Histol Histopathol (2021) 36: 113-122

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

REVIEW **Open Access**

Tumour-associated tissue eosinophilia (TATE) in oral squamous cell carcinoma: a comprehensive review

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Summary. Oral squamous cell carcinoma is the most common head and neck malignancy, characterised by high invasive capacity, lymph node metastasis, and high recurrence rate. Among the morphological features of oral cancer, the tumour-associated tissue eosinophilia has gained growing interest in the last years. Eosinophils are a minor subpopulation of leukocytes, representing 1-3% of all circulating white blood cells. The presence of high levels of eosinophils is associated with several diseases, but their role in cancer pathophysiology is controversial. In particular, an uncertain and contradictory relationship exists between the exact role of tumour-associated tissue eosinophilia and oral cancer development. Many studies have shown that tumour-associated tissue eosinophilia increases both in the progression of oral potentially malignant disorders as well as in the grade and stage progression of oral cancer. Despite this, both negative and positive prognostic outcomes have been associated with eosinophil infiltration. The heterogeneous results may be partially due both to several methodological inconsistencies and to an incorrect interpretation of the physiological role of eosinophils. Therefore, further studies to elucidate the contribution of eosinophil infiltration are needed, focusing on the existence of eosinophil subpopulations regulated by the cancer immune microenvironment. Furthermore, the correct reporting of prognostic marker research is encouraged, in order to ensure the reproducibility and the comparability of the results from different studies.

Key words: Oral squamous cell carcinoma, Oral cavity, Tumour-associated tissue eosinophilia, Eosinophils, Prognosis

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DOI: 10.14670/HH-18-250

Introduction

Oral squamous cell carcinoma (OSCC) is the most common head and neck malignancy, with 400,000 new cases diagnosed annually worldwide, accounting for 2% of all cancers (Shield et al., 2017). This tumour can develop in all regions of the oral cavity from potentially malignant disorders or directly from apparently normal mucosa (Sopka et al., 2013). OSCC is characterised by highly aggressive clinical behaviour, with high incidence of locoregional recurrence and node metastasis and, in more advanced stages, distant metastases and worse prognosis. In the advanced stages, this cancer shows, in addition, the frequent occurrence of chemo/radio-resistance, accounting for the poor prognosis of most cases, with a dismaying 5-year survival rate still restricted to 50-60%.

The recently released 8th edition of the American Joint Committee on Cancer (AJCC) Staging Manual has introduced several modifications improving the prognostic accuracy of the system (Amin et al., 2017). However, despite these important advancements, some evidence shows that the AJCC system still needs to be improved (Kano et al., 2018; Mascitti et al., 2018). Therefore, it is necessary to find new prognostic markers in order to better stratify those patients who could benefit from more specific therapies. Several molecular biomarkers have been proposed as prognostic and predictive markers for OSCC, but the variable results and the high cost currently hinders their clinical utility (Blatt et al., 2017; Santarelli et al., 2017).

In the last years attention has been focused on the tumour microenvironment (Schiavoni et al., 2013). In particular, the study of morphological features of tumour tissue, such as the proportion of tumour cells relative to surrounding stroma or the spatial organization of tumour-infiltrating lymphocytes in the tumour microenvironment, could be a valuable source of information (Almangush et al., 2018; Heikkinen et al., 2019; Mascitti et al., 2020). Among the morphological features of OSCC, some have gained growing interest,

such as the presence of eosinophil infiltration in the tumour microenvironment, known as tumour-associated tissue eosinophilia (TATE) (De Paz et al., 2019). Nevertheless, the exact role of eosinophils in OSCC is not yet elucidated and conflicting results have been reported. The aim of this review is to conduct an extensive and critical review of the literature regarding the clinical and prognostic role of eosinophil infiltration in OSCC.

Eosinophils and their functions

Eosinophils are a minor subpopulation of leukocytes that has been found in the blood of all vertebrates, representing 1-3% of all circulating white blood cells (Chusid, 2018). Following development and differentiation processes, eosinophils are physiologically distributed in the blood and in several tissues (Wen and Rothenberg, 2016). In particular, eosinophils are able to invade tissues attracted by eotaxin and other eosinophil-specific chemokines, which are constitutively produced in physiological conditions and markedly upregulated in pathological states (O'Sullivan and Bochner, 2018). Eosinophils were discovered by several scientists around the mid-nineteenth century, although Paul Ehrlich definitively identified these cells by using eosin in 1879 (Kay, 2015). Although the existence of these cells has been proven for nearly 150 years, only in the last few decades has the role of eosinophils in human health and disease emerged. Their role in modulating multiple components of the immune system has been significantly explored, demonstrating bidirectional interactions between eosinophils and Th2 helper cells. Eosinophils can also affect the survival of bone marrow plasma cells and the regulation of IgA production. Furthermore, eosinophils can act both as antigen-presenting cells and as effector cells of the innate immune system (Wen and Rothenberg, 2016). For these reasons, the eosinophils seem to be a critical component in a complex regulatory network, modulating both inflammatory and immune responses in order to maintain tissue homeostasis in both healthy and pathological states (Lee et al., 2010). Eosinophils exert their functions through the so-called eosinophil secretome, defined as the complete set of proteins secreted into the extracellular space, including cytotoxic granules, cytokines, chemokines, and growth factors (Sakkal et al., 2016). The main proteins secreted by these cells are the eosinophil cationic protein (ECP), the major basic protein, the eosinophil derived neurotoxin, and the eosinophil peroxidase. Furthermore, a wide range of both pro-inflammatory and anti-inflammatory cytokines and chemokines can be released, allowing these cells to respond efficiently to many different stimuli (Hogan et al., 2008; Sakkal et al., 2016).

Eosinophilia, defined as high levels of eosinophils, both at systemic and tissue levels, is associated with several diseases, such as allergies, asthma, gastrointestinal disorders, and vasculitis (Valent et al., 2012; Diny et al., 2017). Indeed, these cells have been related to the underlying pathogenesis and to the prognosis of several diseases. On the contrary, the role of eosinophils in cancer pathophysiology is controversial. Despite being recognised for over 120 years, both negative and positive prognostic outcomes have been associated with TATE (Przewoski, 1896; Davis and Rothenberg, 2014; Sakkal et al., 2016). Furthermore, despite numerous studies reporting the presence of TATE in several tumour types, including OSCC, there are few clues as to the mechanistic role of eosinophils in cancer biology (Fig. 1) (Lowe et al., 1981).

Tissue eosinophilia in oral squamous cell carcinoma

The first investigation of TATE in OSCC was conducted by Lowe and Fletcher in 1984, with the aim to evaluate the expression patterns of this parameter (Lowe and Fletcher, 1984). Histological sections of 275 squamous cell carcinomas, including 136 OSCCs, were examined for the presence of TATE in close proximity to or within the tumour, classified as moderate or massive according to the number of eosinophils per 10 high-power field (HPF). The results of the study showed the presence of TATE in 15 OSCC patients (11.6%). Interestingly, almost all cases showing massive TATE (7 out of 9) were found in OSCCs, demonstrating for the first time that the oral cavity seems to be particularly affected by eosinophilic infiltration.

After this descriptive report, the study of TATE in OSCC was almost neglected for nearly twenty years. Indeed, the first studies investigating the clinical significance of TATE in OSCC were published by a Brazilian group between 2002 and 2003. In particular, Dorta et al. conducted the first morphometric study to evaluate the presence of TATE in 125 specimens of Stage II and III OSCC (Dorta et al., 2002). For each case, the number of observed eosinophils per 75 HPF was divided by the total area, obtaining the mean number of eosinophils per mm^2. 45.6% of patients showed eosinophilic infiltrate, demonstrating an association between intense TATE and better 5-year disease-free survival (DFS) and overall survival (OS). In 2003 the same group compared the accuracy of haematoxylin & eosin (H&E) staining with the accuracy of immunohistochemistry (monoclonal anti-CD15 antibody) in assessing the eosinophilic infiltrate in OSCC (Lorena et al., 2003a). The results showed that there was no significant difference between H&E and CD15 staining, suggesting that immunohisto-chemistry is not necessary for the identification or the measurement of TATE in OSCC.

One of the most critical aspects related to the evaluation of TATE is the method of eosinophil counting. This aspect was examined for the first time in 81 OSCC specimens by comparing the classical method (in which the eosinophils are counted per 10 HPF) with the density method (in which only the area containing

the highest eosinophil numbers is counted for each case) (Alkhabuli and High, 2006). Findings from this study showed that the density method seems to be more related with TATE function, although the authors suggested that a good correlation between the two methods could be achieved if the cut-offs of the classical methods are modified. More recently, a Finnish group evaluated different approaches to count TATE in OSCC by comparing the density method with a modified version of the classical method (Peurala et al., 2018). Contrary to the previous study, the results showed an excellent interobserver concordance by the classical method.

Several studies were conducted with the aim to investigate the role of TATE in tumour invasion. Due to the peculiar histological features of TATE in oral cavity, an Italian group proposed the eosinophilic-rich squamous cell carcinoma as a possible new microscopic entity of OSCC (Falconieri et al., 2008). In particular, the presence of tissue eosinophilia was associated with invasive OSCC, suggesting that eosinophilic aggregates in small biopsies of superficial lesions could be a reliable indicator of invasive tumours. Another study investigated the association between TATE and tissue invasiveness in 43 primary OSCC specimens (Oliveira et al., 2009). Most of the patients with Stage III and IV showed a significant intense TATE, particularly in close association with striated damaged muscle fibres, although no significant association between TATE and survival outcomes was found. The distribution of eosinophil infiltrates among different tumour subsites was reported in a study of 76 OSCC specimens (Tadbir et al., 2009). In particular, three areas were analysed: the stroma subjacent to the epithelium, the intratumoral stroma, and the invasive tumour front. Although TATE had no correlation with prognostic outcomes, this study confirmed that the eosinophilic infiltrate mainly affects the invasive tumour front. The presence of TATE in different tumour subsites was recently associated with the depth of invasion, a parameter used to measure the invasiveness of OSCC regardless of any exophytic component (Jain et al., 2019). In 87 OSCC specimens the intratumoral and peritumoral TATE were evaluated, showing an association between eosinophil infiltration and increasing tumour infiltration depth. Another finding was the association between the eosinophil count and the infiltration of muscle fibres, suggesting a role of eosinophils in the degradation of muscular fibres damaged by malignant cell invasion through the

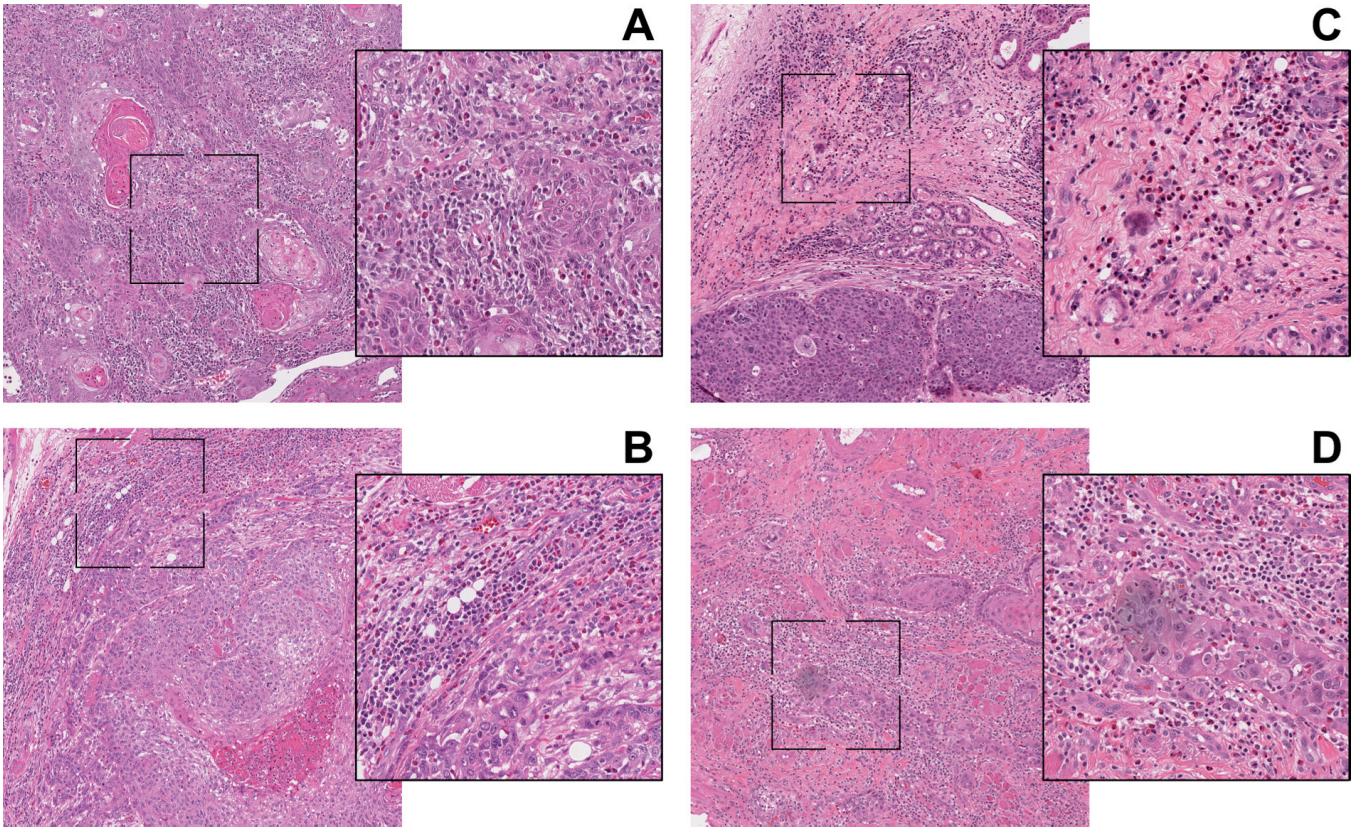

Fig. 1. Histological views of eosinophil infiltration in OSCC: H&E stained sections showing the presence of TATE at different distances from the invasive front (**A-D**). The insets show a part of the same images at higher magnification. Eosinophils can be easily identified by the presence of pink-red cytoplasm and fine granules stained by eosin.

secretion of ECP (Sugihara et al., 2001).

Some studies focused on the significance of TATE in predicting lymph node metastases in patients with OSCC. In the first report, 71 patients with early stage OSCC were divided into two groups based on the number of eosinophils/mm^2 observed (absent/mild TATE group and intense TATE group) (Oliveira et al., 2012). Although no prognostic difference was found between these two groups, intense TATE was associated with occult lymph node metastases, suggesting that tissue eosinophilia could be used as a predictive factor for lymph node involvement. These results were confirmed in a small OSCC cohort study, in which the presence of intense TATE was associated with locoregional recurrences (Rakesh et al., 2015).

Jain et al. compared for the first time the eosinophil infiltration in oral dysplastic lesions with OSCC specimens (Jain et al., 2014). The results showed an initial recruitment of eosinophils also in dysplastic lesions, although the eosinophil count was significantly lower compared to OSCC. Furthermore, TATE in OSCC cases without metastases was found to be higher than those in metastatic OSCC, suggesting a possible prognostic role in oral cancer. Another study focused on dysplastic lesions, investigating if tissue eosinophilia could be used as a marker of malignant transformation in oral leukoplakia (Madhura et al., 2015). The eosinophil count was found to be directly proportional to the degree of dysplasia, suggesting that a high eosinophil infiltration could be associated with higher risk of stromal invasion. These results were confirmed by a recent report suggesting that TATE could be considered an indicator of invasion in oral cancer (Martinelli-Klay et al., 2018). Indeed, the morphometric study of 99 oral specimens (51 oral intraepithelial neoplasia and 48 OSCC) showed that the intensity of TATE was significantly associated with the severity of disease. In particular, a significant increase in tissue eosinophilia in invasive OSCC was reported, although the eosinophil count was not related to the degree of dysplasia.

It is known that conventional histological grading of OSCC correlates poorly with prognostic outcomes. For this reason, numerous efforts have been made to improve the prognostic power of this parameter by including other histological risk factors. The presence of TATE in different histological grades of OSCC has been recently analysed, showing discordant results. Indeed, in the first study intense TATE was found to be associated with well-differentiated OSCC (Bankur et al., 2016). On the contrary, Deepthi et al. reported an association between the increased level of TATE and a higher histological grade (Deepthi et al., 2019).

The most significant research on TATE in OSCC was recently conducted in a large cohort of oral tongue squamous cell carcinoma (De Paz et al., 2019). Eosinophil infiltration was quantified at the invasive tumour front, incorporating the results of TATE expression into a nomogram. 259 surgical specimens were divided in two groups based on the number of eosinophils/mm^2 observed (low and high TATE groups). Higher levels of TATE were associated with several parameters, including high pT classification, Stage III and IV, and tumour depth. Significantly, multivariate analysis demonstrated that TATE was an independent predictor of survival; in particular, high TATE levels were associated with worse 5-year OS. Another finding of this study was a correlation between TATE and tumour-associated blood eosinophilia. Notably, this was the first report of an association between tissue and circulating eosinophil count in OSCC. Indeed, only one case of an OSCC patient with TATE and blood eosinophilia has been reported so far (Horie et al., 2007).

Numerous studies have tested the sensitivity of several staining techniques different from H&E, with the aim to find other reliable diagnostic tools for eosinophil detection. Special stains such as carbol chromotrope and Congo red have been particularly investigated for detection of eosinophil infiltration due to their property to bind with these cells. Carbol chromotrope staining is a less known stain, reported for the first time by Lendrum, that showed the ability to selectively detect eosinophils. Lendrum's carbol chromotrope staining was tested for the first time in a pilot study on a small cohort of OSCC specimens, suggesting the reliability of this staining method and a favourable prognosis in patients with TATE (Debta et al., 2011). However, these results were not confirmed by another group, in which the patients with increased tumour size were more frequently associated with high eosinophil count (Peter et al., 2015). Another study using carbol chromotrope in 70 OSCC specimens found a weak correlation between the TATE in tumour primary site and the number of eosinophils in metastatic lymph nodes (Jain et al., 2018). Furthermore, TATE was significantly higher in non-metastatic OSCC compared to metastatic tumours, suggesting a relationship between eosinophilic infiltrate and less aggressive tumour behaviour. A comparison between carbol chromatrope and H&E was recently conducted in a small cohort of OSCC patients (Vaibhav et al., 2018). Patients with Stage IV OSCC showed a significant intense TATE, mainly at the invasive front. Carbol chromatrope was also found to have higher specificity than H&E and to be more accurate in quantifying TATE.

Congo red staining is an organic compound used in most laboratories for staining amyloid in tissue sections, although it has demonstrated the ability to detect eosinophils. A comparative analysis of Congo red, H&E, ad EMR1 marker was conducted for the first time with the aim to evaluate eosinophil infiltrate in oral cavity specimens (Kargahi et al., 2015). In particular, 20 cases of normal oral mucosa, 20 cases of dysplastic lesion, and 20 cases of OSCC were investigated. Congo red staining showed a significantly higher number of eosinophils than H&E, although EMR1 seemed to be a more sensitive marker. Nevertheless, all staining techniques confirmed a progressive rise in eosinophil count when going from normal to cancerous tissue. On the contrary,

another study on a small cohort of OSCC specimens reported that H&E staining technique was significantly more sensitive in detecting TATE than Congo red (Yellapurkar et al., 2016). However, no significant prognostic value of TATE in OSCC was found. A recent report focused on the presence of TATE in oral dysplastic lesions and in different histological grades of OSCC (Deepthi et al., 2019). The results confirmed those reported by previous studies, showing a higher number of eosinophils in OSCC than in dysplastic lesion. Furthermore, an increased level of TATE was associated with a higher histological grade. Finally, the only comparison reported in the literature between Congo red and carbol chromatrope in OSCC showed a comparable ability to detect TATE (Vaibhav et al., 2018).

Although the exact role of TATE in OSCC has not yet been elucidated, their participation in the anti-tumour response it is likely to be mediated by the release of cytotoxic proteins. The presence of eotaxin, a chemokine involved in the selective recruitment of eosinophils, was investigated in OSCC cases with and without TATE (Lorena et al., 2003b). An intense expression of eotaxin was reported in eosinophils, although a slight expression of this chemokine was also reported in normal and malignant epithelial cells. These results suggest that the release of eotaxin from eosinophils in OSCC may represent an important pathway of local eosinophil accumulation.

Due to the role of granule-derived cytotoxic proteins in the eosinophil-tumour interactions, the anti-tumour effect of ECP was studied in two OSCC cell lines (SCC-4 and SCC-25) (De Lima et al., 2015). After exposure for 72h to ECP, even at low concentration, OSCC cells showed several morphological changes and a significant reduction in cell viability. Another study investigated the frequency of ECP-gene polymorphism 434(G>C), a single nucleotide polymorphism associated with an altered eosinophil function, and its relationship with TATE in OSCC (Pereira et al., 2010). In particular, 157 OSCC patients and 165 healthy subjects were analysed with PCR analysis, but no significant difference between the two groups was found. Although no significant differences on survival rates was reported, OSCC patients with intense TATE and ECP 434(G>C) polymorphism seemed to have a tendency towards a poor prognosis.

Eosinophils role in OSCC: current limitations and future perspectives

Overall, the results of the studies regarding the presence of TATE in OSCC are highly heterogeneous and controversial, describing an uncertain and contradictory relationship between the role of eosinophils and oral cancer development (Table 1). Indeed, many studies agree in showing that the presence of TATE increases both in the progression of oral potentially malignant disorders as well as in the grade and stage progression of OSCC. These results contrast with those regarding the prognostic role of eosinophil infiltration in OSCC. Indeed, although initial studies had suggested a favourable prognostic role of TATE in OSCC patients (Dorta et al., 2002; Debta et al., 2011), many reports failed to demonstrate a prognostic significance of this morphologic parameter (Oliveira et al., 2009; Tadbir et al., 2009; Yellapurkar et al., 2016; Peurala et al., 2018). The main reason for this disagreement may lie in the inadequate sample size used in most studies. Notably, some recent studies have suggested a poor prognostic role of high TATE in OSCC. In particular, a multivariate analysis of eosinophil infiltration in a cohort of 259 oral tongue squamous cell carcinomas demonstrated the association of TATE with worse OS (De Paz et al., 2019). The heterogeneous

Table 1. OSCC outcomes related to TATE detected by histopathological analysis.

Parameter	Outcomes associated with high TATE	References
Pathological stage	Increasing Stages (III-IV)	Oliveira et al., 2009; Vaibhav et al., 2018; De Paz et al., 2019
	Increasing pT levels	Peter et al., 2015; De Paz et al., 2019
	Increasing tumour infiltration depth	Martinelli-Klay et al., 2018; De Paz et al., 2019; Jain et al., 2019
	Higher risk of lymph node metastases	Oliveira et al., 2012
	Lower risk of lymph node metastases	Jain et al., 2014, 2018
Dysplastic lesions	High degree of dysplasia	Kargahi et al., 2015; Madhura et al., 2015
Grading	Poorly differentiated OSCC (G3)	Deepthi et al., 2019
	Well-differentiated OSCC (G1)	Bankur et al., 2016
Prognosis	Better OS/DFS	Dorta et al., 2002; Debta et al., 2011
	Worse OS/DFS	De Paz et al., 2019
	No correlation with prognostic outcomes	Oliveira et al., 2009, 2012; Tadbir et al., 2009; Yellapurkar et al., 2016
	Higher risk of locoregional recurrences	Rakesh et al., 2015
Other parameters	Muscle involvement	Oliveira et al., 2009; Jain et al., 2019
	Alcohol/tobacco consumption, male sex	De Paz et al., 2019

TATE, tumour-associated tissue eosinophilia; OSCC, oral squamous cell carcinoma; OS, overall survival; DFS, disease-free survival.

results may be due to several methodological inconsistencies; in particular, four critical points can be identified (Table 2). The first point is the method of eosinophil counting, which may change depending on the approach used: the classical method (in which the mean number of eosinophils per 10 HPFs is used) and the density method (in which only the area containing the highest eosinophil numbers is counted for each case) (Fig. 2). The second critical point is the approach used to classify the degree of eosinophilic infiltration. Indeed, the cut-off values for classifying OSCC patients with TATE vary considerably, both for the number of cut-off used (single, double, or multiple cut-offs) and for their values, although the values applied in many studies are 10 and 100 eosinophils per HPF (Lowe and Fletcher, 1984; Alkhabuli and High, 2006; Peurala et al., 2018; Vaibhav et al., 2018). The third point is the number of HPF evaluated for each patient, influencing the accuracy of TATE estimation. Indeed, the number of HPF evaluated for each OSCC specimen ranged from 10 to 75 in different studies. Finally, the number of eosinophils can be reported in absolute terms or as the number of cells per total area (in mm^2). Other parameters, such as the use of visual or automated software for performing TATE counts, the use of H&E or other staining techniques, and the thickness of slide sections could also influence the results. Also, the magnification (mainly ×400 or ×800) used might influence the number of eosinophils detected in each field. Furthermore, several studies omitted relevant data, such as the number of HPF analysed or the cut-off used, hindering the comparison of results obtained from different authors (Pereira et al., 2011). Taken together, all these limitations prevent the possibility of conducting a metanalysis of TATE in OSCC.

Another reason that might explain the inconsistencies reported in the literature regarding the role of TATE in OSCC derives from an incorrect interpretation of the physiological role of eosinophils. Indeed, several studies indicate that eosinophils can both promote and hinder tumour growth (Martinelli-Klay et al., 2009). In particular, tumour immune response seems to be dependent both on tumour-infiltrating lymphocytes and on other leukocytes within the tumour microenvironment (Sakkal et al., 2016). Eosinophils are able to induce tumour cell death, probably through cell-cell interactions, and under specific conditions could also act as anti-tumour antigen-presenting cells (Gatault et al., 2012; Varricchi et al., 2018). In spite of this, eosinophils are known to induce Th2 cells, disturbing the Th1/Th2 cytokine polarization and hindering an effective anti-tumour response (Ellyard et al., 2007). Furthermore, eosinophils produce several pro-angiogenic factors, suggesting a possible role in promoting tumour angiogenesis (Varricchi et al., 2018). As shown for M1 and M2 macrophages, a possible explanation could be the existence of different functional subpopulations of eosinophils, called E1 and E2, based on the balance of Th1/Th2 cytokine expression patterns (Sakkal et al., 2016; Varricchi et al., 2018). These findings suggest that the role of eosinophils in cancer development and progression should be included in the context of a wider scenario, concerning the role of myeloid cell infiltrate in solid tumors. Indeed, tumor-infiltrating myeloid cells are an integral component of the tumour microenvironment and have an important role in regulating cancer progression and survival (Neophytou et al., 2020). Tumour associated macrophages are the most extensively studied myeloid cells in OSCC, suggesting that the differential polarization of macrophages in the tumour microenvironment is linked to different prognosis. Indeed, a recent metanalysis demonstrated a poor prognostic outcome in OSCC patients showing high levels of M2 macrophages (Troiano et al., 2019). Mast cells are another group of myeloid-derived cells that are involved in tumour development. These cells accumulate in the tumor microenvironment near the blood vessels, although their prognostic role has not yet been clarified. Indeed, it is possible that the tumor microenvironment can polarize mast cells toward anti-tumorigenic (MC1) or pro-tumorigenic (MC2) phenotype, depending on the

Table 2. Main parameters and their determination used for eosinophil counting that are reported in literature.

Parameter	Modalities
Eosinophil counting approaches	Classical method (mean number of eosinophils per 10 HPF) Density method (highest eosinophil density per surface area) Other methods (e.g. the mean value different representative tumour areas)
Cut-off values used	Single cut-off (low/high TATE): 61; 67; 100; 175 Double cut-offs (low/moderate/high TATE): 5-15; 10-30; 21-68; 10-83; 26-83; 10-100; 50-120 Multiple cut-offs: 0-29-72; 4-10-21-45-89
Counting parameters	n. of HPF evaluated: 10; 15; 20; 25; 75 n. of eosinophils reported: per HPF; per mm^2 Magnification: ×400; ×800
Staining technique	H&E; carbol chromotrope; red Congo

TATE, tumour-associated tissue eosinophilia; HPF, high-power field; H&E, haematoxylin and eosin.

tumour types (Varricchi et al., 2019). Regarding OSCC, recent immunohistochemical studies suggest a positive prognostic role of mast cell accumulation at the invasive front (Attramadal et al., 2016; Brockmeyer et al., 2017; Dantas et al., 2017). Even short-lived myeloid cells like neutrophils seem to play a role in tumour biology. As for other myeloid cells, the existence of different functional subpopulations of neutrophils has been proposed: N1 neutrophils, showing antitumor cytotoxic activity, and N2 neutrophils, associated with pro-tumorigenic activity (Fridlender et al., 2009). Recent studies confirmed these hypotheses showing an association between tumour-associated neutrophils and poor prognostic outcomes in OSCC (Magalhaes et al., 2014; Wang et al., 2014; Glogauer et al., 2015).

Another possibility has been proposed by Lee et al. as "LIAR hypothesis" (i.e. Local Immunity And/or Remodelling/Repair), suggesting to rethink the "end-stage effector cell paradigm" of the eosinophils (Lee et al., 2010). In particular, the LIAR hypothesis suggests that the eosinophil accumulation in tumour tissues is the result of both a large pool of dying cells, which provide the initial stimulus for eosinophil recruitment, and a tissue microenvironment with a high number of proliferating cells, which supplies the necessary survival and differentiation factors for eosinophil accumulation. Therefore, eosinophils might be mainly involved in the remodelling of host connective tissue in response to destruction caused by the invasion of tumour cells (Lorena et al., 2003b). The LIAR hypothesis seems to be confirmed by Pereira et al. reporting a stable accumulation of eosinophils in the invasive tumour front and not near the necrotic areas (Pereira et al., 2010).

Conclusion

In conclusion, the biological role of TATE in OSCC is still uncertain and further studies to elucidate the contribution of eosinophil infiltration are needed, especially in light of the existence of eosinophil subpopulations regulated by the cancer immune microenvironment. For this reason, it is necessary to identify new reliable markers and improved methods for eosinophil detection and to conduct studies involving eosinophils within the tumour microenvironment. Regarding the histopathological studies in OSCC, it is necessary to conduct prognostic studies with a larger sample size, comparing the different methods used to quantify eosinophil infiltration. Furthermore, the correct reporting of prognostic marker research is encouraged,

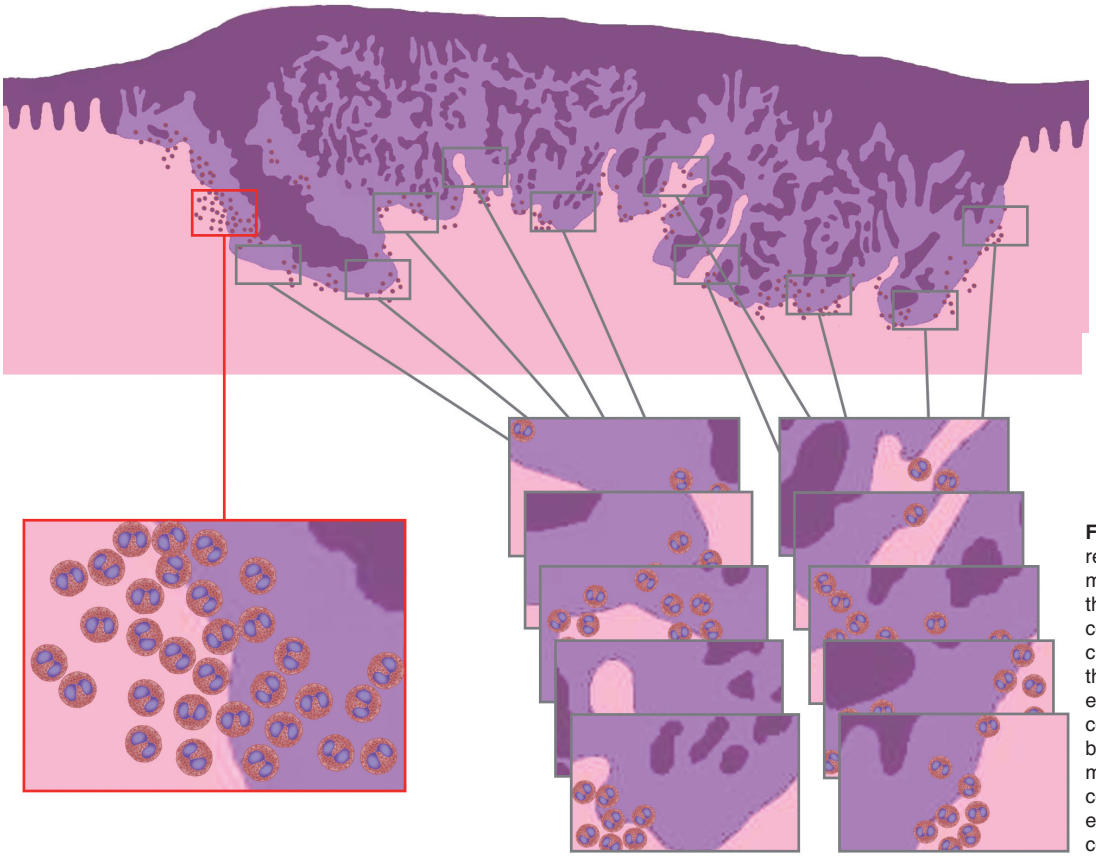

Fig. 2. Schematic representation of the two main approaches reported in the literature for eosinophil counting in OSCC: the classical method, in which the mean number of eosinophils per 10 HPF is counted (right side, grey boxes) and the density method, in which the area containing the highest eosinophil numbers is counted (left side: red box).

in order to ensure the reproducibility and the comparability of the results from different studies. In this regard, specific tools such as the Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) or the Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD) checklists are strongly recommended.

References

Alkhabuli J.O. and High A.S. (2006). Significance of eosinophil counting in tumor associated tissue eosinophilia (TATE). Oral Oncol. 42, 849-850.

Almangush A., Heikkinen I., Bakhti N., Makinen L.K., Kauppila J.H., Pukkila M., Hagstrom J., Laranne J., Soini Y., Kowalski L.P., Grenman R., Haglund C., Makitie A.A., Coletta R.D., Leivo I. and Salo T. (2018). Prognostic impact of tumour-stroma ratio in early-stage oral tongue cancers. Histopathology 72, 1128-1135.

Amin M.B., Edge S.B. and American Joint Committee on Cancer. (2017). Ajcc cancer staging manual, 8th ed. ed. Springer. Switzerland.

Attramadal C.G., Kumar S., Gao J., Boysen M.E., Halstensen T.S. and Bryne M. (2016). Low mast cell density predicts poor prognosis in oral squamous cell carcinoma and reduces survival in head and neck squamous cell carcinoma. Anticancer Res. 36, 5499-5506.

Bankur R., Rodrigues C., Anjaly D., Gopinathan P.A. and Bankur P.K. (2016). Quantitative analysis of tumor-associated tissue eosinophilia in different histological grades of oral squamous cell carcinoma. Indian J. Dent. Res. 27, 463-467.

Blatt S., Kruger M., Ziebart T., Sagheb K., Schiegnitz E., Goetze E., Al-Nawas B. and Pabst A.M. (2017). Biomarkers in diagnosis and therapy of oral squamous cell carcinoma: A review of the literature. J. Craniomaxillofac. Surg. 45, 722-730.

Brockmeyer P., Kling A., Schulz X., Perske C., Schliephake H. and Hemmerlein B. (2017). High mast cell density indicates a longer overall survival in oral squamous cell carcinoma. Sci. Rep. 7, 14677.

Chusid M.J. (2018). Eosinophils: Friends or foes? J. Allergy Clin. Immunol. Pract. 6, 1439-1444.

Dantas R.C.M., de Souza R.O., Valverde L.F., Vidal M.T.A., Sales C.B.S., Sousa L.P., Dos Santos J.N., Ramos E.A.G. and Gurgel Rocha C.A. (2017). Evaluation of mast cell density in the tumor microenvironment in oral epithelial dysplasia and oral squamous cell carcinoma. Appl. Immunohistochem. Mol. Morphol. 25, e83-e88.

Davis B.P. and Rothenberg M.E. (2014). Eosinophils and cancer. Cancer Immunol. Res. 2, 1-8.

De Lima P.O., Dos Santos F.V., Oliveira D.T., De Figueiredo R.C. and Pereira M.C. (2015). Effect of eosinophil cationic protein on human oral squamous carcinoma cell viability. Mol. Clin. Oncol. 3, 353-356.

De Paz D., Chang K.P., Kao H.K., Lao W.W., Huang Y.C., Chang Y.L. and Huang Y. (2019). Clinical implications of tumor-associated tissue eosinophilia in tongue squamous cell carcinoma. Laryngoscope 129, 1123-1129.

Debta P., Debta F.M., Chaudhary M. and Wadhwan V. (2011). Evaluation of prognostic significance of immunological cells (tissue eosinophil and mast cell) infiltration in oral squamous cell carcinoma. J. Cancer Sci. Ther. 3, 201-204.

Deepthi G., Kulkarni P.G. and Nandan S.R.K. (2019). Eosinophils: An imperative histopathological prognostic indicator for oral squamous cell carcinoma. J. Oral Maxillofac. Pathol. 23, 307.

Diny N.L., Rose N.R. and Cihakova D. (2017). Eosinophils in autoimmune diseases. Front. Immunol. 8, 484.

Dorta R.G., Landman G., Kowalski L.P., Lauris J.R., Latorre M.R. and Oliveira D.T. (2002). Tumour-associated tissue eosinophilia as a prognostic factor in oral squamous cell carcinomas. Histopathology 41, 152-157.

Ellyard J.I., Simson L. and Parish C.R. (2007). Th2-mediated anti-tumour immunity: Friend or foe? Tissue Antigens 70, 1-11.

Falconieri G., Luna M.A., Pizzolitto S., DeMaglio G., Angione V. and Rocco M. (2008). Eosinophil-rich squamous carcinoma of the oral cavity: A study of 13 cases and delineation of a possible new microscopic entity. Ann. Diagn. Pathol. 12, 322-327.

Fridlender Z.G., Sun J., Kim S., Kapoor V., Cheng G., Ling L., Worthen G.S. and Albelda S.M. (2009). Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "n2" tan. Cancer Cell 16, 183-194.

Gatault S., Legrand F., Delbeke M., Loiseau S. and Capron M. (2012). Involvement of eosinophils in the anti-tumor response. Cancer Immunol. Immunother. 61, 1527-1534.

Glogauer J.E., Sun C.X., Bradley G. and Magalhaes M.A. (2015). Neutrophils increase oral squamous cell carcinoma invasion through an invadopodia-dependent pathway. Cancer Immunol. Res. 3, 1218-1226.

Heikkinen I., Bello I.O., Wahab A., Hagstrom J., Haglund C., Coletta R.D., Nieminen P., Makitie A.A., Salo T., Leivo I. and Almangush A. (2019). Assessment of tumor-infiltrating lymphocytes predicts the behavior of early-stage oral tongue cancer. Am. J. Surg. Pathol. 43, 1392-1396.

Hogan S.P., Rosenberg H.F., Moqbel R., Phipps S., Foster P.S., Lacy P., Kay A.B. and Rothenberg M.E. (2008). Eosinophils: Biological properties and role in health and disease. Clin. Exp. Allergy 38, 709-750.

Horie N., Shimoyama T., Kaneko T. and Ide F. (2007). Multiple oral squamous cell carcinomas with blood and tissue eosinophilia. J. Oral Maxillofac. Surg. 65, 1648-1650.

Jain M., Kasetty S., Sudheendra U.S., Tijare M., Khan S. and Desai A. (2014). Assessment of tissue eosinophilia as a prognosticator in oral epithelial dysplasia and oral squamous cell carcinoma-an image analysis study. Patholog. Res. Int. 2014, 507512.

Jain S., Phulari R.G., Rathore R., Shah A.K. and Sancheti S. (2018). Quantitative assessment of tumor-associated tissue eosinophilia and mast cells in tumor proper and lymph nodes of oral squamous cell carcinoma. J. Oral Maxillofac. Pathol. 22, 227-233.

Jain D., Tikku G., Bhadana P., Dravid C. and Grover R.K. (2019). The impact of peritumoral retraction clefting and intratumoral eosinophils on overall survival in oral squamous carcinoma patients. Pathol. Oncol. Res. 25, 183-189.

Kano S., Sakashita T., Tsushima N., Mizumachi T., Nakazono A., Suzuki T., Yasukawa S. and Homma A. (2018). Validation of the 8th edition of the ajcc/uicc tnm staging system for tongue squamous cell carcinoma. Int. J. Clin. Oncol. 25, 844-850.

Kargahi N., Razavi S.M., Deyhimi P. and Homayouni S. (2015). Comparative evaluation of eosinophils in normal mucosa, dysplastic mucosa and oral squamous cell carcinoma with hematoxylin-eosin, congo red, and EMR1 immunohistochemical staining techniques. Electron Physician 7, 1019-1026.

Kay A.B. (2015). The early history of the eosinophil. Clin. Exp. Allergy 45, 575-582.

Lee J.J., Jacobsen E.A., McGarry M.P., Schleimer R.P. and Lee N.A. (2010). Eosinophils in health and disease: The liar hypothesis. Clin. Exp. Allergy 40, 563-575.

Lorena S.C., Dorta R.G., Landman G., Nonogaki S. and Oliveira D.T. (2003a). Morphometric analysis of the tumor associated tissue eosinophilia in the oral squamous cell carcinoma using different staining techniques. Histol. Histopathol. 18, 709-713.

Lorena S.C., Oliveira D.T., Dorta R.G., Landman G. and Kowalski L.P. (2003b). Eotaxin expression in oral squamous cell carcinomas with and without tumour associated tissue eosinophilia. Oral. Dis. 9, 279-283.

Lowe D. and Fletcher C.D. (1984). Eosinophilia in squamous cell carcinoma of the oral cavity, external genitalia and anus--clinical correlations. Histopathology 8, 627-632.

Lowe D., Jorizzo J. and Hutt M.S. (1981). Tumour-associated eosinophilia: A review. J. Clin. Pathol. 34, 1343-1348.

Madhura M.G., Gajalakshmi S., Kumar B.V., Suma S., Sarita Y. and Shweta R.D. (2015). Role of tissue eosinophils in oral leukoplakia: A pilot study. J. Oral. Maxillofac. Pathol. 19, 286-290.

Magalhaes M.A., Glogauer J.E. and Glogauer M. (2014). Neutrophils and oral squamous cell carcinoma: Lessons learned and future directions. J. Leukoc. Biol. 96, 695-702.

Martinelli-Klay C.P., Mendis B.R. and Lombardi T. (2009). Eosinophils and oral squamous cell carcinoma: A short review. J. Oncol. 2009, 310132.

Martinelli-Klay C.P., Lombardi T., Mendis B., Soares E.G., Salvado F., Courvoisier D.S. and Mauricio P. (2018). Tissue eosinophilia in oral intraepithelial neoplasia as a probable indicator of invasion. Oral Dis. 24, 103-108.

Mascitti M., Rubini C., De Michele F., Balercia P., Girotto R., Troiano G., Lo Muzio L. and Santarelli A. (2018). American joint committee on cancer staging system 7th edition versus 8th edition: Any improvement for patients with squamous cell carcinoma of the tongue? Oral Surg. Oral Med. Oral Pathol. Oral Radiol. 126, 415-423.

Mascitti M., Tempesta A., Togni L., Capodiferro S., Troiano G., Rubini C., Maiorano E., Santarelli A., Favia G. and Limongelli L. (2020). Histological features and survival in young patients with hpv negative oral squamous cell carcinoma. Oral Dis. 26, 1640-1648.

Neophytou C.M., Pierides C., Christodoulou M.I., Costeas P., Kyriakou T.C. and Papageorgis P. (2020). The role of tumor-associated myeloid cells in modulating cancer therapy. Front. Oncol. 10, 899.

O'Sullivan J.A. and Bochner B.S. (2018). Eosinophils and eosinophil-associated diseases: An update. J. Allergy Clin. Immunol. 141, 505-517.

Oliveira T.D., Tjioe K.C., Assao A., Sita Faustino S.E., Lopes Carvalho A., Landman G. and Kowalski L.P. (2009). Tissue eosinophilia and its association with tumoral invasion of oral cancer. Int. J. Surg. Pathol. 17, 244-249.

Oliveira D.T., Biassi T.P., Faustino S.E., Carvalho A.L., Landman G. and Kowalski L.P. (2012). Eosinophils may predict occult lymph node metastasis in early oral cancer. Clin. Oral Investig. 16, 1523-1528.

Pereira M.C., Oliveira D.T., Olivieri E.H., Rogatto S.R., Carvalho A.L., Landman G. and Kowalski L.P. (2010). The 434(g>c) polymorphism in the eosinophil cationic protein gene and its association with tissue eosinophilia in oral squamous cell carcinomas. J. Oral Pathol. Med. 39, 56-62.

Pereira M.C., Oliveira D.T. and Kowalski L.P. (2011). The role of eosinophils and eosinophil cationic protein in oral cancer: A review. Arch. Oral. Biol. 56, 353-358.

Peter C., Shashidara R., Haragannavar V., Pradeep S., Sridhara S., Gopalkrishna A., Poojary S., Nayak S. and Sushanth A. (2015). Assessment of tumor associated tissue eosinophilia (tate) in oral squamous cell carcinoma using carbol chromotrope stain. Int. J. Odontostomatol. 9, 91-95.

Peurala E., Tuominen M., Loyttyniemi E., Syrjanen S. and Rautava J. (2018). Eosinophilia is a favorable prognostic marker for oral cavity and lip squamous cell carcinoma. APMIS 126, 201-207.

Przewoski E. (1896). Ueber die locale eosinophilie beim krebs nebst bemerkungen über die bedeutung der eosinophilen zellen im allgemeinen. Centralblatt für Allgemeine Pathologie und Pathologische Anatomie 5, 177-191.

Rakesh N., Devi Y., Majumdar K., Reddy S.S. and Agarwal K. (2015). Tumour associated tissue eosinophilia as a predictor of locoregional recurrence in oral squamous cell carcinoma. J. Clin. Exp. Dent. 7, e1-6.

Sakkal S., Miller S., Apostolopoulos V. and Nurgali K. (2016). Eosinophils in cancer: Favourable or unfavourable? Curr. Med. Chem. 23, 650-666.

Santarelli A., Mascitti M., Rubini C., Bambini F., Giannatempo G., Lo Russo L., Sartini D., Emanuelli M., Procaccini M. and Lo Muzio L. (2017). Nuclear survivin as a prognostic factor in squamous-cell carcinoma of the oral cavity. Appl. Immunohistochem. Mol. Morphol. 25, 566-570.

Schiavoni G., Gabriele L. and Mattei F. (2013). The tumor microenvironment: A pitch for multiple players. Front. Oncol. 3, 90.

Shield K.D., Ferlay J., Jemal A., Sankaranarayanan R., Chaturvedi A.K., Bray F. and Soerjomataram I. (2017). The global incidence of lip, oral cavity, and pharyngeal cancers by subsite in 2012. CA Cancer J. Clin. 67, 51-64.

Sopka D.M., Li T., Lango M.N., Mehra R., Liu J.C., Burtness B., Flieder D.B., Ridge J.A. and Galloway T.J. (2013). Dysplasia at the margin? Investigating the case for subsequent therapy in 'low-risk' squamous cell carcinoma of the oral tongue. Oral Oncol. 49, 1083-1087.

Sugihara R., Kumamoto T., Ito T., Ueyama H., Toyoshima I. and Tsuda T. (2001). Human muscle protein degradation in vitro by eosinophil cationic protein (ecp). Muscle Nerve 24, 1627-1634.

Tadbir A.A., Ashraf M.J. and Sardari Y. (2009). Prognostic significance of stromal eosinophilic infiltration in oral squamous cell carcinoma. J. Craniofac. Surg. 20, 287-289.

Troiano G., Caponio V.C.A., Adipietro I., Tepedino M., Santoro R., Laino L., Lo Russo L., Cirillo N. and Lo Muzio L. (2019). Prognostic significance of cd68(+) and cd163(+) tumor associated macrophages in head and neck squamous cell carcinoma: A systematic review and meta-analysis. Oral Oncol. 93, 66-75.

Vaibhav S.L., Priya P.L., Sonam C.K., Supriya K., Garima Y., Sabeer S., Juvale P. and Javir G. (2018). Evaluation of tumor-associated tissue eosinophilia in different stages of oral squamous cell carcinoma using special stains: An in vitro histopathological study. J. Contemp. Dent. Pract. 19, 579-586.

Valent P., Gleich G.J., Reiter A., Roufosse F., Weller P.F., Hellmann A., Metzgeroth G., Leiferman K.M., Arock M., Sotlar K., Butterfield J.H., Cerny-Reiterer S., Mayerhofer M., Vandenberghe P., Haferlach T., Bochner B.S., Gotlib J., Horny H.P., Simon H.U. and Klion A.D. (2012). Pathogenesis and classification of eosinophil disorders: A review of recent developments in the field. Expert Rev. Hematol. 5,

157-176.
Varricchi G., Galdiero M.R., Loffredo S., Lucarini V., Marone G., Mattei F., Marone G. and Schiavoni G. (2018). Eosinophils: The unsung heroes in cancer?. Oncoimmunology 7, e1393134.
Varricchi G., de Paulis A., Marone G. and Galli S.J. (2019). Future needs in mast cell biology. Int. J. Mol. Sci. 20.
Wang N., Feng Y., Wang Q., Liu S., Xiang L., Sun M., Zhang X., Liu G., Qu X. and Wei F. (2014). Neutrophils infiltration in the tongue squamous cell carcinoma and its correlation with ceacam1 expression on tumor cells. PLoS One 9, e89991.
Wen T. and Rothenberg M.E. (2016). The regulatory function of eosinophils. Microbiol. Spectr 4.
Yellapurkar S., Natarajan S., Boaz K., Baliga M., Shetty P., Manaktala N., Prasad M. and Ravi M. (2016). Tumour-associated tissue eosinophilia in oral squamous cell carcinoma- a boon or a bane? J. Clin. Diagn. Res. 10, ZC65-68.

Accepted September 28, 2020