and tryptase positive mast cells in both tested groups of cancer. Interactions between these cells are not widely investigated, especially in the context of tumoral tissues [41]. According to literature data, mast



cells influence dendritic cell migration, maturation, and function by releasing TNF- $\alpha$  (tumor necrosis factor), histamine, and prostaglandin E2 and D2. It has been shown that the relationship between mast cells and DCs may contribute either to pro-inflammatory and anti-tumoral activity by release of IL-12 and IL-6 by DCs, or to immunosuppression and pro-tumoral function via IL-10 production, among others [36, 42].

In the context of tumor immunity, NKT cells are usually associated with antitumor responses, and the number of NK cells is positively associated with the prognosis and the survival time [39, 43]. In our study, among all four investigated immune cells, NK cells were the most scanty population of infiltrating cells. In accordance with previous findings, in our study, the number of NK cells was also higher in the OSCCBP group compared to the OSCCPP group, but the difference was not statistically significant. A possible explanation for the lack of significance may be the small number of tested cases, but it is also possible that technical reasons may be responsible for our results.

We revealed statistically significant correlations between the number of NK cells and Langerhans cells in both tested groups of cancer. We assume that NK cells may have a prognostic role due to their close interaction with antigen-presenting cells such as Langerhans cells. It has been shown that DCs mediate NK cell activation during innate immune responses, e.g. by improving survival, interferon  $\gamma$  secretion and cytotoxic activity of NK cells. On the other hand, NK cells have the ability to promote DCs' maturation and aid them to initiate adaptive immunity (e.g. against tumoral cells) via T-cell stimulation and differentiation. Interaction between these two immune cell populations is still under investigation [44, 45, 46].

Several studies have demonstrated correlations between tumor-associated macrophages, mast cells and microvessel density in different malignancies [47, 48, 49, 50]. In recent years, many findings have confirmed that mast cell accumulation correlates with increasing density of microvessels [51]. Mast cell activity facilitating tumorigenesis includes production of factors that support the process of forming a new vascular system within the site of tumor formation. Mast cell proangiogenic factors include VEGF (vascular endothelial growth factor), bFGF (basic fibroblast growth factor), TNF- $\beta$  (transforming growth factor  $\beta$ ), TNF- $\alpha$ , IL-8, metalloproteinases, tryptase and chymase. Our results also indicated a statistically significant association between the presence of mast cells and the process of angiogenesis. We found a negative correlation between the microvessel density and the number of tryptase positive mast cells in the OSCCPP group and a positive correlation between these parameters in the OSCCBP group. Further studies of the molecular mechanism of actions of

mast cells are needed to better understand their role in angiogenesis.

It is well known that monocytes are continually recruited into tumors, differentiate into TAMs, accumulate in hypoxic areas, and may induce angiogenesis through secretion of angiogenic cytokines [52, 53]. Tumor-associated macrophages secrete numerous proangiogenic factors, such as VEGF, bFGF, and MMP9 (matrix metalloproteinase), which are all associated with tumor angiogenesis and metastasis [25]. The strong correlation between TAM infiltration and microvessel density described in both tested groups of cancer is in concordance with current knowledge in this field. Moreover, recent studies have shown that tumor and immune cells including TAMs release lymphangiogenic factors into the tumor microenvironment [54]. Thus, macrophages seem to be essential angiogenic and metastatic promoters that act both to prepare sites for metastatic cells and to promote extravasation and growth of metastases [55].

Here, we demonstrated a correlation between the number of Langerhans cells and density of tumor vessels. Data concerning the relationship between dendritic cells and angiogenesis are rather scanty. Martinet *et al.* suggested that DCs may contribute to the formation of tumor vessels in human breast tumors through lymphotoxin- $\beta$  production [56]. These authors observed that the density of DC clusters was strongly correlated with the density of tumor vessels and favorable clinical outcome. Moreover, it has been shown in ovarian carcinoma that tumor-associated plasmacytoid DCs are able to induce angiogenesis *in vivo* by producing TNF- $\alpha$  and IL-8 [57]. On the other hand, recent studies have shown that VEGF inhibits the maturation and function of dendritic cells, and its expression negatively correlated with the number of DCs in tumors [58]. VEGF is thus suggested to be associated not only with the enhancement of angiogenesis, but also with a decline of local immune response in tumors. Decreased number of DCs in the OSCCPP group and a significant correlation between the number of langerin positive cells and the density of microvessels are coherent and could support this hypothesis. Although previous results suggest a relationship between DCs and angiogenesis within tumors, we cannot exclude the alternative possibilities that DCs arriving via tissue lymphatics may preferentially be attracted to pre-existing vessels. Further studies of the relationship between the number and activity of Langerhans cells and microvessel density are needed to better understand their role in oral carcinogenesis.

To the best of our knowledge, this is the first study considering the extent of tumor infiltration by four types of immune cells and microvessel density. The present study revealed an association between in-

creased number of macrophages, decreased number of mast cells, Langerhans dendritic cells as well as NK cells and poorer prognosis of OSCC patients. Moreover, our findings suggest that the infiltrating cells (macrophages, Langerhans and mast cells) may be involved in angiogenesis. Further research is required to determine the underlying mechanisms by which these immune cells play a role in oral tumorigenesis. In particular, the understanding of the importance of these cells for microvessel development may serve as a potential therapeutic strategy.

*This work was supported by a grant of the Medical University of Lodz, no. 503/6-038-01/503-61-002. The authors declare no conflict of interest.*

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