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Review

Oral cancer: changing the aim of the biopsy in the age of precision medicine. A review

Il cancro del cavo orale: ridefinizione del ruolo delle biopsie nell'era della medicina di precisione. Review della letteratura

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SUMMARY

Oral cancer is a heterogeneous disease that develops through a complex, multi-step process. Precision medicine should help to better understand its molecular basis, integrate traditional classifications and have a positive impact on cancer management. To apply this information in clinical practice, we need to define its histology and identify biomarkers expressed by the tumour that provide useful information for planning tailored treatment. The most reliable information currently derives from evaluation of biomarkers on post-operative samples. To plan personalised treatment, oncologists need to assess these markers on biopsy samples. We reviewed the recent literature and identified 6 of 184 publications that compared markers measured on biopsy and post-operative samples or assessed their predictivity for the development of lymph node metastases. Data from these studies suggest that markers measured on biopsy samples can provide useful indications for tailoring treatments. However, due to their heterogeneity and low level of evidence, these results need to be confirmed by clinical studies on a large population to standardise and validate biomarkers in biopsies and to assess their reliability in other diagnostic mini-invasive procedures such as radiomics and liquid biopsy.

KEY WORDS: surgical biopsy, liquid biopsy, radiomics, depth of invasion, precision medicine

RIASSUNTO

Il cancro orale è una malattia eterogenea che origina ed evolve con un processo complesso e multifasico. La medicina di precisione permette di pianificare un trattamento personalizzato sulla base delle caratteristiche biologiche e molecolari delle singole neoplasie. Le informazioni oggi più affidabili sono fornite dalla valutazione post-operatoria dei biomarcatori, ma per pianificare un trattamento personalizzato è necessario valutare questi marcatori sulla biopsia. Per questo abbiamo rivisto la letteratura dell'ultimo quinquennio ed abbiamo identificato 6/184 articoli che valutano i marcatori sulla biopsia confrontandone i valori con quelli misurati sul pezzo operatorio di ciascun paziente o valutandone la predittività per lo sviluppo di metastasi linfonodali. I dati che emergono da questi studi suggeriscono che la valutazione dei marcatori sul campione bioptico potrebbe fornire indicazioni utili per programmare trattamenti personalizzati. Tuttavia, a causa della eterogeneità e del basso livello di evidenza dei lavori considerati, questi risultati devono essere confermati da studi clinici su un'ampia popolazione per standardizzare e validare i biomarcatori e la loro affidabilità in altre procedure mini-invasive, ad esempio radiomica e biopsia liquida.

PAROLE CHIAVE: *biopsia chirurgica, biopsia liquida, radiomica, profondità di invasione, medicina di precisione*

Introduction

Cancer of the oral cavity is the eighth most common cancer worldwide: GLOBOCAN reported an estimated 354,900 new cases in 2018; about 90% of these tumours are oral squamous cell carcinomas (OSCCs) with a 5-year survival of

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< 60% [1]. OSCCs can arise from the oral tongue, which is the most common sub-site and has the worse prognosis [2-4], followed by the floor of mouth, buccal mucosa, alveolar mucosa, and hard palate. Historically, OSCC affects older people, mainly men, smokers and alcohol drinkers. Recent clinical and epidemiological studies have reported an overall decline in the incidence of tobacco-related OSCCs, probably due to the anti-smoking campaigns [5-7], but an increasing trend of tongue and oropharyngeal cancers in younger patients, women and individuals with no exposure to traditional risk factors [8-10]. While infection with human papillomavirus accounts for an increasing number of patients with oropharyngeal cancers, risk factors, especially viruses, in OSCC non-smokers and non-drinkers, are unclear [11,12]. Head and neck cancer (HNC), including OSCC, is a heterogeneous disease that develops through a complex, multi-step process involving genetic alterations, growth regulation, apoptosis, angiogenesis, invasion and metastasis; moreover, it is influenced by the individual's genetic predisposition and environmental exposure to carcinogens [13,14]. This heterogeneity is confirmed in daily practice by the observation that patients with clinically similar tumours have different treatment responses and outcomes [15,16].

Precision medicine

To address this heterogeneity, the Cancer Genome Atlas project [17], classifications based on molecular pathology [18], and the Precision Medicine Initiative [19] have been proposed. These efforts should help to better understand the molecular basis of cancer, integrate traditional classifications and have a positive impact on cancer management [20]. Given this complexity, both the diagnosis and therapy of OSCCs should be planned by a highly specialised multidisciplinary team that is able to globally assess the disease and patient [21,22]. Such personalised diagnostic and treatment strategies which precisely target molecular alterations refer to Precision Medicine or Genomics-Driven Cancer Medicine [23-25]. To apply precision medicine in clinical practice, we need to identify biomarkers expressed by tumours that reflect their individual characteristics [26-28].

Tissue biomarkers validated on post-operative surgical specimens

Many prognostic/predictive biomarkers have been taken into account and tested, such as c-erbB2, EGFR (Epidermal Growth Factor Receptor), tumour infiltrating lymphocytes, c-met, etc., and currently the most reliable information on high-risk patients derives from assessment of tissue biomarkers on post-operative samples. In 2004, two trials by the European Organization for Research and Treatment of Cancer (EORTC), and the United States Radiation Therapy Oncology Group (RTOG) compared adjuvant radiation alone *vs* chemoradiation in advanced head and neck cancers. The results showed that the addition of concurrent chemotherapy was associated with better oncologic outcomes compared to radiation alone in a subset of high risk patients. They evaluated pathological classification of the primary tumour, positive margins, tumour depth, positive nodes, extranodal extension (ENE), lympho-vascular invasion (LVI), perineural invasion (PNI) and histology grade [29,30]. A subsequent comparative analysis of data pooled from these two trials showed that ENE and/or microscopically involved surgical margins were the only risk factors for which the impact of post-operative chemoradiotherapy was significant in both trials [31]. Cooper et al. in 2012 confirmed these data by evaluating the long-term results of patients enrolled in the RTOG study. They found that at 10 years, local recurrence and disease-free survival rates were significantly better only in patients included in the chemo-radiotherapy arm for ENE and/or microscopically involved margins [32]. Subsequently, other studies confirmed these data and proposed risk classes for effective post-operative therapeutic planning [33-36]. The 8th edition of the TNM staging system [20] suggests that HPV and depth of invasion (DOI) as well as specific biomarkers expressed by HNCs should be assessed. OSCCs are rarely related to HPV, while DOI is useful to evaluate biological behaviour, because prognosis worsens when the tumour is deeper [37,38]. In the past, the DOI of early-stage tongue tumours (T1-T2) was considered a predictor of occult lymph node metastases, as in skin carcinomas, and was used to plan neck dissection [39,40]. Before the publication of the TNM, 8th Edition, the terms "*Tumour Thickness (TT)"* and "*Depth of Invasion (DOI)"* were often considered synonymous. However, DOI, as defined in the AJCC (American Joint Committee on Cancer) Cancer Staging Manual 8th Edition, has a better predictive value than TT [38,41]. Recent studies based on this definition suggest that DOI > 4 mm (range: 3.4 mm-6.6 mm) is a strong predictor of locoregional recurrence, and survival together with two or more markers such as PNI, LVI and Worst Pattern Of Invasion (WPOI-5) [42-44] which have been integrated into the 8th TNM edition. In addition, several studies have highlighted tumour budding (BD) and tumour-stroma ratio (TSR), considered in other non-TNM-based staging systems, as predictive factors for lymphatic diffusion and disease-free survival (DFS) [15,45-47]. Table I shows the definition and purpose of these markers [2,15,37,38,42,45, 46,48-57].

To plan personalised treatment, oncologists need to assess these markers, including DOI, on biopsy samples according to the AJCC TNM (8th edition) recommendations [38]. Herein, we review the literature in order to evaluate the

Table I. Pathological markers predictive of loco-regional recurrence and prognosticators.

Marker	Definition	Notes
Depth of Invasion (DOI)	**DOI** is measured from the level of the basement membrane of the closest adjacent normal mucosa. A "plumb line" is dropped from this plane to the deepest point of tumour invasion [38,42]	Included in T1-3 Categories for Oral Cavity Cancer, TNM Staging Manual 8th Edition [37] The T category increases with every interval of 5 mm
Tumour Thickness (TT)	**TT** is measured from the surface of the tumour to the deepest point of invasion. In exofitic and ulcerated lesions TT is measured from the imaginary line reconstructing the intact mucosa to the deepest point of invasion [42,48] **TT1** is measured from the level of adjacent mucosa to the deepest point of tumour invasion [49] **TT2** is the distance from the bottom of most adjacent dysplastic abnormal **rete pegs** to the deepest point of invasion [49] **TT3** is measured as distance from the epithelial junction of the most adjacent dermal papillae to the deepest point of tumour invasion [49]	As defined before publication of the 8th TNM Edition [42,48] or defined as an alternative to the proposed classification [49] **Rete pegs**: epithelial extensions in the connective tissue underlying the mucosa [49]
Radiological Depth of Invasion (rDOI)	**rDOI** is measured by drawing a perpendicular line from the **reference line** to the deepest point of the tumour [50]	Radiological definition of DOI is also reported in TNM 8th Edition [37]. The **reference line** connects the junction of the tumour surfaces and of the normal mucosa on both sides [50]
Tumour Budding (TB)	**TB** is defined as the presence of either isolated single cells or small-cell clusters comprising fewer than five cells scattered in the stroma ahead of the invasive tumour front [15,45,48,51-54]	TB is speculated to be the result of interactions between cancer cells and tumour microenvironments. It is expression of loss of cohesion and active invasive cellular movement [55]. It is considered the first step in metastasis of a solid tumour
Pattern of Invasion (POI)	**POI** at the tumour-host interface of oral cancer is graded 1 to 5.	Tumour dispersion is assessed at the advancing tumour edge. The most common WPOI-5 phenotype is tumor dispersion through soft tissue. Dispersed extratumoral peri-neural invasion, or extratumoral lymphovascular invasion, also can qualify for classification as WPOI-5 [37]
Worst Pattern of Invasion (WPOI)	**WPOI-5** (**POI Grade 5**) consists of dispersed, discontinuous growth pattern, with a defined tumour dispersion cutoff of 1 mm [37,54]	
Tumour-Stroma Ratio (TSR)	**TSR** defines the interactions between cancer cells and intra-tumoural stroma, which is the main component of the microenvironment [46,56,57]	These interactions are important for both cancer initiation and progression: the proportion of this stroma acts as a key regulator in cancer biology and could provide strategies for biological cancer treatment
Peri Neural Invasion (PNI)	**PNI** is defined as the tumour cell infiltration in any layer of the nerve sheath or tumour in close proximity involving more than one-third of the nerve circumference [49]	PNI should be subclassified as either intratumoral or extratumoral, and as focal or multifocal. Extensive multifocal PNI is usually extratumoral and frequently associated with a "strand-like" tumour phenotype [37]
Lympho Vascular Invasion (LVI)	**LIV** is defined as the detection of tumour epithelial cells within or attached to the endothelial cell lining of the vascular space [49]	LIV should be reported as either intratumoral or extratumoral, as well as focal or multifocal [37]

concordance between the values of tissue biomarkers measured in both pre-operative and post-operative samples from the same patient.

Methods

For this purpose, we reviewed the recent literature published in English (January 2015-December 2019) by consulting the PubMed, Medline, Web of Science, Embase and Cochrane databases. We used the following keywords (strings): oral biopsy and depth of infiltration/DOI; oral biopsy and tumour/tumor budding; oral cancer diagnosis and depth of infiltration/DOI; oral cancer diagnosis and tumour/tumor budding. Only peer-reviewed papers were considered. We thus identified 184 papers (Fig. 1); 90 of these were duplicates. The remaining 94 were evaluated based on the abstracts. *Inclusion criteria* were: A) histologically proven squamous cell carcinomas (SCC) of the oral cavity; B) both prospective and retrospective studies; C) papers that compared biopsy and post-operative samples from the same patient; D) papers that evaluated predictivity of biomarkers on biopsy specimens; E) articles based on a retrospective series whose biopsy and post-operative slides were reviewed by the authors. *Exclusion criteria*: a) reviews, case reports, abstracts; b) histology other than SCC; c) studies that did not review histologi-

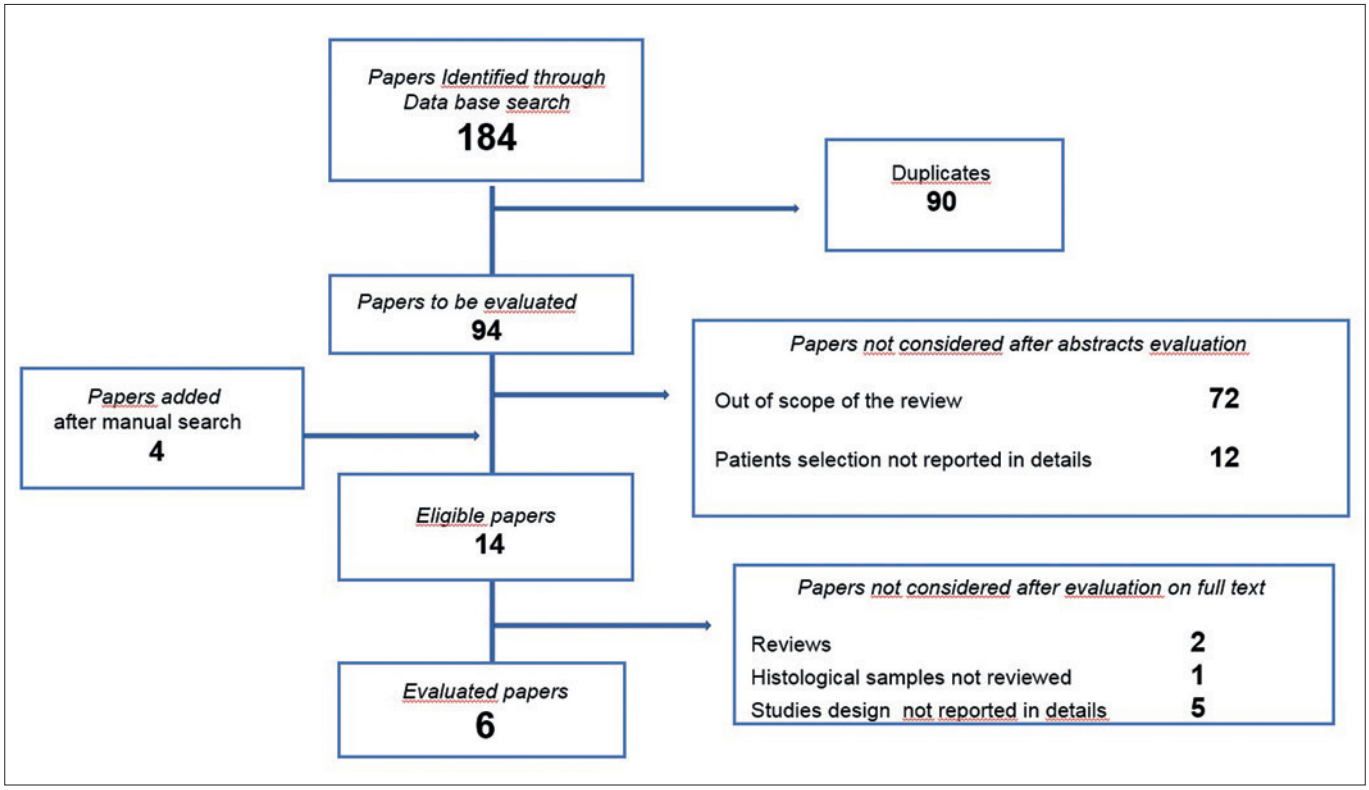

Figure 1. Flow chart showing selection of papers.

cal samples. As a result of this analysis, we discarded 72 papers unrelated to our aim; for 12 studies, criteria for patient selection or study design were not reported in detail. Fourteen papers (the 10 selected articles and 4 others identified through a manual search) were evaluated on the complete text. Following this analysis, 8 more papers were considered unsuitable for our purpose as they were reviews or the study design did not fall within the selection criteria.

Results

All six papers included were retrospective, and were divided into two groups based on the aim of the study (Tab. II) [48,49,58-61]:

Comparison of tissue markers on biopsy and post-operative samples. Three papers [58-60] compared the values of biomarkers measured on patient's biopsy and post-operative samples from the same patients in order to evaluate their concordance and reliability. Almangush et al. [58] reviewed the slides of 100 patients (81 = Stage I-II) with SCC of the mobile tongue treated with surgical excision. They measured TT and found that mean values were 4.1 mm (0.5-10 mm) in biopsy samples *vs* 6.3 mm (0.5-23 mm) in the corresponding post-operative specimens. They also evaluated TB (Tab. I) and their prognostic score (BD Model, Tab. III), and documented a sensitivity and specificity of pre-operative *vs* post-operative values of 59% and 100%, respectively, showing a 83% pre-operative/post-operative agreement. Seki et al. [59], among 91 OSCC patients treated with surgery alone or preoperative chemotherapy and resection, analysed 44 undergoing exclusive surgery. They evaluated TT, TB Score (TBS, Tab. III), infiltrative pattern (INF, Tab. III) and LVI, and found that the depth of infiltration measured on biopsies was lower than that on post-operative samples, while there was a good correlation of budding score in pre-treatment and post-treatment samples. Seki Soda et al. [60] recently analysed the relationship between TBS in biopsies and in resected specimens of 248 patients with OSCC and the effect of pre-operative chemotherapy on TBS. The authors did not report staging, but pointed out that 141 of 248 patients received preoperative chemotherapy. The mean TBS in biopsy and resected specimens showed no significant difference between patients with and without preoperative chemotherapy. Moreover, they found that pre-operative chemotherapy is effective in lowering the budding score and suppressing relapses.

Table II. Evaluation of pathological markers on biopsy and postoperative samples in the 6 papers selected (continues on page 113)

Author	Study design Time period	Site Stage or TNM teatment	No. patients Biopsy technique	Aim of the study
Almangush, 2018 [58]	Retrospective 1981-2016	Mobile oral tongue Stage I-II = 81% Stage III-IV = 19% Surgery = 100%	100 Incisional biopsy	Analysis of the sensitivity and specificity of BD scores on biopsy and postoperative samples
Seki, 2016 [59]	Retrospective 2009-2013	Tongue, floor of the mouth T1 = 43% T2-T4 = 57% Preop CT = 52% Surgery = 48%	91 Not described	Detection of histopathologic parameters predictive for lympho nodal metastases in preoperative biopsy specimens
Seki Soda, 2019 [60]	Retrospective 2009-2015	Oral cavity Stage not reported Preop CT = 57% Surgery = 43%	248 Incisional biopsy	Evaluation of relashionship between tumour budding score in biopsy and resected specimens and the effect of pre-op chemotherapy on tumour budding
Seki, 2017 [48]	Retrospective 2009-2014	Mobile tongue, buccal mucosa, palate, lip T1-T2 = 76% T3-T4 = 24% Preop CT and resection +/- ND = 53% Resection+/- ND = 47%	209 Incisional biopsy	Evaluation of the relashionship between tumour budding in biopsy specimen and lymphnode metastases
Nayanar, 2019 [61]	Retrospective 2014-2016	Oral cavity (lips, buccal mucosa, tongue, hard palate, retromolar trigone, floor of the mouth) Stage II = 48% Stage III = 20% Stage IV = 32% Wide excision with Neck dissection	160 Incision or wedge biopsy	Identification of clinical and histopathological predictors of lymphnode metastases Development of a predictive scoring system
Sahoo, 2020 [49]	Retrospective 2014-2016	Oral cavity (GB sulcus, tongue, floor of the mouth, retromolar and maxilla) cT1/T2 Resection and elective neck dissection	150 Excisional biopsy	Comparison of prognostic performance of TT and of DOI (TT2 and TT3) in predicting lymphnode metastases. Predictive potential of PNI and LVI

Predictive value of pathological markers in biopsy samples. Four papers [48,49,59,61] evaluated whether markers assessed on biopsy samples were predictive of lymph node metastases (Tab. II). Seki et al. [59] examined 33 patients with tongue and floor SCC treated with tumour resection and neck dissection. They found that BD score ≥ 3 and TT > 3 mm was significantly predictive of nodal metastases, overall survival (OS) and relapse-free survival (RFS), while TT alone measured on biopsy samples was not. The same team [48] evaluated TBS on a larger series of 209 OSCC patients (76% cT1) treated with resection or with pre-operative chemotherapy and resection, without stratifying the patients according to treatment modalities. They found that ≥ 3 buds in the biopsy samples was predictive of lymph node metastases, and that TBS was significantly correlated with INF, Grading and LVI ($p < 0.01$). The authors pointed out that the TB cut-off point needs to be defined, and that the evaluation of TBS and TT needs to be standardised. Nayanar et al. [61] analysed 160 patients with oral cancer (Stage II = 48%) treated with wide excision

Table II. Evaluation of pathological markers on biopsy and postoperative samples in the 6 papers selected (follows from page 112)

Markers	Results	Notes
TB TT BD model score	BD model score preo-op *vs* post-op - Sensitivity 59.1% - Specificity = 100% - Agreement pre/post op = 83% Depth values (mm) pre-op (mean) = 4.1 (0.5-10 mm) post-op (mean) = 6.3 (0.5-23 mm)	17% = non-representative biopsies (badly fragmented, too superficial, technical artifacts) TB and TT (Tab. I) Budding model score (Tab. II)
Histologic Grading TT INF LIV Budding score	Tumour depth: higher in resection specimens than in biopsy samples. It is predictive of lymph node metastases on resection specimens, not on biopsy samples Budding score: good correlation between biopsy and postop specimens BD = > 3 and Tumour depth > 3 mm significantly predictive of lymph node metastases, OS and RFS BD = > 3 and INFa/INFb: predictive of early or late lymph node metastases or late in many cases	TT (Tab. I) INF (Tab. I) Budding score (Tab. IIb) Comparison between TB, TT, and INF status on lymph node metastases was assessed on 33 patients who underwent resection and neck dissection
Budding score	Mean budding scores: no significant differences in both groups; but tended to increase in resected patients without preop CT Preoperative CT-S1 treatment is effective for suppressing relapse and lowering the budding scores	Budding score (Tab. II)
TB INF Tumour grade TT	TB => 3 predictive of Lymphnode metastases and poor prognosis P < 0.01 (mainly T1-T2 cN0) The budding score (high) together with INF and LIV were found to be independent risk factors for lymphnode metastases at multivariate analyses Strong correlation (p < 0.01) between the budding score and tumour grade, tumour depth, INF, and blood vessels invasion TB in early OSCC can be used as a reliable parameter for stratifying patients with different risks of lymph node metastases	Authors describe biopsy technique; Tumour budding can be detected using routine haematoxylin and eosin-stained slides or cytokeratin-stained slides. Results may differ significantly depending on the staining used
Shape of rete pegs Pattern of invasion Depth of invasion (TT) Histologic differentiation Site of cancer	Risk score (4-18) Score 4: predictive of N+ = 5% Score 18: predictive of N+ = 91%	The risk score needs to be tested and validated in other patient populations Rete pegs (Tab. I) Risk score (Tab. II)
TT1, TT2, TT3, DOI PNI, LVI	TT2 and LVI correlate with lymph node metastases	TT1, TT2, TT3, DOI (Tab. I)

and neck dissection and proposed a predictive score based on selected pathological features (rete pegs, pattern of invasion, TT, histologic differentiation) and site of tumour (buccal mucosa, tongue, other). The score ranged from 4 to 18; the authors found that a score of 4 is predictive of risk for lymph node metastasis of 5%; for scores of 10, 14 and 18, the risk was 31%, 67% and 91%, respectively. Sahoo et al. [49] studied 150 cT1-T2 patients treated with resection and neck dissection for OSCC. They compared three measurement modalities of the depth of invasion: TT1, TT2 (DOI) and TT3 (alternative DOI); according to the authors, TT2 corresponds to DOI, as defined in the TNM 8th Edition [37,38]. They found that TT2 (DOI) and LVI correlate with nodal metastases.

Discussion

To our knowledge, this is the first comprehensive review correlating biological tumour characteristics between biopsy and corresponding pathologic specimen with an evidence-based method. Unfortunately, the studies that met

Table III. Different scores for prognostic evaluation.

Author	Definition	Score
Almangush, 2018 [58]	BD Model Score (BDM): N° of buds and depth of invasive front (IF, mm)	0: < 5 buds at the IF and depth < 4 mm 1: =/> 5 buds at the IF or depth > 4 mm 2: =/> 5 buds at the IF and depth > 4mm
Seki, 2017 [48]	Tumour Budding Score (TBS): Number of buds	Low: < 3 cells Intermediate: 3-4 cells High: => 5 cells
Seki, 2017 [48]	INF (Infiltrative Pattern): Cancer growth and mode of invasion	INFa: Expanding growth with a distinct border from surrounding tissue; INFb: Intermediate pattern between INFa and INFc; INFc: Infiltrative growth with no distinct border from the surrounding tissue
Nayanar, 2019 [61]	Risk Score: Sum of single score of clinical and pathologic parameters	Shape of rete pegs: Slender and fused = 1, Bulbous and uniform = 2; Irregular = 3 Pattern of invasion: Pushing = 1; Minimally invasive = 2; Frankly invasive = 5 Depth of invasion (TT, mm): 0-3 = 1; > 3 = 3 Histogic differentiation: Well = 1; Moderate = 3; Poor = 5 Site of cancer: Buccal mucosa = 0; Tongue = 1; Others = 2

the inclusion criteria had a level of evidence of 3 and 4 according to the AJCC on Cancer Levels of Evidence classification [20]. It is worth stressing a criticality common to all: namely that they differ in the design of the study, selection of patients, oral subsite of cancer and in choice of pathological markers such as DOI, TT and budding score; for this reason, the results are not comparable. Furthermore, many authors have measured the depth of the invasion differently than what is foreseen in the TNM 8th Edition, even when published after its release. In this regard, the paper of Driven et al. that quantified the impact of the stage migration on patients' prognosis using TT and DOI in a retrospective cohort of 456 OSCC patients is interesting. They found similar stage category and prognosis regardless of the measurement used [62]. This review highlights several other critical issues.

Biopsy technique

The patients evaluated by Almangush [58], Seki [48] and Seki-Soda [60] underwent incisional biopsy; those studied by Nayanar [61] had an incisional or wedge biopsy; those in the study of Sahoo [49] were treated with an excisional biopsy. The authors did not provide more details on the procedures applied to perform the incisional/excisional biopsy. This information is important since the reliability of the biopsy sample strictly depends on the quality and quantity of the tissue taken. Seki et al. [59] did not specify the biopsy modalities in their first report, but in a later study aimed to evaluate the relationship between TBS assessed in OSCC biopsy specimens and lymph node metastases (LNM), recommending that incisional biopsy be performed peripherally to the cancer so as to obtain a significant amount of non-necrotic tissue from the centre of the tumour [48].

Depth of biopsy

It should also be stressed that the biopsy sample should be deep enough to possibly include the entire tumour infiltration front and underlying healthy tissue [48]. Reliability of biopsy (incisional, wedge, punch, excisional) is influenced by the depth of the sample because it is unlikely that specimens always include tumour stroma on the invasive front, which is adequate to evaluate the relationships between the various components of the tumour microenvironment (TME) [48,58,59]. In fact, tissue biomarkers are the morphological expression of the TME, or the field in which the immune system and the tumour interplay. Inside TME cancer cells, non-tumour cells and stroma are in a continuous and dynamic relationship, and any change within the TME may reflect changes of the balance between the immune system and tumour [63]. In this context, the role of biopsy is not only diagnostic, but also predictive, since biopsies should include the invasive front, the behaviour of tumour cells in the TME interface and biomarkers that provide useful information to guide the therapeutic choice [15,64,65-67]. Leite et al. [66], in a recent preliminary study on biopsy samples from 56 OSCC cases, highlighted the possible importance of the correlation between grading, predominant mode of invasion and TB intensity. The authors classified the mode of invasion in 4 degrees, similar to the proposal of Shimuzu [54]; they also graded TB as low-intensity (5 buds in one single x200 power field) and high- intensity (≥ 5 buds in

one single x200 power field). The majority of cases studied (66.1%) were high-intensity TB; all cases with the worst mode of invasion showed high-intensity TB, while no association was found between TB and histopathological grading. They concluded that both TB and mode of invasion in OSCC diagnostic specimens could help to select patients who could benefit from more aggressive treatment. This paper has some critical issues, such as the biopsy performed in the central part of the tumour, which is rich in necrotic tissue that must be eliminated, and the lack of follow-up. Therefore, these promising data require confirmation on larger series with a longer follow-up. A large biopsy on the surface (at least 8 mm) and depth (at least 5 mm) could allow an assessment of the microenvironment in early cancers, but does not solve the problem [48,64]. In this regard, Dhanda [64] compared TT measurement on biopsies and the corresponding post-operative samples reported in the medical records of 93 patients operated on for oral cancer; we have not included this paper in our review according to the exclusion criteria (point c) because those specimens were not revised by a pathologist. However, measurements reported in medical charts provide interesting information because in this series 20% of tumours had a tumour depth > 10 mm on post-operative specimens; on biopsies only 28% of samples had a thickness greater than the DOI calculated on the post-operative specimens. They therefore suggested performing a 10 mm deep punch biopsy, which should potentially include the invasive front in up to 80% of the cases analysed in this paper [64].

Additional biopsy related issues

It should also be pointed out that 17% of biopsy samples are not representative of the cancer due to technical errors such as fragmented, superficial, non-orientable samples and artifacts in the preparation of slides [58]. For this reason, how the specimen is sectioned and oriented is important: slides are evaluable if sections are perpendicular and include all mucous, submucosal and muscle layers. On the contrary, tangential sectioning does not allow the evaluation of infiltration depth [68]. In addition, biopsy and surgical procedures can sometimes make it difficult to measure the depth of invasion on post-operative specimens. This critical point is underlined by Berdugo who analysed the post-operative samples of 239 glossectomies [69]: 5% had only a minimal residual of cancer and in 14% no tumour was found. He also emphasised that in 21/183 (11.5%) pT2 OSCCs the DOI was underestimated due to positivity of deep resection margins. Finally, it should be kept in mind that a large surgical biopsy may create an inflammatory reaction which alters the microenvironment and could promote the regional spread of cancer [70,71].

Changing the aim of biopsy

To plan the best treatment of a tumour, one must know its histopathological diagnosis, site, size and biological aggressiveness. Currently, the risk of developing lymph node metastases can be evaluated by measuring biomarkers such as Grading, PNI, LVI, positive resection margins, depth and mode of invasion and tumour budding on post-operative specimens. On the basis of this information, high-risk patients can be identified for treatment with post-operative radiotherapy or chemoradiotherapy. It is hoped that this predictive and prognostic information will be available for all patients, even those candidates for non-surgical therapies, at the end of the diagnostic process, so as to personalise the therapy for each. This review documents the possibility to reliably test these tissue biomarkers on biopsy samples. However, it is necessary that the biopsy technique and the methods of evaluation of biomarkers are standardised and validated in clinical trials on large case series before they can be used in clinical practice. For these reasons, the role of biopsy is changing and evaluation of a biopsy sample will allow not only to define the morphology of a tumour, but also to guide the therapeutic choice.

Clinical and imaging evaluation of depth of infiltration

The 8th edition of TNM suggests that the DOI of oral cancer is assessed pre-operatively with clinical and imaging investigations [38]. It should be emphasised that clinical evaluation is subjective, depends on the experience and ability of each physician and is limited by anatomical conditions such as trismus and/or the patient's intolerance to palpation for pain [72]. Recently, prospective studies comparing pre-operative clinical and magnetic resonance imaging (MRI) evaluation [72], or MRI alone [50,73,74] with post-operative DOI have documented a good correlation of these investigations, especially in tumours with infiltration > 5 mm. On the contrary, other authors have reported an overestimation of T2 lesions and underestimation of T4 cancers when clinical T staging is compared with histopathological data [75,76]. These papers also highlight several critical issues related to imaging (MRI/computed tomography, CT) such as artifacts due to movement and dental fillings; the evaluation of peritumoural oedema and inflammation, and timing between imaging and biopsy (first imaging or biopsy? If imaging is performed after biopsy, how long does it take to get reliable information from MRI/CT?) [50,76,77]. Tumour shrinkage of formalin-fixed post-operative specimens may interfere with comparing the DOI between MRI pre-operative imaging and post-operative specimens [72,78]. A recent meta-analysis [79] documented a high correlation of TT measured by intraoral ultrasound and histopathology mainly in

T1-2 tongue cancers ($p < 0.001$), although it is operator-dependent and often overestimates exophytic tumours [80,81].

Future directions

Other non-invasive diagnostic modalities are being considered to support evidence-based clinical decision-making, in particular radiomics and liquid biopsy.

Radiomics is an emerging translational field of research. Thanks to the extraction of mathematically defined parameters from routine medical images it is possible to generate large-scale sets of information that can be correlated with OS and treatment-related toxicity and can also be used to identify new biomarkers to be implemented in daily clinical practice [82]. Currently, there are few but promising published studies on the application of radiomics in oral cancer with CT or MRI [83-85]. Probably, if the role of radiomics will be confirmed with standardised methodology on a large number of patients, these results would help to promote cancer treatment towards personalised precision medicine [82,86].

Liquid biopsy is particularly promising as an alternative to surgical biopsy. It allows assessment of biomarkers in fluids (serum, saliva, urine, etc.) such as circulating tumour cells (CTC), DNA and RNA fragments (miRNA), exosomes that originate from cancerous cells, and their characteristics, particularly mutations and number of somatic alterations [87-90]. Liquid biopsy theoretically provides the individual molecular profile of each cancer over time, and could allow physicians to diagnose a tumour, plan tailored therapy and monitor cancer evolution [91,92]. Unfortunately, no circulating biomarker has yet been validated for routine use in clinical practice for OSCC, because alongside the promising potentials, there are also considerable critical issues [93,94]. For example, the levels of circulating DNA fragments derived from the tumour (cfDNA) represent a small fraction (up to 10%) of the total cfDNA and variations between patients related to cancer stage, tumour vascularisation, tumour burden and apoptosis rate can compromise the accuracy of the tests [92,95-97].

Conclusions

The traditional role of biopsy is evolving; in the future, a single procedure will allow to define the histology of a tumour and to identify predictive and prognostic biomarkers. The data emerging from this review of the literature, even if heterogeneous and fragmented, suggest that the evaluation of markers on biopsy samples, mainly the DOI and growth characteristics of the invasive front (INF, TB*)* could provide useful indications for planning tailored treatments. Minimally invasive procedures such as liquid biopsy are also promising, although only preliminary results are currently available which require further confirmation. Further clinical studies are needed to standardise and validate clinical and imaging evaluation, surgical and liquid biopsy technique, sampling of specimens, and choice of biomarkers and their assessment before introducing these diagnostic and predictive modalities in daily clinical practice.

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