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Making routine head and neck pathology Easy: From dissection to diagnosis

Jasmine Siaw [a], Timothy Fielder [a], Sebastian Senff [b], Carsten E. Palme [c,d], Jonathan R. Clark [c,d], Caroline L. Cooper [b,e,*], Ruta Gupta [a,d]

[a] Department of Tissue Pathology and Diagnostic Oncology, Royal Prince Alfred Hospital, NSW Health Pathology, Sydney, Australia
[b] Anatomical Pathology, Pathology Queensland, Princess Alexandra Hospital, Woolloongabba, Queensland, Australia
[c] Department of Head and Neck Surgery, Chris O'Brien Lifehouse, Sydney, Australia
[d] Faculty of Medicine and Health, University of Sydney, Sydney, Australia
[e] Faculty of Health, Medicine and Behavioural Sciences, The University of Queensland, St Lucia, Brisbane, Australia

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ABSTRACT

Resections from the mobile tongue, the oropharynx and neck dissections constitute a large proportion of routine head and neck pathology workload. Histologically detected proximity to margins and prognostic factors like depth of invasion, perineural or lymphovascular invasion, or extranodal extension guide adjuvant radiotherapy and/or chemotherapy.

This review discusses practical approaches to macroscopic examination of the various types of tongue and oropharyngeal resections and neck dissections. Differential inking and radial sections demonstrate proximity of tumour to margins. The macroscopic examination and sampling should then be directed towards identifying remaining adverse prognostic features including the maximum extent of invasion at the primary site and extranodal extension, nodal matting or soft tissue deposits in the neck dissections.

The diagnostic challenges differ in the tongue and oropharynx. The diagnosis of tongue SCC is relatively easy, however, precursor oral epithelial dysplasia can be challenging. Architectural and cytologic clues assisting in identifying dysplasia and practical clues distinguishing it from reactive changes are discussed. In contrast, dysplasia is not diagnosed in the oropharynx. p16 immunostaining and detection of human papillomavirus (HPV) play a critical role in the diagnosis and prognosis of oropharyngeal SCC. Nuances in the implementation and interpretation of p16 immunostaining and HPV assays are discussed.

1. Introduction

Head and neck cancers comprise morphologically and prognostically different cancers grouped together due to their anatomic location. The most common cancer in the head and neck is squamous cell carcinoma (SCC). The aetiology, risk factors, morphology, management and prognosis of SCC vary significantly based on the site of origin. For example, solar, ultra-violet driven cutaneous SCCs are more common in elderly men with Fitzpatrick Skin types 1–3. These carry one of the highest tumour mutation burdens amongst human malignancies and show high response rates to immune check point inhibitors [1,2]. In contrast, SCCs arising from the oral or laryngeal mucosa have traditionally been more common in older men with lifetime exposure to smoking and alcohol. More recently, a trend of increasing incidence of mucosal SCC in young patients without significant exposure to smoking is emerging globally [3]. Furthermore, Human Papillomavirus (HPV) and Epstein-Barr virus (EBV) associated SCCs arise from the oropharynx and nasopharynx, respectively, and occur in younger patients. The criteria for tumour grading and staging vary among the different SCCs, placing a premium on appropriate diagnosis of the type of head and neck SCC.

Within the head and neck, oral cancer accounted for an estimated annual economic burden of AUD $ 2.1 billion in 2019 though no pathology costs were considered [4]. SCC arising in the anterior two thirds of the tongue and HPV associated carcinoma arising in the oropharynx including the posterior third of the tongue together constitute the most common head and neck cancers globally [5]. Handling these specimens can be challenging for trainees and general surgical pathologists. Herein we discuss three important areas of routine diagnostic head and neck pathology and provide practical and resource efficient insights. We begin with the practical nuances of macroscopic examination of tongue and oropharyngeal resections along with the accompanying neck dissection specimens. A primer on the diagnosis of oral epithelial

* Corresponding author. Anatomical Pathology, Pathology Queensland, Princess Alexandra Hospital, Woolloongabba,Queensland,Australia.
E-mail address: caroline.cooper@health.qld.gov.au (C.L. Cooper).

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dysplasia follows and lastly, we discuss practice points in the diagnosis of HPV-associated SCC.

2. Macroscopic resection of tongue and oropharyngeal resections

An underlying common factor in all head and neck malignancies is the anatomic complexity of the region that makes achieving oncologically complete resections difficult. Histologically detected margin involvement, and adverse prognostic factors such as perineural, lymphovascular involvement or nodal metastases guide adjuvant radiation or chemotherapy. Macroscopic examination and block selection to optimise the detection of these adverse prognostic factors is critical for guiding appropriate treatment decisions.

Surgical approaches of tumours in the tongue vary with tumour size and depth. Smaller, more superficial tumours are removed as a laser resection. In the oropharynx, the tumours can be removed through a transoral robotic surgery (TORS). Larger tumours require partial or subtotal glossectomies. The type of the specimen influences the best approach to macroscopic examination; however, the principles remain the same across all these specimen types.

2.1. Prosection (Fig. 1A–D)

1. Confirm the specimen type and laterality.
2. Orient the specimen according to the provided orientation markers and laterality.
    - Orienting sutures are generally required for smaller specimens (Fig. 1A) and should be a routine practice for the surgical teams to provide these for optimum clinicopathologic correlation.
    - For larger specimens where the tip of the tongue or the floor of mouth are included (Fig. 1B, supp fig 1), these can provide anatomic orientation as described by Slootweg et al. [6].
        - The dorsal surface of the mobile tongue contains roughened papillae.
        - The lateral tongue is lined by smooth, thin mucosa.
        - The anterior two-thirds of the tongue are separated from the posterior third by circumvallate papillae that form a distinct row anterior to the sulcus terminalis.
        - In the posterior third, the surface becomes cobblestoned from underlying lymphoid tissue (Supp Fig. 2).
3. Photographs of intact specimens showing the relationship of tumour to lateral, medial, posterior and deep aspects are optimal as these can facilitate histological reporting and multidisciplinary discussions.
4. Measure the specimen in three dimensions (medial to lateral, anterior to posterior and superior to inferior).
5. Describe the external appearance of the mucosa and the tumour.
6. Differentially ink the left and right resection margins of the specimen.
7. Serially section perpendicular to the long axis of the specimen at 3 mm intervals.
    - Laying out the 3 mm slices sequentially helps with macroscopic inspection of the tumour, its extent of invasion and proximity to margins.
    - Photography of the cut surface of the slices including close up images of any close or involved margins.
8. Describe the cut surface of the tumour.
    - Measure the tumour in three dimensions (anterior to posterior; right to left and superficial to deep).
9. Radial sections demonstrating the distance of the tumour from anterior, posterior, lateral, medial and deep margins is recommended as it allows (Fig. 1C):
    - Histologic measurement of the distance of the tumour to the margins.
    - Assessment of patterns of invasion, especially for highly infiltrative tumours with worst pattern of invasion 5.
    - Evaluation of adverse prognostic features such as lymphovascular or perineural invasion at the periphery of the tumour or in extratumoural location.
10. Block allocation should be optimised towards identifying the greatest extent of tumour invasion and proximity to all margins.

2.2. Neck dissection

These specimens may be received enblock unoriented, oriented with sutures, pinned on a corkboard, or as separated levels in different jars.

Inking of the neck dissection specimens remains controversial, as presence of tumour at the soft tissue margins cannot be revised. Nevertheless, it remains a risk for local recurrence and positive margins

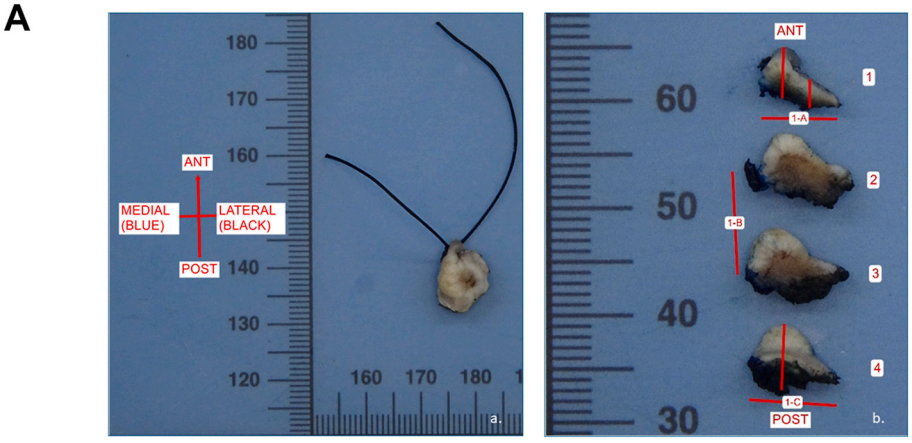

Fig. 1A. Laser excision of right lateral tongue. (a) Suture anterior. There is an exophytic, firm grey area with a central ulcer on the mucosal surface. Medial is inked blue and lateral is inked black. (b) Serially sliced from anterior to posterior. The tumour is keratinaceous with an exophytic growth. It measures 10 mm medial to lateral x 10 mm anterior to posterior x 3 mm thick. The anterior and posterior edges are embedded radially. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

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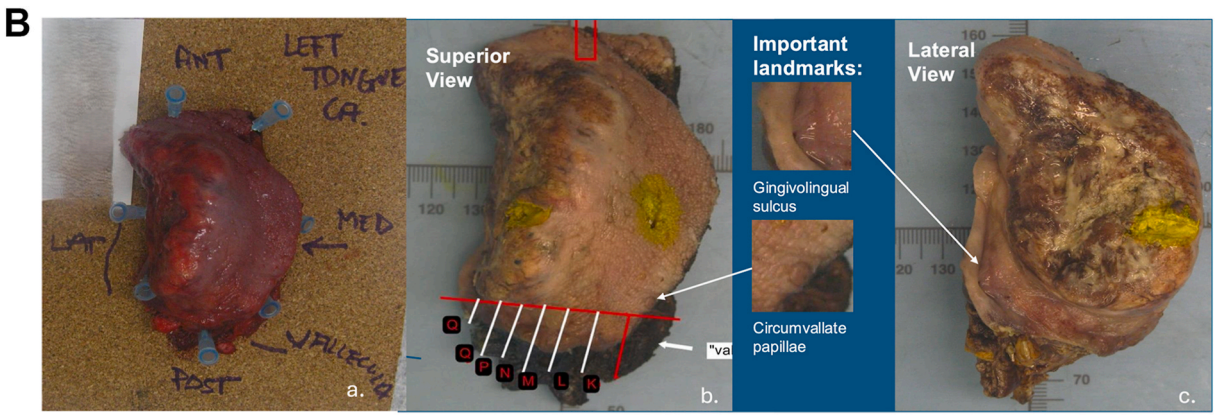

Fig. 1B. Hemiglossectomy, left tongue. (a) This hemiglossectomy is received pinned and oriented. (b and c) The anatomic landmarks of the tongue can be identified as shown.

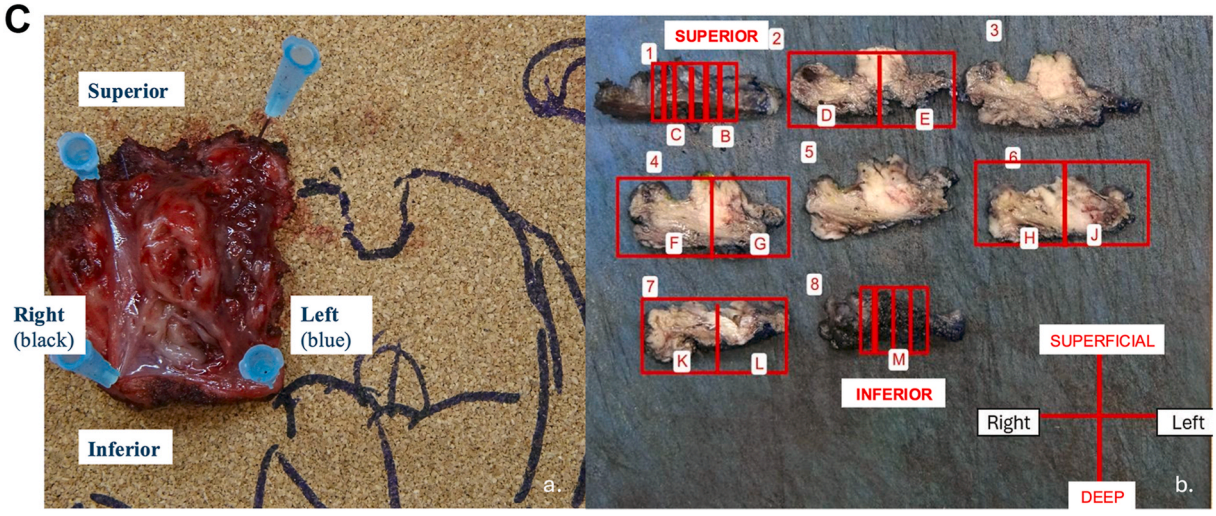

Fig. 1C. Transoral robotic surgery (TORS) of right oropharynx. (a) A TORS specimen oriented on a corkboard on an anatomic diagram. (b) For tongues (Fig. 1B) and oropharyngeal resection specimens, the specimen is ideally serially sliced perpendicular to the long axis and sections are taken to demonstrate proximity to margins and the greatest extent of invasion.

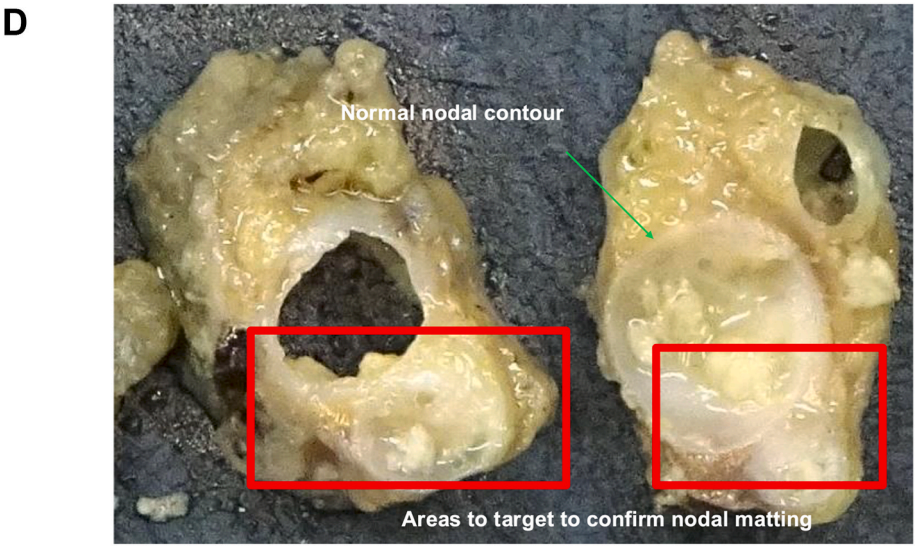

Fig. 1D. Extranodal extension. For macroscopically involved lymph nodes, serial slicing can assist with selection of blocks to histologically assess and confirm extranodal extension, matting of multiple nodes and involvement of structures such as skeletal muscles or major vessels.

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can guide decisions for adjuvant radiotherapy [7].

Individual lymph nodes are best identified by palpation. Dermody et al. have shown that histologic examination of <4 lymph nodes per neck dissection level is associated with poor disease free and disease specific survival. Thus, all attempts should be made to retrieve at least 4 lymph nodes in each level of the neck dissection. If these are not found, it is advisable to submit the entire neck dissection level for histologic examination [8]. Fat clearing solutions can sometimes assist in lymph node retrieval as done for colorectal cancers where the mesenteric adipose tissue can be significantly bulky. However, use of these solutions adds an additional day to processing and may not be an efficient alternative to sampling the entire fat in neck dissections submitted as separate levels with limited amount of adipose tissue.

Macroscopically uninvolved lymph nodes should be submitted entirely for histologic examination.

a. Lymph nodes smaller than 5 mm can be submitted intact for histologic examination.
b. Lymph nodes larger than 5 mm should be sectioned at 3 mm interval to optimise the identification of occult metastases or minor extranodal extension.

Macroscopically involved lymph nodes should be sectioned perpendicular to their long axis.

a. Photography of the cut surface of the macroscopically involved lymph node is useful (Fig. 1D).
b. Sample areas suspicious for:
   a. Extranodal extension
   b. Interface of adjacent nodes to identify nodal matting (defined as confluence of two or more nodes due to the tumour extending from one lymph node into another. Overlapping synonyms including fusion, adhesion, confluence and conglomeration should be reserved for two lymph nodes with fibroinflammatory thickening of their capsules without histological evidence of tumour crossing the tissue between the lymph nodes) [9].
   c. Infiltration into adjacent structures including skeletal muscle or major blood vessels [9].
c. If extranodal extension is not macroscopically apparent, consider submitting the entire lymph node for histopathologic examination. This often depends on the size of the involved node and in these instances, the photographs of the cut surface can be extremely useful for macroscopic and histologic correlation for optimal assessment of extranodal extension.

2.3. *Macroscopic evaluation of post neoadjuvant treatment specimens*

Standardised, internationally accepted guidelines are currently lacking for these specimens. Institutional best practice protocols should be followed until these are developed, and ideally, macroscopic examination should be directed towards identifying the maximum extent of residual viable tumour. It is well established that it can be difficult to delineate residual viable tumour from fibroinflammatory response of the tumour bed. Thus, it may be prudent to embed the entire tumour bed if $\leq$ 20 mm in size or embed at least 1 block per 10 mm for larger tumour beds. Photography of the intact and cut slices of the tumour is critical in these instances (Supp Figs. 3 and 4).

Histological evaluation of SCC of the tongue with associated adverse prognostic features is well described with limited inter or intra observer variability. On the other hand, precursor oral epithelial dysplastic (OED) lesions can provide significant diagnostic challenges, particularly in small biopsies, that are addressed here [8].

3. Oral epithelial dysplasia

The World Health Organisation (WHO) 5th edition of Head and Neck Tumours defines oral epithelial dysplasia (OED) as a spectrum of architectural and cytological epithelial changes arising in a range of oral potentially malignant disorders (OPMDs) [10]. OPMDs clinically manifest as leukoplakic and erythroplakic lesions and are relatively common with a prevalence of up to 10 % in some populations [11]. Many cases of OED are associated with the use of tobacco, areca nut and alcohol, and lichenoid inflammation in older women. The risk of OED progressing to SCC varies significantly (3–50 %) and OED grading assists in stratifying malignant transformation risk [10].

Diagnosis of OED relies on morphological evaluation of architectural and cytological abnormalities, either in combination or alone (summarized in Table 1) [10,12]. The currently accepted grading of dysplasia is three tier, mild, moderate, or severe. This depends partly on how many epithelial layers show atypia—from the basal third (mild) to full thickness (severe) (Fig. 2a–c). However, this layer-based model is imperfect. In some cases, cytological atypia limited to the basal third or simply the basal zone may still justify a designation of severe dysplasia when features such as bulbous rete ridges, basal cell budding, disorganization, and marked pleomorphism are pronounced (Fig. 2 d). Some authors propose a simplified binary (low/high grade) system, though further validation is needed [13].

Of note, the terminologies of 'severe', 'high grade' dysplasia and carcinoma *in situ* (CIS) are often used interchangeably and carry similar prognosis, although the WHO recommends against the use of CIS.

Several benign or reactive conditions can mimic OED, causing diagnostic challenges particularly in small biopsies. Accurate distinction relies on careful assessment of overall morphology, distribution of atypia, and associated stromal features. Conditions including reactive or inflammatory atypia (e.g., due to trauma, infection, candidiasis, or ulceration), oral lichen planus, pseudoepitheliomatous hyperplasia, verrucous and papillary lesions and atrophic mucosa can pose difficulties (Fig. 3a–d).

Table 1
Morphologic features in OED.

Architectural features	Cytological features
Abrupt change from normal to abnormal epithelium	Abnormal variation in cell size and shape
Irregular stratification	Variation in nuclear size and shape
Loss of normal polarity or basal disorganization	Nuclear membrane irregularities
Basal budding	Hyperchromasia
Early keratinisation	Increased nuclear:cytoplasmic ratio
Extension of changes along minor salivary gland ducts	Single cell keratinisation
	Suprabasal mitoses
	Karyorrhexis and apoptosis

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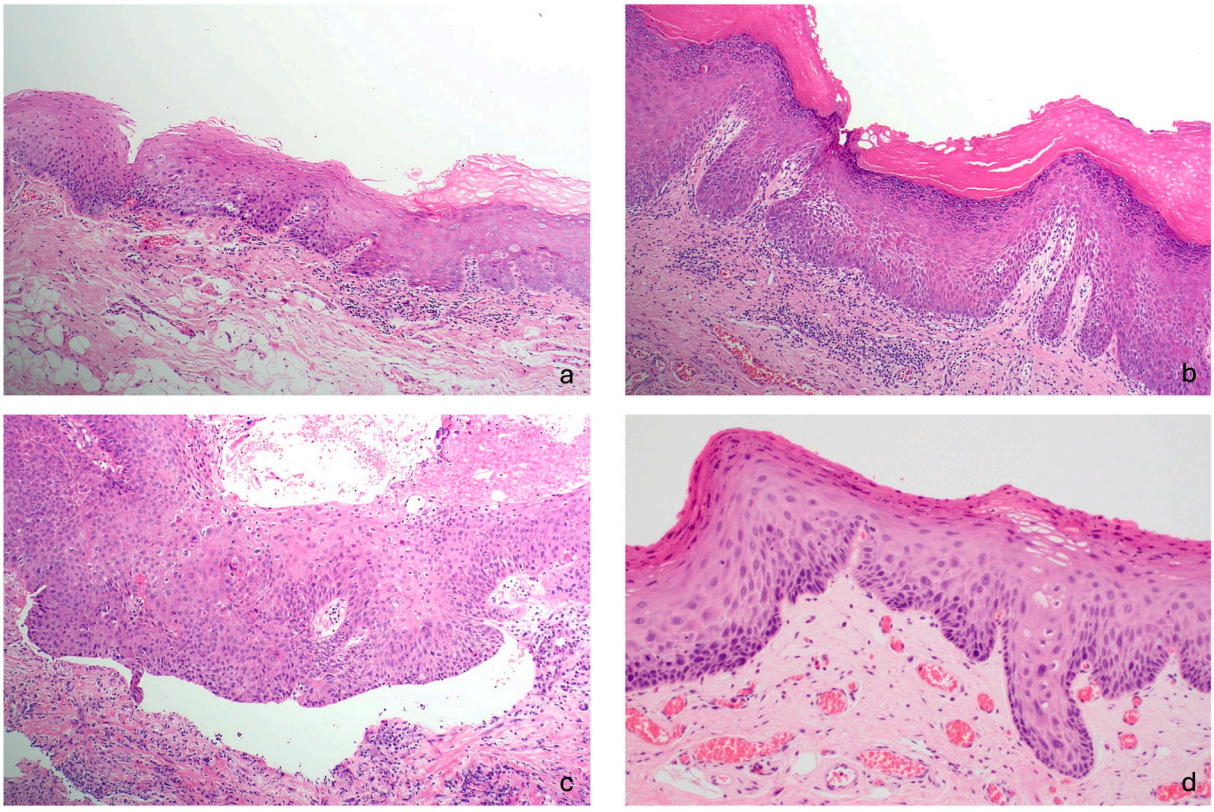

Fig. 2. Grades of OED. (a) H&E x 100 demonstrating low grade squamous dysplasia, with atypia in the lower third. (b) H&E x 100 demonstrating moderate grade dysplasia and keratosis. (c) H&E x 100 demonstrating high grade dysplasia in an area of ulceration and inflammation, the biopsy is disrupted. (d) H&E x 100 demonstrating high grade dysplasia with severe cytological atypia limited to the basal third of the squamous epithelium, sufficient to warrant a diagnosis of high grade.

Careful attention to the diagnostic features outlined above and interpretation in the clinical context can be helpful [14]. However, in poorly oriented, small, superficial, inflamed and crushed biopsy fragments, diagnosis is often challenging. This can be further compounded in post treatment biopsies. In general, the edge of dysplasia shows an abrupt transition, in contrast to reactive atypia which usually merges imperceptibly, and a biopsy taken at the junction of clinical normal and abnormal mucosa can be helpful. Clues to pseudoepitheliomatous hyperplasia include exuberant down-growing proliferation of epithelium into the submucosa, with only occasional dyskeratotic cells, and relatively uniform nuclei with smooth nuclear membranes. In general, reactive change is uniform without nuclear membrane irregularities. Also, the cytologic atypia associated with reactive changes generally does not extend along minor salivary gland ducts. Often the distinction between reactive atypia and low-grade dysplasia is the most challenging, however this may not be clinically significant as treatment most often involves careful clinical follow up.

Immunohistochemistry (IHC) can support the assessment of OED [15]. Commonly used markers include Ki-67 for proliferative activity, p53 for aberrant tumour-suppressor protein accumulation, and p16 for cell-cycle dysregulation. These are proposed to help distinguish reactive atypia from true dysplasia, clarify borderline cases, and improve diagnostic confidence when architectural or cytological features are subtle (Table 2) [16].

However, IHC has notable limitations. No single marker is specific or sensitive enough to diagnose or grade OED reliably, and results often show overlap between dysplastic and nondysplastic lesions [17]. Interpretation can vary between observers, and staining patterns may be influenced by technical factors. IHC also increases cost and processing time. Consequently, IHC is considered an adjunct, not a replacement, for conventional histopathological evaluation and in practice has limited use. Artificial intelligence (AI) based tools are a promising future step towards enhancing the accuracy and objectivity of assessing and grading OED and are currently under evaluation but not yet in routine practice [18,19].

Morphologic evaluation is key in the assessment of OED in the oral cavity with limited practical utility of IHC. In contrast, p16 IHC plays an important role in the diagnoses of oropharyngeal SCC (OPSCC). However, several factors influence the evaluation of p16 IHC in OPSCC as outlined below.

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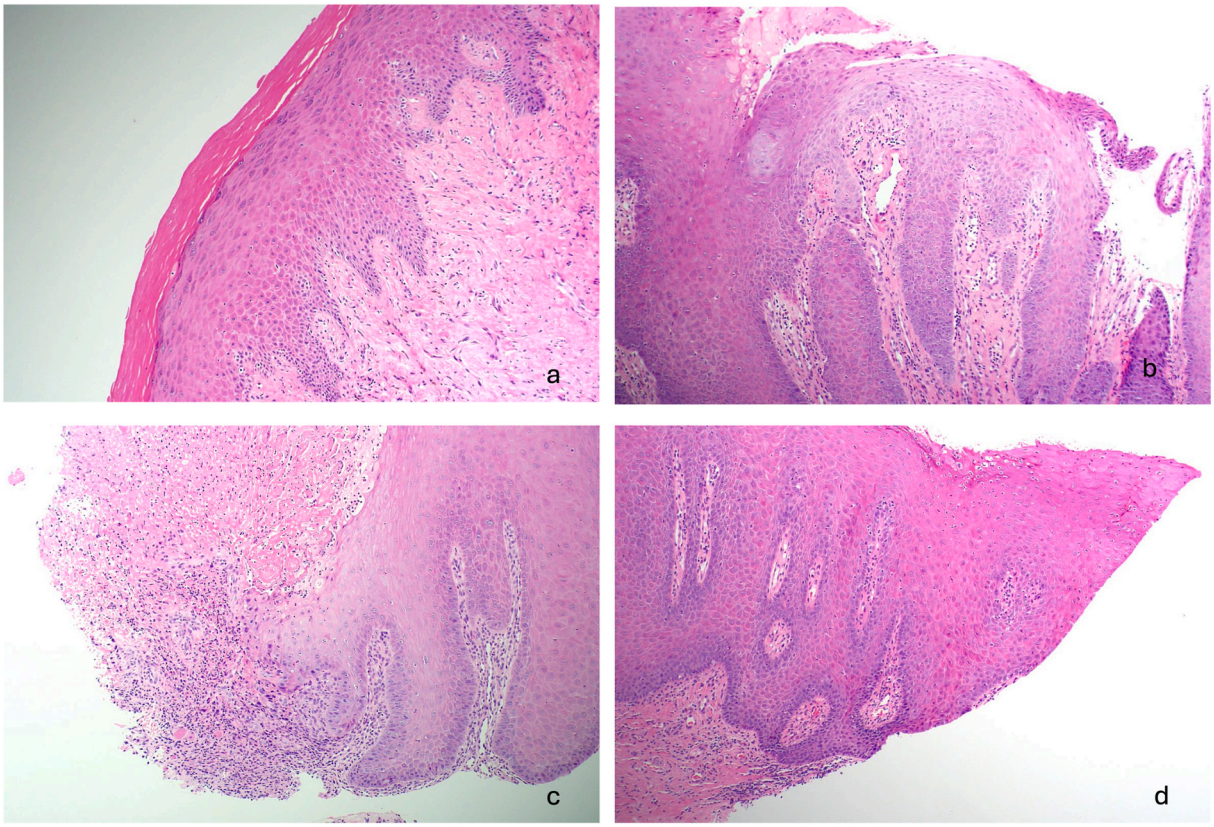

Fig. 3. Mimics of OED. (a) H&E x 100 clinical leukoplakia, biopsy shows keratosis but no dysplasia. (b) H&E x 100 reactive squamous hyperplasia adjacent to an ulcer. (c) H&E x 100 reactive atypia at the edge of an ulcer. (d) H&E x 100 lichenoid inflammation and keratosis with Candida.

Table 2
Immunohistochemical Expression of p53, and Ki-67 in normal oral mucosa and OED.

	p53 Expression	Interpretation (p53)	Ki-67 Expression	Interpretation (Ki-67)
Normal Oral Squamous Mucosa	Weak to absent staining; if present, confined to **basal/parabasal** layers	Indicates normal, low p53 turnover and absence of mutation-associated accumulation	Ki-67 positivity limited to **basal layer**	Reflects normal proliferative compartment
Dysplastic Oral Squamous Mucosa (mild to severe)	Increased p53 staining; can be **strong and extend into suprabasal layers**; in severe dysplasia may show diffuse nuclear accumulation	Suggests increased p53 activity or mutation-related stabilization	Increased Ki-67 staining with **expansion into suprabasal layers**; intensity and distribution increase with dysplasia severity	Indicates proliferation beyond basal layer, correlating with degree of dysplasia

4. HPV associated SCC of the head and neck

The link between high-risk human papillomavirus (HPV) infection and OPSCC is well established along with its rising incidence in North America, Northern Europe and Australia [20–22]. HPV-OPSCC often presents with nodal metastases as primary lesions can be small or clinically and radiologically occult or never found [23]. Patients with HPV-OPSCC also have better outcomes compared to stage-matched HPV-negative oral SCC [24,25]. While management of HPV-OPSCC is similar to HPV-negative OPSCC outside of clinical trials, there is emerging evidence that adjuvant treatment may be de-escalated in HPV-OPSCC [25–28].

The accurate and consistent determination of HPV status in OPSCC is therefore crucial for prognosis, therapeutic stratification, and clinical trial enrolment. Available tests to determine HPV status include p16 IHC, which serves as a surrogate marker of genomic incorporation of high-risk HPV (HR-HPV) (Fig. 4). Commonly used p16 clones include E6HV, JC8, G175-405 and 6H12; while most of the literature is based on the E6HV clone, comparative studies have found similar performance between different clones [29,30]. IHC clones should be validated in-house and appropriate external control tissue (known p16-positive tumour tissue to serve as a positive control and normal tonsillar tissue to serve as a negative control (Supp Fig. 5a–c) should be used. The threshold for positivity is moderate to strong nuclear and cytoplasmic staining in 70 % of tumour cells (Fig. 4c, Supp Fig. 5d) [31]. In addition, there are HPV-specific tests including HR-HPV RNA *in situ hybridization* (ISH) and HPV polymerase chain reaction (PCR).

The key principles in HPV testing in head and neck carcinomas can be summarized in the following key points.

A. Who should be tested?
   Only SCCs in sites with a meaningful association with HPV should undergo testing
   - *Universally undergo HPV testing by p16 IHC:*
     - SCCs of the oropharynx (palatine tonsil, lingual tonsil/base of tongue, uvula, soft palate and oropharyngeal walls)
     - Cervical lymph nodes with metastatic squamous cell carcinoma of unknown primary origin.
   - *Not undergo testing:*
     - Non-squamous carcinomas

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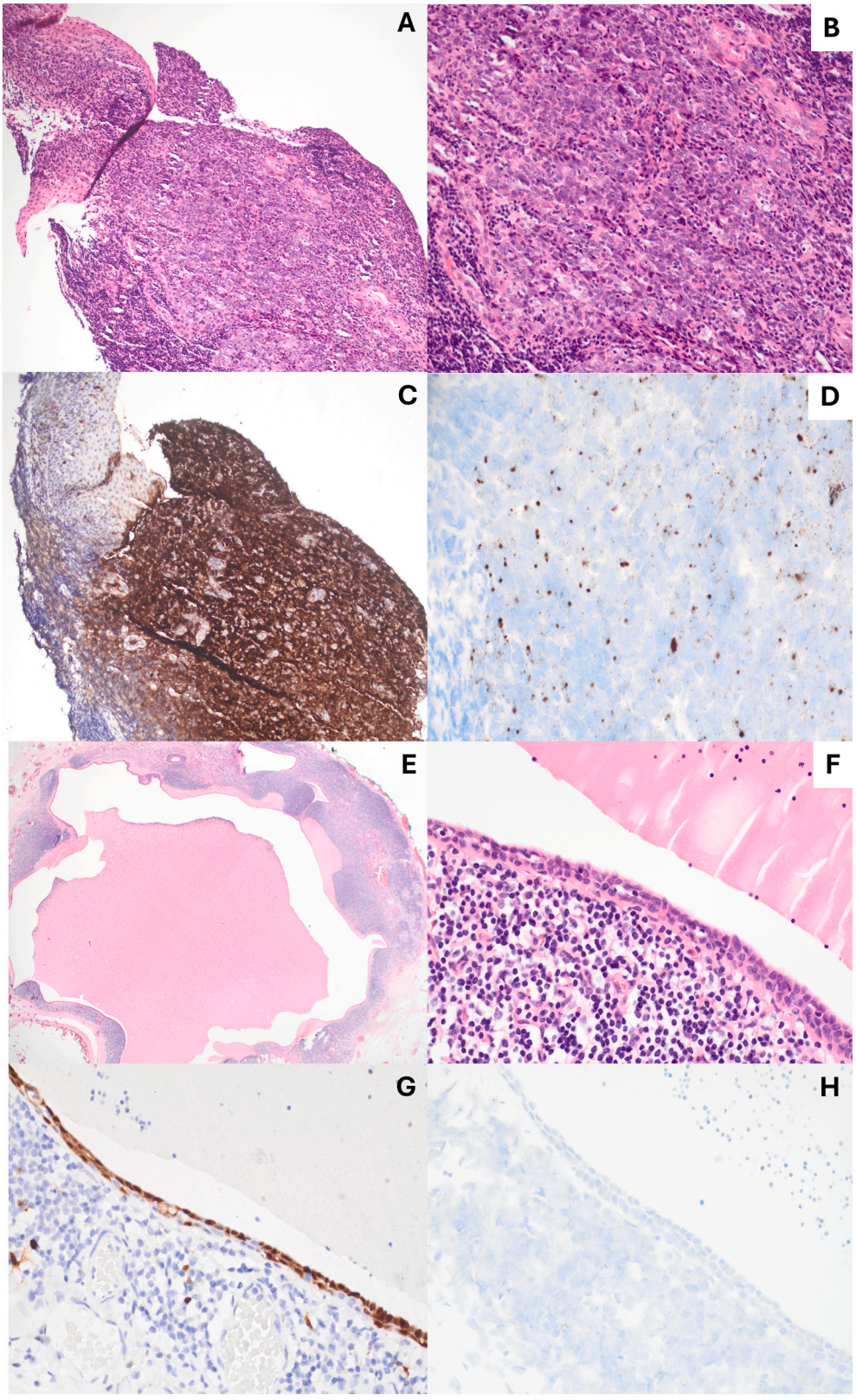

Fig. 4. Case examples of p16 IHC and HPV ISH. (a) H&E x 40. Tonsillar biopsy showing loss of crypt and follicular architecture. (b) H&E x 200. At higher magnification, there are sheets of cells with minimal cytoplasm and mildly enlarged hyperchromatic nuclei. Mitoses are present. Keratinisation is not seen. (c) Positive p16 IHC showing strong nuclear and cytoplasmic staining in >70 % of tumour cells. (d) Positive HR-HPV RNA-ISH with positive granular and punctate staining in tumour cell nuclei. (e and f) H&E of a cystic squamous intranodal lesion. (g) Equivocal p16 IHC showing moderate staining in about 60 % of epithelial cells. (h) Negative HR-HPV RNA-ISH.

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- Carcinomas in non-oropharyngeal sites (including oral cavity, hypopharynx, larynx, nasopharynx and sinonasal tract) [32–34].
- Special considerations:
  - All EBV negative nasopharyngeal carcinomas can be tested with p16. Correlation with radiologic findings to ascertain the extent of the lesion is also useful in these instances.
  - Sinonasal tract SCC should undergo testing. A subset of tumours are associated with high-risk HPV and emerging data appears to show favourable prognosis [31].
  - p16 testing helps in identification of HPV related multiphenotypic sinonasal carcinoma.

B. How should these be tested?
- *p16 IHC*
  - preferred primary test for HPV testing but is not sufficient in all situations.
- *HPV-specific testing is recommended in addition to p16 IHC in:*
  - OPSCC cases with equivocal p16 staining (strong staining in 50–70 % of tumour cells or weak diffuse/extensive staining), as OPSCCs with discordant p16 and HPV-specific test results show worse clinical behaviour than conventional HPV-associated OPSCC [35,36].
  - Geographic regions with low prevalence of HPV-associated OPSCC;
  - Keratinising OPSCC;
  - Large tumours involving other sites as well as the oropharynx;
  - Soft palate, uvular sites (i.e. non-tonsillar or base of tongue) cancers;
  - Metastatic p16 positive cervical lymph node SCC of unknown primary to help confirm a clinically occult oropharyngeal primary site;
  - P16-positive sinonasal SCC;
  - Cytology, cell block specimens, due to difficulty establishing reliable thresholds for p16-positivity in relatively low cellularity specimens [34,37].

C. The preferred HPV-specific testing methods are RNA-ISH or DNA PCR.

For optimal coverage of high-risk HPV types, the College of American Pathologists recommends that ISH assays include HPV types 16, 18, 26, 33, 35 and 58 [31].
- *HR-HPV RNA ISH testing is ideal:*
  - Detects transcriptionally active high-risk HPV (E6/E7 mRNA) and allows direct visualization of RNA in tumour cells.
  - HPV RNA-ISH is considered positive (Fig. 4d) when any of the tumour cells show positive dot-like nuclear staining [34,38].
- *HR-HPV DNA ISH testing is not recommended:*
  - Inferior specificity and sensitivity compared to RNA-ISH [37].
- *HPV DNA PCR:*
  - Highly sensitive but not entirely specific as it also detects biologically irrelevant HPV and must be interpreted in conjunction with p16 IHC.

D. Important diagnostic notes and pitfalls for OPSCC:
- *"Carcinoma in situ" terminology should not be used in OPSCC of tonsillar epithelium.*
  - The reticulated epithelium of the tonsils and the base of tongue lack a continuous basement membrane to allow interaction with inflammatory cells as part of the immune defence system [39].
  - OPSCC can metastasise when limited to surface or crypt mucosa.
- *OPSCC should not be graded or designated as 'poorly differentiated'*
  - Conventional tumour grading does not predict outcomes in HPV-associated OPSCC [40].
- *p16 positivity alone is not diagnostic of OPSCC:*
  - Seen in >30 % of cutaneous HNSCC [41],
  - Seen in other malignancies including, NUT carcinoma [42], INI1 or SMARC deficient carcinomas [43], melanoma, lymphoma, small cell carcinoma that may have morphologic overlap with OPSCC, particularly on cytology.
  - A squamous IHC marker such as p40 should always be performed if there is doubt as to the tumour lineage.
  - IHC for NUT, INI1, SMARC should also be considered.
  - HPV-specific testing is recommended on p16-positive nodal metastases.
  - HPV-specific testing is negative in inflamed branchial cleft cysts (Fig. 4e-h).

5. Conclusion

SCC of the Head and Neck region is commonly encountered by Anatomical Pathologists, as diagnostic biopsies where histological features are key to correct diagnosis as in OED, in circumstances where appropriate use of biomarkers is necessary to guide treatment, as in HPV related OPSCC, and in larger and often complex resection specimens where accurate orientation and block selection is critical to determine the need for further treatment. HPV status is important for prognostic and potentially for treatment purposes in OPSCC and sinonasal SCC. HPV testing should only routinely be performed in these specific locations. p16 IHC is the mainstay of testing with additive benefit from HPV-specific (ideally RNA ISH) testing in the circumstances outlined above. We have highlighted some of the nuances, features, and pitfalls, in evaluating these specimens.

CRediT authorship contribution statement

Jasmine Siaw: Writing – original draft, Writing – review & editing. **Timothy Fielder:** Writing – original draft, Writing – review & editing. **Sebastian Senff:** Writing – original draft, Writing – review & editing. **Carsten E. Palme:** Writing – review & editing. **Jonathan R. Clark:** Writing – review & editing. **Caroline L. Cooper:** Supervision, Writing – original draft, Writing – review & editing. **Ruta Gupta:** Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors confirm that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humpath.2025.106030.

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