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Emerging histopathological parameters in the prognosis of oral squamous cell carcinomas

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Summary. Oral squamous cell carcinoma (OSCC) is the most common oral malignancy, representing 90% of all malignant neoplasms in the head and neck region. Patients with this aggressive tumor have an overall 5-year survival rate of approximately 50%, which drops to less than 30% when tumors are diagnosed at advanced clinical stages. Over decades, several studies provided high-level evidence of the impact of histopathological features on treatment guidelines and prognosis of OSCC. The 8th American Joint Committee on Cancer (AJCC) TNM staging system recognized the importance of depth of invasion to the T category and extranodal extension to the N category for OSCC. This review provides the current knowledge on emerging histopathological parameters identified as potential biomarkers for OSCC, such as depth of invasion, tumor thickness, the pattern of invasion, inflammatory profile, and tumor-stroma ratio, evaluating their clinical relevance on patient outcomes. Analysis, limitations, and potential biological mechanisms are highlighted and discussed. Assessing and reporting these markers are cost-effective and can be incorporated into daily practice.

Key words: Oral cancer, Histopathological parameters, Prognosis, Depth of invasion, Tumor thickness, Pattern of invasion, Inflammatory profile, Tumor-stroma ratio

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Introduction

Oral squamous cell carcinoma (OSCC) is the most common type of oral cancer, accounting for up to 90% of all head and neck malignant neoplasms (Sung et al., 2021). Recent global statistics indicate that lip and oral cavity cancers represent the 16th most common malignant neoplasm worldwide, with almost 377,000 new cases per year, making it a significant public health problem (Miranda-Filho and Bray, 2020). A higher prevalence is observed in males aged 50 years and older, though an increasing number of cases in women and in the younger age group has been observed in recent years (Coletta et al., 2020; Warnakulasuriya and Kerr, 2021). Like other malignancies, OSCC occurs due to a multistep process characterized by distinct genetic and epigenetic alterations (Guan et al., 2019; Georgaki et al., 2021). Regardless of the factors, whether exogenous (e.g., smoking, alcohol, oncogenic HPV) or endogenous (e.g., genetic predisposition), a fundamentally common feature in oral carcinogenesis is the gradual accumulation of defects, changes that together initiate the phenotypic (clinical and microscopic) transformation from normal to the dysplastic epithelium (oral potentially malignant disorders) and, finally, to invasive carcinoma (Georgaki et al., 2021).

With new therapeutic modalities such as targeted therapy and immunotherapy still restricted to recurrent and/or metastatic cases, surgery with or without neck dissection and adjuvant radiochemotherapy are the standard care for OSCC (Chinn and Myers, 2015; Warnakulasuriya and Kerr, 2021). Classically, clinical measures of tumor location and extension (size, volume) and growth and invasive properties (e.g., surgical margins, lymph node metastasis, extranodal extension and lymphovascular and neural invasion) have been used

to guide the treatment approaches. However, despite advances in cancer treatment, the 5-year mortality rate ranges from 40 to 50%, without any improvement in recent decades (Chinn and Myers, 2015; Wong and Wiesenfeld, 2018). Thus, identifying possible prognostic markers is necessary for managing cancer patients and predicting the tumor's biological behavior (Almangush et al., 2017; Pansini et al., 2021).

Histopathological parameters, such as the pattern of invasion, tumor budding, tumor-stroma ratio (TSR), and tumor-infiltrating lymphocytes (TILs), have been proposed as prognostic indicators of OSCC (Dolens et al., 2021). Recently, the 8th American Joint Committee on Cancer (AJCC) TNM staging system for oral cavity cancers included the depth of invasion (DOI) of the primary tumor in the T category and extracapsular infiltration in lymph nodes as a critical parameter to define clinical staging, thus recognizing the importance of histopathological features (Amin et al., 2017).

This review provides the current knowledge on emerging histopathological parameters identified as potential biomarkers in OSCC, evaluating their impact on prognosis. Analysis, limitations, and potential biological mechanisms are highlighted and discussed.

Depth of invasion (DOI) and tumor thickness: do these different parameters provide better prognostic data?

DOI and tumor thickness are measured in different ways, though both are supposed to represent invasion depth. DOI is measured by drawing a perpendicular line from the basement membrane of the adjacent intact mucosa to the deepest point of invasion (Amin et al., 2017), whereas tumor thickness is measured from the surface of the invasive OSCC to the deepest point of invasion (Edge and Compton, 2010). Before the 8th edition of the AJCC TNM staging system, many studies did not include a clear definition of measurement or simply defined each parameter inconsistently (Kane et al., 2006; Huang et al., 2009; Melchers et al., 2012). As a result, the prognostic significance related to tumor thickness and DOI in OSCC has been discussed over the years. The cut-off values for DOI and tumor thickness reported in the literature also vary, ranging from 1.5 to 10 mm (van Lanschot et al., 2020).

Although the insertion of the DOI in the clinical staging has shown promising results and points to a personalized direction of the OSCC according to its morphological and molecular characteristics, it must be emphasized that only the long-term follow-up of prospective studies can provide clear evidence of the impact of the insertion of the DOI in the clinical staging in the prognosis of the cases diagnosed as OSCCs. Tumor thickness can be greater than DOI in exophytic tumors and smaller in endophytic/ulcerated growth patterns (Fig. 1). Tumor thickness has been shown to be less predictive of lymph node metastasis (Shim et al., 2010; Amin et al., 2017; van Lanschot et al., 2020). Thus, DOI is not the same as tumor thickness, and the two are not interchangeable (Dirven et al., 2017; Salama et al., 2021).

A few studies have compared DOI and tumor thickness (Dirven et al., 2017; Liu et al., 2020; Salama et al., 2021). In a study developed by Dirven et al. (2017), 26 (5.7%) patients had a different T category when using thickness instead of depth. However, the outcome of the patients, represented by both disease-specific survival and overall survival, was quite similar if based on DOI or tumor thickness. Liu et al. (2020) observed that DOI and tumor thickness significantly correlated with each other and the nodal spread of disease. The cut-off points with the best prediction of lymph node metastasis were 4.5 mm for DOI and 8 mm for tumor thickness, recognizing these features as distinct parameters that need to be reported appropriately (Liu et al., 2020). However, Lee et al. (2021) pointed out that tumor thickness was not a significant prognostic factor for early-stage OSCC, whereas DOI was associated with disease-free survival. Ideally, prospective multi-center studies are needed to define the optimal cut-off for each parameter and to identify whether there is a significant difference in the prognostic value when analyzing them.

The results of the International Consortium for Outcome Research, a multi-institutional study involving 3,149 patients, demonstrated that every 5 mm increase in DOI is enough to upstage the T category (<5 mm: T1, 5-10 mm: T2, and >10 mm: T3/T4) (Ebrahimi et al., 2014). Almangush et al. (2018a) reclassified the cases of early-stage oral tongue cancers (tumors ≤4 cm in diameter) with a basis in 4 mm and compared the prognostic value with 5 mm of the 8th edition of the AJCC TNM staging system. For this group of tumors, DOI set at a cut-off of 4 mm provided better survival prediction than 5 mm. In the recent large meta-analysis (Dolens et al., 2021), the DOI cut-off point varied among studies, and the majority applied either 4- or 5-mm. Pooled analyses confirmed that both cut-off were significantly associated with poor outcomes, though the HR values were higher, with less heterogeneity and pooled lower confidence intervals for the cut-off of 4 mm (Dolens et al., 2021). Although the results of these studies successfully demonstrated an association between DOI and prognosis, they did not clear the biological mechanisms.

Several studies indicate DOI as an important predictor of lymph node metastasis in OSCC (Barriere et al., 2015; Xu et al., 2020; Salama et al., 2021). Among other factors, metastasis involves (1) intravasation (invasion into the bloodstream and/or lymphatic system), (2) immune system evasion, (3) extravasation (exit from the bloodstream to a potential new site of tumor development), and (4) establishment (Hapach et al., 2019; Neinavaie et al., 2021). The biological characteristics of the neoplastic cell will also be necessary. For example, epithelial-mesenchymal transition (EMT), especially in more invasive areas of the tumor, produces a phenotype characterized by greater motility that more easily invades adjacent tissues,

including those that form the walls of blood vessels (Barriere et al., 2015). Thus, intravasation involves tumor dispersal mechanisms (Joosse et al., 2015) and produces cancer cells that enter the bloodstream as circulating tumor cells. Several studies pointed to DOI as an independent risk factor for recurrence and worse survival (Almangush et al., 2018a; Xu et al., 2020; Salama et al., 2021), and the combination of DOI with tumor budding and inflammatory response revealed promising results on OSCC prognostic assessment (Almangush et al., 2015; Yu et al., 2019; Domingueti et al., 2021).

As a limiting factor, DOI is usually determined only a few days after excisional surgery based on the histopathological evaluation of the surgical specimen. Although there is no reliable method to estimate DOI before or during the initial surgery, it can be measured on diagnostic incisional biopsies; however, these measurements are often not representative of the specimen and generate conflicting results (Alsaffar et al., 2016; Mao et al., 2019; Xu et al., 2020). However, a previous study demonstrated that representative incisional biopsies can provide important prognostic morphological parameters, with such findings being in consonance with post-operative resection samples (Bello et al., 2021).

Although DOI cut-off values adopted by the AJCC are universal for all sites in the oral cavity, the different anatomical areas of the mouth may need different and specific cut-off to improve its prognostic potential. In this context, studies should be designed to assess whether DOI cut-off values need to be modified based on the specific intraoral locations.

The pattern of invasion: prognostic relevance and clinical significance

The tumor invasion front represents the deepest area of the tumor, where it is expected to observe poor cellular differentiation and cell dissociation. Several molecular events of importance for tumor growth occur at the invasion front, such as gain or loss of adhesion molecules, secretion of proteolytic enzymes, increased cell proliferation and angiogenesis (Bryne, 1998; Almangush et al., 2018b; Dolens et al., 2021). Thus, the invasive front of the tumor has become an area of interest to study the biological behavior of the tumor.

The pattern of invasion and tumor budding (TB) are two important parameters that determine the risk of lymph node metastasis in OSCC (Almangush et al., 2018b; Bjerkli et al., 2020). The worst pattern of invasion (WPOI) refers to the dissociative infiltration capacity of neoplastic cells at the tumor/host interface and includes five types (WPOI -1 to -5), though the

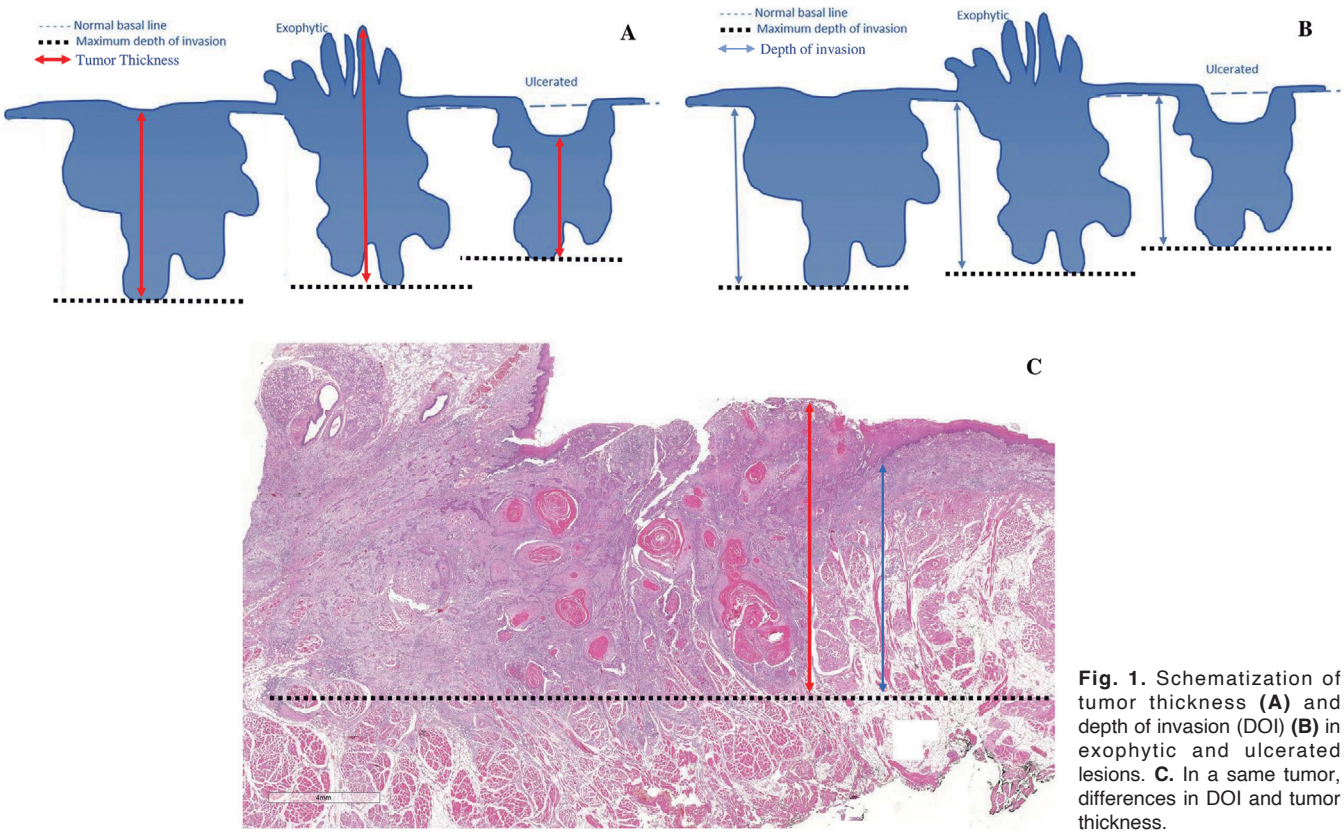

Fig. 1. Schematization of tumor thickness **(A)** and depth of invasion (DOI) **(B)** in exophytic and ulcerated lesions. **C.** In a same tumor, differences in DOI and tumor thickness.

studies in the literature trend to divide WPOI in 2 groups: cohesive pattern (grouping WPOI-1, -2 and -3) and infiltrative pattern (grouping WPOI-4 and -5) (Almangush et al., 2014). Evidence showed that patients with WPOI-4 and -5 have higher rates of mortality, locoregional recurrence, and occult neck metastasis compared to WPOI1-3 patients (Fu et al., 2021; Mishra et al., 2022). In two recent meta-analyses, WPOI proved to be a valuable prognostic marker for patients with OSCC (Dolens et al., 2021; Elseragy et al., 2022).

TB is defined as the presence of single tumor cells or small clusters of up to 5 cells in the tumor stroma (Fig. 2). This is inherent to the mechanism of carcinogenesis: loss of cell cohesion and high motility of tumor cells (Wang et al., 2011). It is believed that TB is strongly linked to EMT. EMT is a biological process that converts an epithelial cell into a highly motile cell with a mesenchymal phenotype, a hallmark of invasion and subsequent metastasis (de Morais et al., 2023). Wang et al. (2011) suggested that tumor buds may represent cells undergoing EMT, as indicated by the reduced expression of E-cadherin and increased vimentin expression in tongue SCC. Furthermore, aldehyde dehydrogenase 1 (ALDH1), a cancer stem cell marker, was elevated in the budding area compared to other areas, suggesting that tumor buds in OSCC have a phenotype like cancer stem cells, conferring migratory and invasive properties (Marangon Junior et al., 2019). Several studies have reported TB as an independent prognostic factor (Bjerkli et al., 2020; Togni et al., 2022). A meta-analysis study published by Almangush et al. (2018b) included 16 articles that evaluated the prognostic value of tumor sprouting in OSCC. The group showed a significant association between TB and advanced clinical staging and poorer survival.

There are two methods for analyzing the pattern of invasion and TB: manually or digitally using hematoxylin-eosin (H&E)-stained or immunohistochemically (IHC) stained (pan-cytokeratin) slides (Shimizu et al., 2018). The most used method is manual evaluation using H&E-stained slides. In OSCC, the most accepted method, proposed by Wang et al. (2011), is sample analysis under a low magnification (×200 magnification) where TB is counted in the field that has the highest number of buds. Further, the samples are categorized as high-density budding (5 or more buds) and low intensity budding (<5 buds). This classification is widely accepted in studies of OSCC (Almangush et al., 2018b; Bjerkli et al., 2020; Dourado et al., 2020).

Although H&E staining is highly recommended for the analysis of TB, a method that can be easily used during the routine by professional pathologists (Lugli et al., 2017), the evaluation in H&E sections cannot be reliable and then the use of IHC is necessary for a better visualization, especially in cases with an excessive peritumoral inflammatory infiltrate (Almangush et al., 2018b). It should be emphasized that TB is considered a low-cost histological parameter that can be easily

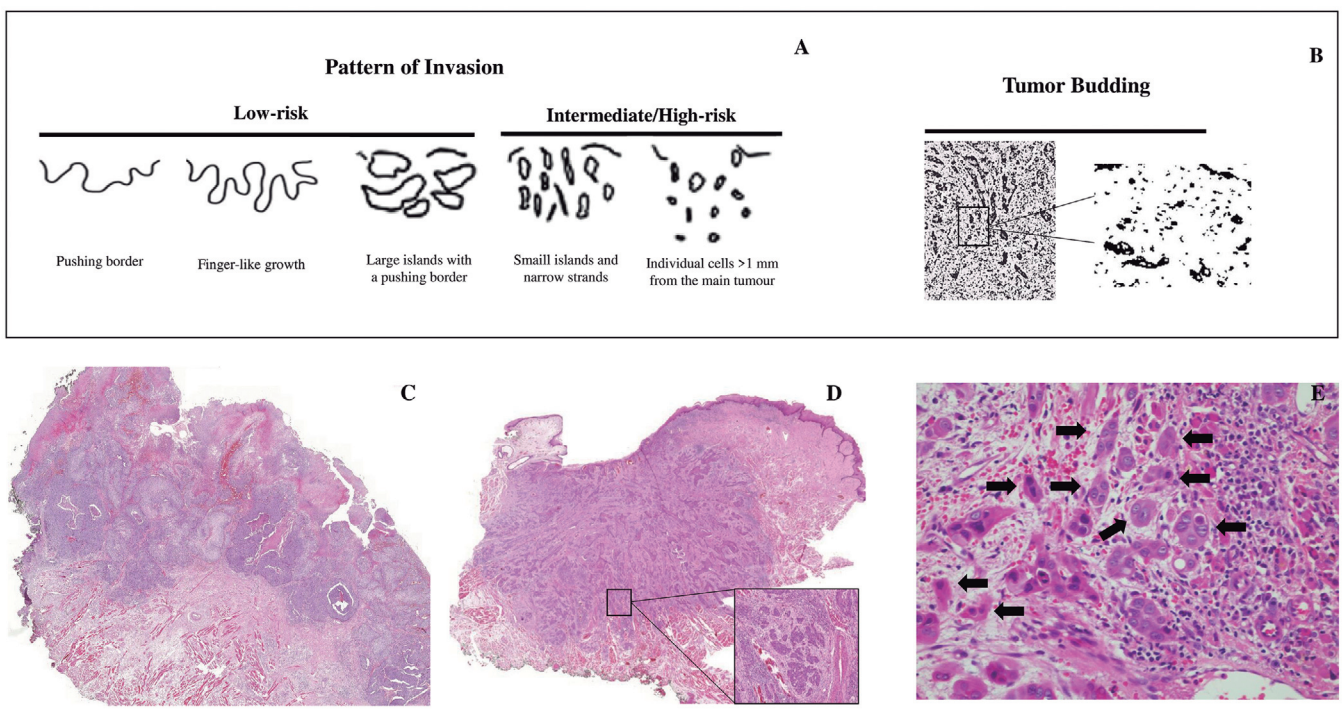

Fig. 2. Diagrammatic representation of the pattern of invasion **(A)** and tumor budding **(B)** in OSCC. Representative tumors composed by large tumor islands **(C)** and small tumor islands **(D)**. **E.** Arrows indicate tumor buds at the invasive lesion.

assessed by pathologists, with good interobserver agreement (Almangush et al., 2018b).

Another point that must be discussed is that TB and WPOI are analyzed in surgical specimens, which comprise the entire depth of the tumor tissue, allowing easy assessment of the front of invasion. However, Seki et al. (2017) evaluated this parameter in incisional biopsy specimens and demonstrated positive correlation with postoperative TB count and survival rates. A recent systematic review showed that the presence of preoperative TB has significant prognostic value for lymph node metastasis, overall survival, and disease-free survival. According to Almangush et al. (2019), this analysis may be especially beneficial if we can predict tumor aggressiveness preoperatively and apply it to therapeutic considerations. It should be noted that, if TB needs to be assessed in preoperative biopsy specimens, surgeons should include the deepest area of the tumor to clearly visualize the invasion front, which may not be feasible in all cases analyzed. An appropriate incisional biopsy results in a better described lesion and significantly increases the likelihood that the best therapeutic strategy will be chosen upfront thanks to the evaluation of many tumor/stromal prognostic characteristics (Bello et al., 2021). Bello et al. (2021) suggest that clinicians should take representative deep biopsies (>5 mm), thus enabling the analysis of important morphological parameters.

Inflammatory profile of the tumor microenvironment: identification of prognostic markers in tumor stroma

Bidirectional communication between tumor cells and their microenvironment is critical for cancer progression (Orr, 1938). Thus, it emerges that tumors are not simply collections of disordered malignant cells but rather maladaptive organisms composed of tumor and different cell populations supporting cancer growth (Tarin, 2012; Binnewies et al., 2018) (Fig. 3). Major structural and functional changes occur at the interface between tumor cells and adjacent stromal cells (Shan et al., 2022). In 1989, Bryne et al. suggested that the tumor invasion front should be evaluated in OSCC using the degree of keratinization, nuclear pleomorphism, invasion pattern, and inflammatory infiltrate as parameters. Based on this system, it was suggested that cells at the invasion front exhibit molecular characteristics that differ from those in areas of the tumor surface. These interactions at the invasion front are crucial for cancer dissemination, which directly impacts the prognosis.

T lymphocytes play a central role in anti-tumor immune response and are the dominant element in the tumor microenvironment (Hiam-Galvez et al., 2021). The tumor-specific antigen can activate highly specific CD8+ T cells, CD4+ T cells and tumor-specific antibodies (Muenst et al., 2016). In OSCC, tumor cells may have the support of immune cells to promote tumor

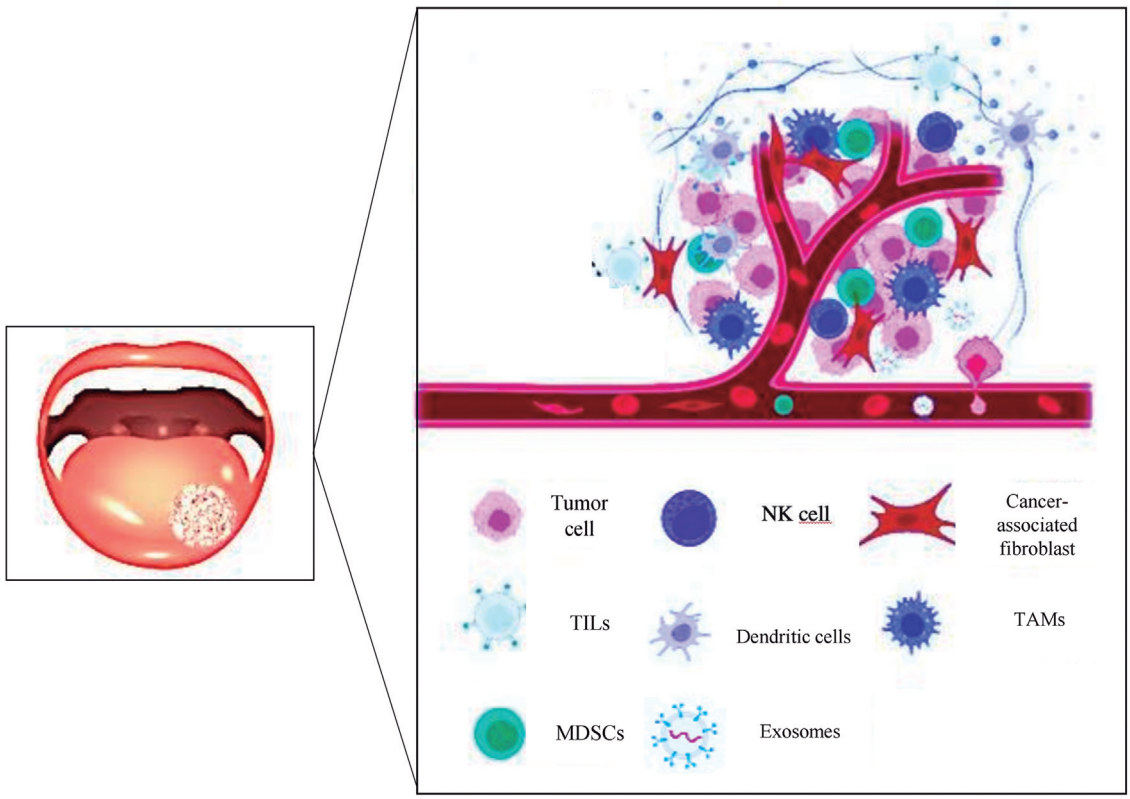

Fig. 3. Tumor microenvironment in OSCC. The diagram highlights tumor-infiltrating lymphocytes (TILs), cancer-associated fibroblasts (CAFs), myeloid-derived suppressor cells (MDSCs), dendritic cells, NK cells, and tumor-associated macrophages (TAMs).

cell proliferation, angiogenesis, and metastasis (Hiam-Galvez et al., 2021). These cells can evade the host's immune system, preventing their own immunogenicity and expression of immunosuppressive signals (Burkholder et al., 2014; Shrihari, 2017; Ronca et al., 2018). The pro-tumor microenvironment is generally characterized by a chronic inflammatory pattern consisting of immunosuppressive cells with pro-tumor activity: regulatory T lymphocytes (Treg cells), M2 macrophages, N2 neutrophils, and myeloid-derived suppressor cells (Curry et al., 2014; Landskron et al., 2014).

More than a decade ago, Klintrup et al. (2005) proposed a method (Klintrup-Mäkinen [KM] grading system) for the classification of the inflammatory reaction in colorectal cancer and showed that high-grade inflammation at the invasive margin was associated with better survival. The KM grading system evaluates generalized inflammatory cell infiltration (Fig. 4). The analysis is performed at the invasion front of the tumor, where the density of inflammatory cells is graded on a 4-point scale: low grade (absence/low density of inflammatory cells) or high grade (a prominent inflammatory reaction that forms a continuous band at the invasive margin). Yu et al. (2019) evaluated OSCC samples by KM grade, and showed that KM grade is an independent prognostic factor.

Current research suggests TILs as a promising prognostic biomarker in several types of tumors, including OSCC (Almangush et al., 2022). TILs can limit or promote tumor growth and metastasis (Hendry et al., 2017). Among the different populations of TILs, including macrophages, dendritic cells and mast cells, TILs are considered a selected population of T cells with higher specific immune reactivity against tumor cells than non-infiltrating lymphocytes (Badalamenti et al., 2019). TILs can be easily quantified in slides stained with H&E, which are already part of the clinical routine, from different subsites of head and neck tumors (Heikkinen et al., 2019, Silva et al., 2023). Therefore, they are likely to be implemented in daily practice.

A standardized method for evaluating TILs was introduced by the International Immuno-oncology Biomarker Working Group (IIBWG) and has shown consistent results (Hendry et al., 2017; Almangush et al., 2021a). In brief, the percentage of stromal TILs present in the stromal area of the tumor and intratumoral TILs (scored as the percentage of tumor islands occupied by lymphocytes) is evaluated. Mononuclear immune cells are scored, while polymorphonuclear leucocytes are excluded. Furthermore, TILs in stromal areas not adjacent to the tumor are excluded. TILs are evaluated in percentages as a rolling score. Almangush et al. (2022) identified that a low number of TILs (<20%) is associated with a low disease-specific survival.

Immune analysis by evaluating the infiltration of TILs is a promising method that can be implemented as a means of prognostic analysis (Heikkinen et al., 2019; Almangush et al., 2021a; Silva et al., 2023). Heikkinen et al. (2019) evaluated the prognostic value of TILs in the stromal and intraepithelial compartments (in the invasive front and the center of the tumor). The authors identified a worse prognosis in cases with low infiltration of stromal TILs in the invasive front of the tumor in the multivariate analysis. Almangush et al. (2021a) reported that the TNM-Immune system, combination of TNM clinical stage and TILs, can independently predict survival and recurrence risk in

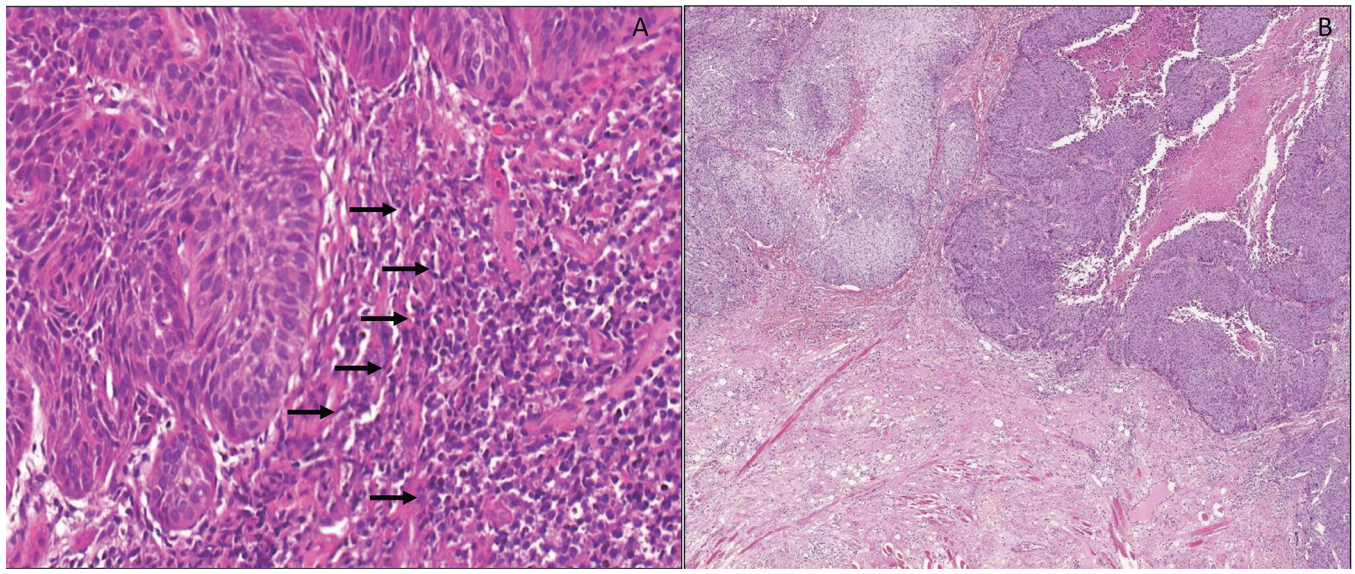

Fig. 4. The assessment of the tumor-infiltrating lymphocytes (TILs) in representative samples. Continuous dense lymphocytic infiltrate is noted at the tumor interface (arrows) **(A)** (H&E), and a tumor with low density of lymphocytes **(B)** (H&E). A, x 200; B, x 40.

patients diagnosed with early-stage OSCC.

Understanding the characteristics of the oral cancer microenvironment can provide essential insights into the biological behavior of the tumor. The control of the neoplastic process depends on the magnitude of the initial immune response and the ability to sustain this response for a prolonged period (Piva et al., 2011). The primary antitumor defense mechanism is the death of cancer cells mediated by CD8 T lymphocytes, also known as cytotoxic T lymphocytes, which are able to identify and kill neoplastic cells that express peptides derived from mutant cellular proteins associated with MHC class I (Pluhar et al., 2015). The participation of CD4 T lymphocytes seems to be related to the production of TNF by macrophages and IFN-γ by the Th1 population. In addition, these lymphocytes may be responsible for increased MHC-I expression by tumor cells, which results in the sensitization of CD8 T lymphocytes and consequent lysis of tumor cells mediated by the perforin/granzyme system or by the binding of Fas present on tumor cells to Fas-L (CD95) of lymphocytes (Hiam-Galvez et al., 2021). However, the immunosuppressive property of CD4 T cells has been associated with a low survival rate in patients with ovarian cancer (O'Higgins et al., 2018).

Taken together, the results show that, more important than the presence/intensity of the inflammatory infiltrate, the profile of inflammatory cells present at the tumor invasion front dictates the biological behavior of the tumor, and these cells serve as essential markers of prognosis and future therapeutic targets. Immunotherapy is a new strategy for tumor therapy, which applies biotechnology and immunological methods to improve the specific immune response to the tumor (Mohan et al., 2019; Mei et al., 2020). Tumor immunotherapy was awarded as the most important scientific breakthrough by Science in 2013 due to its excellent efficacy and innovation (Guan et al., 2019). In 2020, the Nobel Prize in Chemistry was awarded to French microbiologist Emmanuelle Charpentier and American biologist Jennifer Doudna for their "development of genome editing methods." Immunotherapy has outstanding application value in tumor therapy, including adoptive cell immunotherapy, antibody-based therapy, cytokine therapy, tumor vaccines therapy, and gene therapy.

Tumor-stroma ratio (TSR): the crosstalk between neoplastic cells and the associated stroma contributes to the functional and structural support of the tumor microenvironment

TSR, defined as the proportion of tumor tissue relative to surrounding stromal tissue at the invasive front, has been recently introduced as a valuable prognostic feature in many solid tumors (Almangush et al., 2018c; Morais et al., 2022). The TSR, evaluated in H&E-stained sections, was proposed for the first time by Mesker et al. (2007) for patients with colorectal cancer and has now been extended to other types of cancer, including OSCC (Morais et al., 2022). In such a grading system, tumors are divided into "stroma-rich" and "stroma-poor" according to the cut-off point of TSR of 50%, and stroma-rich tumors are associated with a poor prognosis (Fig. 5).

Almangush et al. (2021b), in a meta-analysis, identified an association between TSR and prognostic factors in head and neck cancers, such as tumor stage,

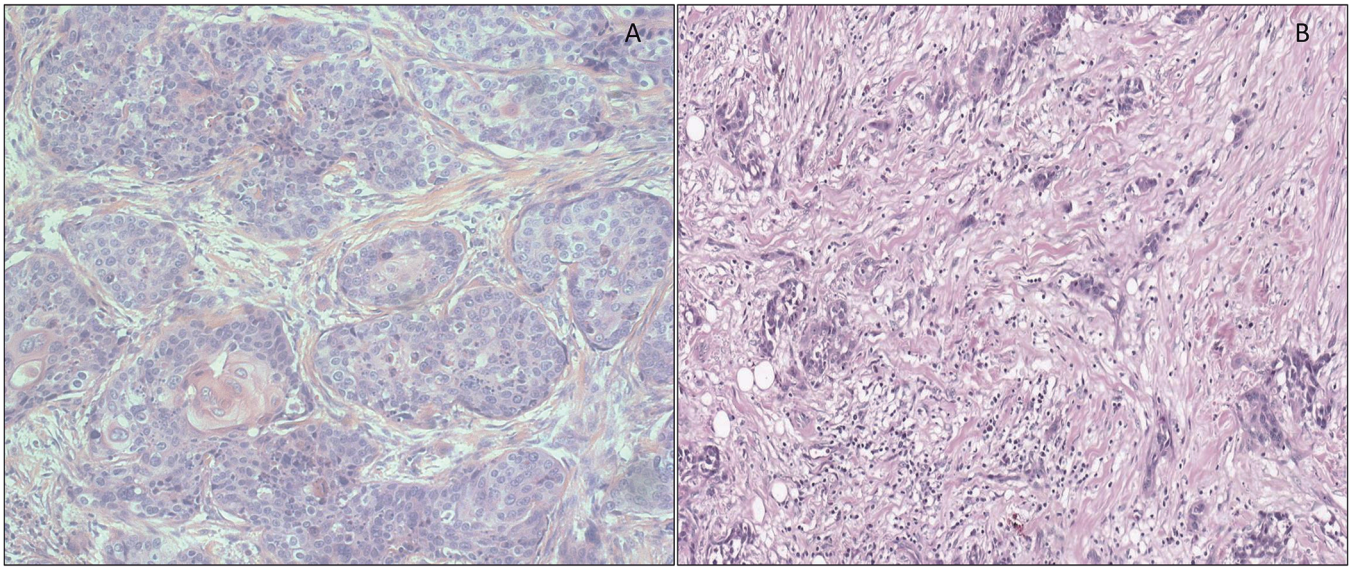

Fig. 5. The assessment of tumor-stroma ratio (TSR) at the invasive front of tumor. Stroma-poor tumor represented by a ratio <50% **(A)** and stroma-rich tumor with stroma occupied by ≥50% of fibrosis in the connective tissue **(B)**.

perineural invasion, TB and poor lymphocytic response. A recent study conducted by Dourado et al. (2020), showed that the combination of TSR and TB provided a risk stratification model with discrimination capability to predict the prognosis of patients with OSCC in the tongue. This outcome is probably related to the combination of independent prognostic parameters significantly increasing the prognostic power (Dourado et al., 2020). Silva et al. (2023) also identified that stroma-rich tumors were significantly and independently associated with poor cancer-specific survival and disease-free survival. The underlying mechanisms that connect TSR with more aggressive biological behavior are still not fully understood. However, among the many postulated theories, the one involving cancer-associated fibroblasts (CAFs) is receiving attention (Qiu et al., 2023).

CAFs, that produce the components of the desmoplastic stroma, are shown to have a role in tumor progression in different types of cancer (Marsh et al., 2012). During the stages of progression, CAFs serve as promoters of growth and invasive process after their activation by different factors secreted by the tumor, such as fibroblast activation protein, smooth muscle α-actin, platelet-derived growth factor, basic fibroblast growth factor, and interleukin 6 (Ping et al., 2021). Furthermore, the activation of CAFs influences a wide range of events, including the induction of EMT, secretion of growth factors, tumor metabolic reprogramming, preparation of the metastatic niche, and resistance to therapy (Ping et al., 2021; Asif et al., 2021). Stromal cells also promote metastasis by enhancing angiogenesis and lymphangiogenesis, thus significantly negatively affecting prognosis (Shan et al., 2021).

As the main component of the tumor micro-environment, the stroma is essential for maintaining epithelial tissues and their malignant counterparts. Naturally, the stroma could act as a barrier to tumorigenesis and invasion, restricting the proliferation of tumor cells in normal tissue (Shan et al., 2022). However, cancer-related stromal components can actively facilitate neoplastic cell growth, differentiation, and motility (Wu et al., 2016). Indeed, the stroma surrounding malignant neoplastic cells is not passive but instead plays a dynamic role in the support and nutrition of tumor parenchyma (Tarin., 2013). The crosstalk between neoplastic cells and the associated stroma contributes to the functional and structural support of the tumor microenvironment, leading to tumor progression and metastasis (Haga et al., 2021; Shan et al., 2022). Furthermore, highly aggressive tumor cells explore the tumor microenvironment, transforming the surrounding tissue and modifying the metabolism of resident cells (Shan et al., 2022). Thus, tumor-related stroma may be a target for new and alternative treatment strategies for malignant tumors.

In recent decades, investigations of histopathological prognostic markers have been based exclusively on the characteristics of tumor cells. However, tumor-related stroma may be a valuable therapeutic target (Schiavoni et al., 2013). In the case of tumor cells, the development of drug-resistant tumor cell clones can result in treatment failure because of the genetic instability of tumor cells. Given their immutable and stable nature, stromal cells are less likely to develop mutations and drug resistance, resulting in a stable therapeutic effect and thus could be used to predict the prognosis and therapeutic response of malignant diseases. The tumor-related stromal components are complex, including the extracellular matrix (ECM), various cell types, and different secreted factors. As an intermediary, the ECM helps cancer cells communicate with the stroma so that neoplastic cells can colonize the microenvironment and metastasize (Valkenburg et al., 2018). Factors that degrade the ECM, including matrix metalloproteinases (Yin et al., 2021), also facilitate tumor initiation and invasion.

In contrast to using molecular markers, the determination of the TSR is simple and fast. It can be done in routine histological material without the need for additional special techniques and extra costs, thus facilitating the repetition of the assessment. Therefore, the TSR is a convenient and valuable tool for a clinical application that could be an asset to the prognostic determination.

Future directions

OSCC treatment is standardized and involves defined clinical parameters. Ideally, histopathological and molecular parameters should be considered in the decision-making process of OSCC treatment. Nowadays, several groups are trying to develop Artificial Intelligence (AI) tools to incorporate histological characteristics for outcome prediction. Creating AI clinical assistant tools could enhance risk stratification prediction and prognostication. It would be interesting if multicentric and longitudinal studies assessing the impact of histopathological parameters could be performed at early clinical stages where prognostication is challenging. Furthermore, the value of each histological parameter at different OSCC sub-sites should be considered during the study's designs.

It is not a single parameter alone which impacts the patient’s survival, but a combination of biological characteristics, so the development and analysis of multiple histopathological parameters could add more accurate information to the prognosis. The possibility of using less invasive diagnostic methods or biopsies to identify tumors with aggressive behavior is the research focus of several groups worldwide to help clinicians during the decision-making process. Validating the prognostic value in diagnostic biopsies of emerging histopathological parameters should be a priority in future studies. The standardization of analysis methods for each morphological parameter, seeking to select models with greater inter- and intra-rater agreement, should be carried out to increase reproducibility without reducing the prognostic power of the variables.

The analysis of the different histopathological parameters discussed in this article in incisional biopsies may enhance the impact of such markers in the evaluation of the prognosis of patients with OSCC and help in adequate therapeutic planning. However, the biopsy technique and biomarker evaluation methods need to be standardized and validated in clinical trials. In the studies developed, few details are reported about the procedures applied to perform the biopsy (Bruschini et al., 2021). This information is of utmost importance, as the reliability of the specimen from a biopsy strictly depends on the quality and quantity of tissue collected. The biopsy should be deep enough to possibly include the tumor invasion front and underlying healthy tissue. Therefore, the role of incisional biopsy, whose function is mainly to define the diagnosis of a tumor, can be aimed at guiding the appropriate therapeutic choice and analyzing the prognosis of patients with OSCC through the analysis of histopathological markers with prognostic value.

Final considerations

This review provides a comprehensive view of the emerging histopathological parameters associated with the prognosis of OSCC and the main biomolecular mechanisms associated with the oral carcinogenesis process. Promising results regarding combining these markers as a potential tool require further validation in extensive multi-center studies.

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