Pitfalls and procedures in the histopathological diagnosis of oral and oropharyngeal squamous cell carcinoma and a review of the role of pathology in prognosis

Julia Anne Woolgar, Asterios Triantafyllou

Oral Pathology, School of Dental Sciences and Dental Hospital, University of Liverpool, Pembroke Place, Liverpool L3 5PS, United Kingdom

Tel.: +44 151 706 5245 (JAW), +44 151 706 5243 (AT); fax: +44 151 706 5240
E-mail address: jaw@liverpool.ac.uk, A.Triantafyllou@liverpool.ac.uk
Summary Histopathological assessment of formalin-fixed biopsy tissue and surgical resection specimens remains the cornerstone of cancer diagnosis and pathological staging in routine clinical practice. In recent years, standard protocols for reporting head and neck cancer have been widely used and these have improved the general level of the pathological assessment. In this article, we look beyond the standard protocols and deal with potential difficulties and pitfalls in the assessment of incisional biopsy specimens, surgical resection specimens and neck dissections. We draw attention to possible shortcomings and issues requiring clarification. Emphasis is given to precise histopathological definitions, histopathological detection and differential diagnosis. The approach is a practical one - a consideration of common experiences and dilemmas faced by the reporting pathologist, and where possible, we offer guidance and practical tips. The article concludes with a brief consideration of the prognostic value of accurate histopathological staging.

KEYWORDS
Oral cancer;
Oropharyngeal cancer;
Squamous cell carcinoma;
Histopathology
1. Introduction

Although, molecular biology is being increasingly used in the investigation of oral and oropharyngeal squamous cell carcinoma (OSCC), histological assessment of surgically-removed formalin-fixed tissues remains the cornerstone of its diagnosis and pathological staging in routine clinical practice. In recent years, standardised pathological protocols and regular multidisciplinary team meetings (MDTs) have become routine and are essential for a high quality service. Nevertheless, problems may occur at all stages of the histopathological diagnosis and staging, from the processing of received tissues to the generation of the final report. They may result from inherent limitations of histopathological definitions, diagnosis and staging; inaccuracies in histological detection and histological misinterpretation. The present article concentrates on such problems. Our aims were: 1) to illustrate potential pitfalls by considering common experiences during the pathological assessment of the initial and subsequent diagnostic / incisional intra-oral biopsy specimens, surgical resection specimens (Fig. 1) and neck dissections (NDs) (Fig. 2); 2) to draw attention to possible shortcomings and issues needing clarification; and 3) to suggest, where possible, practical solutions, improvements and areas of further research. Finally, the impact of detailed, accurate pathological staging in prognosis prediction of OSCC is considered.

2. Problems related to incisional biopsies and surgical resections of primary OSCCs

Questions and potential problems are expected to arise when the pathologist assesses incisional biopsies and surgical resections of the primary tumour. Some of the questions and experiences overlap, while others merit only brief consideration, and hence, some of the situations can be grouped and addressed in conjunction. Table 1 summarises the groupings and indexes the topics which we discuss.

2.1 Proliferative squamous epithelial dysplasia

Similarly to actinic cutaneous keratosis, we tend to designate a dysplastic lesion as ‘proliferative’ when increased epithelial thickness is present. The late life history of such lesions is uncertain.
2.2 Histological subtypes of OSCC

In addition to the conventional and instantly recognised OSCC, several subtypes presenting varying diagnostic challenges have been recognised. While some are addressed here it was deemed appropriate to discuss the remainder in other sections (see Table 1). It is not our intention to repeat descriptions in standard references and emphasis is given to personal approaches, experience and observations.

The OSCC subtypes often occur alone (‘pure’ forms). However, ‘hybrid’ OSCCs, combining varying ratios of more than one subtypes, are now being increasingly recognised and are attributable to proliferation of individual clones expressing different phenotypes. Cervical node metastases of hybrid OSCCs may consist wholly or predominantly of one subtype.

It has been repeatedly stated that the acantholytic SCC could be misdiagnosed as adenocarcinoma, adenosquamous carcinoma, and mucoepidermoid carcinoma. With awareness and increased experience the prompt recognition of the subtype is not difficult, particularly when it is considered that the acantholytic changes, which result in the formation of pseudolumens / pseudoglandular spaces (Fig. 3), are usually not extensive and asymmetrically distributed, preferentially close to the advancing front of the tumour. The presence of malignant acantholysis within surface dysplastic epithelium and experience with cutaneous SCCs wherein the acantholytic subtype is more often seen would be of further help. The recognition can be confirmed by special staining for mucosubstances, eg. Alcian Blue (AB) pH 2.5 followed by periodic-acid/Schiff (PAS), as these are absent from pseudolumens. Malignant acantholysis usually affects the central part of advancing cellular aggregates and a ‘basal’ layer is preserved (Fig. 3). We have, however, observed tumours where the latter becomes focally affected and dyscohesive. This results in collections of malignant keratinocytes occurring as individual units subadjacent to the advancing front, which can be highlighted by immunohistochemistry for cytokeratins (using a panel of pan-keratin antibodies) (Fig. 4). We do not report these as acantholytic SCCs, but as conventional SCCs with dyscohesive foci at the advancing front. It is not known whether this phenomenon is of prognostic significance, but see section 2.3.

Considerable difficulties could arise when the malignant acantholysis affects most of the tumour and the histology becomes that of individual cells of variable / ‘bizarre’ phenotypes, which are extensively insinuating between normal tissue elements rather than the cellular aggregates with pseudoluminal arrangements seen in typical acantholytic SCC. Increased index of suspicion and immunohistochemistry
for cytokeratins would overcome the difficulties. We are characterising these as non-cohesive SCCs, but we are unable to comment on prognostic significance, because they are rare (see section 2.3).

In the context of non-cohesive SCCs, we have to consider the pseudovascular SCC that can be mistaken for angiosarcoma and the giant cell carcinoma of the mouth. Immunohistochemistry for cytokeratins and endothelial markers would be helpful in recognising the former, whereas the very rare giant cell carcinoma may show multinuclear cells resembling osteoclasts or pleomorphic giant cells. When this presents in the mouth, it is important to exclude a metastasis from, for example, thyroid or lung, the usual sites of giant cell carcinoma.

Aptly named, the basaloid SCC (Fig. 5) is characterised by ‘basaloid’ cell phenotypes and variable peripheral palisading. It can bear a resemblance to ameloblastoma, which could cause confusion for tumours centred on the alveolar processes, and occurs either as pure or hybrid forms (Figs. 5, 6). This could be a confounding factor in studies attempting to ascribe different grades to particular subtypes and it is conventionally accepted that basaloid SCCs are of a worse prognosis (see section 4.1.8).

The diagnostic challenges presented by spindle cell carcinoma (Fig. 7) are well known and, although mandatory, immunohistochemistry is not consistent. Cytokeratin immunoreactivity is often focal or variable (40-85%) and it is essential to use a panel of antibodies. The need for careful search for a surface origin, epithelial dysplasia or foci with discernible squamous / squamoid phenotypes has been emphasised, but an incisional biopsy could be lacking such features and a high index of suspicion to prompt further investigation is a sine qua non for mistakes to be avoided. Myofibroblastic reactions (Fig. 8) and the ‘bizarre’ stromal cells often seen in florid granulation tissue, occasionally following previous biopsy, or at sites following radiotherapy such as brachytherapy, have also been considered as possible diagnoses when biopsies show ‘suspicious spindle cells’. We feel, however, that the recognition of these cells is less difficult than that of malignant keratinocytes showing spindled or ovoid phenotypes and occurring as individual units. Myofibroblasts often have discernible borders, eosinophilic cytoplasm, fascicular arrangement, low mitotic activity and, if any doubt, immunoreactivity for smooth muscle actin (SMA) (Fig. 7b), whereas the ‘bizarre’ stromal cells, in comparison with malignant keratinocytes, are usually elongated and lack rounded / ‘epithelioid’ phenotypes (Fig. 7b).

Intra-oral carcinomas may show a variable component exhibiting features similar to the nasopharyngeal non-keratinising carcinoma with a lympho-
plasmacytic inflammatory infiltrate. When the latter is brisk, the designation ‘lymphoepithelial carcinoma’ may be preferred. The histology is distinctive and comprises sheets and cellular masses composed of cells with ill-perceived borders (resulting in the characteristic ‘syncytial’ arrangement), and vesicular nuclei with obvious nucleoli (Fig. 9).

Apart from the nasal cavity and paranasal sinuses, carcinomas showing a transitional phenotype may arise as primaries in oral / oropharyngeal sites, eg. the base of tongue (Fig. 10a) and tonsil. Keratinisation is absent or minimal (occasional small and poorly-formed keratin pearls or squamous ‘eddies’) and a correct diagnosis should be prompted by the distinctive histology. This comprises multilayered cellular masses in characteristic garland- or ribbon-like arrangements (Fig. 10b), which are usually affected by central comedo-necrosis, surrounded by variably discernible basement membrane and composed of mitotically active non-keratinising cells that, peripherally, may have their long axis oriented vertical to the underlying basement membrane (Fig. 10c). Pure or hybrid forms are seen (Fig. 11).

Finally, the rare pigmented intra-oral carcinomas are histopathological curiosities rather than of clinical significance. They correspond to conventional SCCs that have been ‘colonised’ by dendritic melanocytes synthesising and transferring melanin to adjacent malignant keratinocytes (Fig. 12). The phenomenon is similar to that seen in basal cell carcinomas, adnexal tumours and Bednar’s tumour.

Although many subtypes of SCC are considered rare or very rare in the mouth and oropharynx, we believe that they will become increasingly recognised with centralisation of cancer services and sharing of cases between oral and Ear Nose and Throat (ENT) pathologists. Although currently sub-typing may be considered largely ‘academic’, it is essential that it is accurate and well documented so that information on management and prognosis can be shared (see section 5.1.8).

2.3 Grading systems of conventional OSCC, growth patterns and measurements

The histopathological grading of conventional OSCC as well- (pG1), moderately- (pG2) or poorly differentiated (pG3), enjoys popularity because it is simple. The degree of ‘differentiation’ (resemblance to normal oral / oropharyngeal surface epithelium) is subjectively assessed and in terms of keratinisation, cellular pleomorphism, mitotic activity and nuclear aberrations. Application of the system, which evolved from Broders' original classification, indicates that > 50% of oral and > 90% of oropharyngeal SCCs are moderately differentiated. Increased
discrimination is, however, desirable and the World Health Organisation (WHO) is now recommending that grading by differentiation is supplemented by assessment of the pattern of invasion. Hence, it is important that an incisional biopsy is of sufficient size and depth to include part of the advancing front of the tumour. Ideally, the deep front should be included, but if not, as in large tumours, the peripheral (lateral) front is often sufficiently representative to allow provisional assessment.

Four patterns of invasion have been described depending on the degree of keratinocyte dyscohesion (decreased intercellular cohesion / loosening of keratinocytes). Different regions of the same tumour often show different patterns of invasion, but the most aggressive pattern is recorded. The type-I pattern corresponds to tumours where the constituent cells retain cohesion and are arranged as broad, bands and columns, occasionally bulbous, which tend to penetrate at more or less the same level; this results in a rather defined, although asymmetrical, ‘pushing’ or ‘expansive’ advancing front. The type-II pattern is characterised by the arrangement of malignant keratinocytes as islands or sheets, rounded, irregular or spiky, which are asymmetrically distributed and penetrate at different levels; this results in an irregular tumour silhouette. The type-III pattern resembles type II as regards the tumour silhouette, but dyscohesion, in the form of tiny islands and cords that bud off from the larger islands and sheets, extensively affects the advancing front here (Fig. 13). The type-IV pattern corresponds to the archetypal non-cohesive tumours wherein the irregular advancing front shows malignant keratinocytes infiltrating as individual units because of loss of intercellular cohesion (Figs. 14-16). Except for irregularity, the advancing front becomes variably defined in types III and IV and ‘satellite elements’ ahead of it are often seen. We wonder, however, whether these types should be further sub-typed in terms of the extent of dyscohesion / loss of cohesion. For example, when these processes are mainly affecting the advancing front, the tumours should be categorised as type IIIa and IVa respectively. On the other hand when the processes affect most of, or the entire tumour mass, tumours becoming thus ill-defined and composed of tiny islets and spidery cords or single cells, the grading should be IIIb and IVb. In this vein, the conventional SCCs with dyscohesive foci at the advancing front and the non-cohesive SCCs, which were noted in section 2.2, could be included in the types IVa and IVb respectively. Whether such sub-typing is merely academic or has a prognostic significance remains to be seen.

Multifactorial grading systems, such as that suggested by Anneroth and Hansen and modified by Bryne et al., select the most ‘worrisome’ / ‘unfavourable’ region of the advancing front for assessment, consider features of both tumour cells and the tumour-host interface, and score each feature on a scale of 1 to 4, to give a
total malignancy score ranging from 4 to 24 or 28. Keratinisation, nuclear aberrations (Fig. 13) and mitoses are considered in the assessment of the tumour cells, whereas the tumour-host interface is assessed in terms of the pattern and level of invasion, and the inflammatory-cell reaction. Multifactorial systems are preferable to grading by differentiation, but discrimination is still not ideal. Application of such a system in a series of conventional T2 SCCs of tongue resulted in total scores clustered around a median of 20.5 for tumours with metastasis and 16.5 for tumours without metastasis.\textsuperscript{18}

In comparison with the system introduced by Clark \textit{et al.}\textsuperscript{19} for the histopathological grading of cutaneous malignant melanoma, the conventional OSCC multifactorial systems do not recognise the superficial vascular plexus as a separate level of invasion and do not distinguish between ‘brisk infiltrative’ and ‘brisk non-infiltrative’ patterns of the inflammatory reaction. We have observed both patterns in our material (Figs. 13, 17) and occasionally noted an association of brisk and infiltrative inflammation with extensive / massive apoptosis of tumour cells (Fig. 17). The significance of infiltrative and non-infiltrative inflammation, particular inflammatory-cell types, like eosinophils (Fig. 18), and extent of apoptosis in the histopathological grading of OSCC might be worth pursuing.

Except for the pattern and cellular composition of the inflammatory reaction, the presence or absence of tumour-induced (desmoplastic, ‘reactive’ according to Reed\textsuperscript{20}) stroma has not been considered in the conventional OSCC grading systems. Desmoplastic stroma can be seen in both the primary tumour and metastases and comprises an increased number of stromal cells, for example fibroblasts or myofibroblasts, which are often arranged parallel to the contour of tumour-cell aggregates and are variably mixed with fibrillary collagen or glycosaminoglycans, and often shows little or no inflammation (Figs. 19, 20). Although the formation of tumour-induced stroma (‘desmoplasia’) is pivotal in tumourigenesis and metastasis\textsuperscript{21} it has received little attention in OSCCs.\textsuperscript{22}

Regardless of possible shortcomings we recommend the routine use of multifactorial grading while reporting on conventional OSCCs and we also record desmoplasia, lymphovascular invasion (see section 2.5) and peri- / endoneural invasion (Fig. 21). Lymphovascular and / or neural invasion can be seen in stroma between tumour-cell aggregates and adjacent to or ahead of the main invasive front (see Fig. 9 in Woolgar and Triantafyllou\textsuperscript{23}). However, it is the latter that should be meticulously sought because of its prognostic significance (see section 5.1.4) and inclusion in measurements of the width of the surgical margin (see below).
When assessing the size of an OSCC, the macroscopic appearances and growth pattern need to be considered. Although measurement of tumour volume is the most accurate indicator of the tumour size, the UICC staging procedure started out as a clinical assessment of the extent of disease, and hence, the surface greatest diameter was the obvious indicator of tumour size. The pathological TNM stage uses exactly the same criteria and categories as clinical staging. In assessing the surface diameter histologically, only invasive carcinoma should be included. In addition to measuring the surface diameter and given the importance of the Breslow thickness in the prognosis of cutaneous malignant melanoma, it was gradually appreciated that the tumour thickness of an OSCC should be also recorded. In OSCCs, however, the reconstructed tumour thickness (Fig. 22), which compensates for an exophytic growth component or ulceration and tissue destruction, is considered a better prognosticator than the actual (Breslow) tumour thickness. (Note that in the case of endophytic tumours, the reconstructed thickness coincides with the actual thickness and that ‘depth of invasion’ is an alternative term for reconstructed thickness.) Care should be taken to ensure that any tumour cells (including lymphovascular emboli and neural spread) ahead of the main invasive front are included. The Vernier of the stage of the microscope or a graticule can be used for the measurements.

Occasionally, OSSCs have no obvious invasive front. They consist of thin spidery cords only detectable histologically or correspond to the dyscohesive tumours described in section 2.2. Measuring these tumours can cause practical difficulties as do large tumours, tumours consisting of widely distributed multiple blobs or nodules (Fig. 23) and tumours covering more than one anatomical region or involving anatomical curvatures. In such cases, the final recorded measurements should be based on the macroscopic measurement supplemented by microscopic assessment of immunohistochemical preparations and tissue blocks, which would allow highlighting or reconstruction (see section 3.2) of the tumour silhouette respectively.

Except for surface diameter and reconstructed thickness the distance of tumour to nearest deep and mucosal resection margins should be measured (see sections 3.3 and 5.1.9, and Woolgar and Triantafyllou).

2.4 Deep (unequivocal) vs. superficial (early / minimal) invasion

The diagnosis of OSCC is usually straightforward when there is unequivocal invasion together with cytological atypia. Detecting islands, cords and other
arrangements of squamoid epithelial cells, which are not salivary or odontogenic (see sections 2.6, 2.7 and 2.9), in submucosal tissues such as skeletal muscle, fat or salivary lobules (Fig. 24), or in bone marrow spaces qualifies as unequivocal invasion. This criterion is particularly helpful: 1) when epithelial islands or cords are detected in submucosa below merely dysplastic surface epithelium (Fig. 25); and 2) in the recognition of carcinoma *cuniculatum*. The latter is characterised by tortuous centrally-keratinising columns of proliferated squamous epithelium with minimal cytologic atypia, which are meandering between skeletal muscle fibres or residual bone trabeculae (Fig. 26). When unequivocal invasion is established, an origin with dysplastic surface epithelium confirms the primary nature of the lesion and this should be sought by step and serial sectioning if not immediately apparent. However, this may not be possible in incisional biopsies and extensively ulcerated tumours and re-biopsy could be an option. Additional problems may arise due to insufficient sampling (either at the macroscopic assessment and trimming stage or insufficient sampling of the block) or poor orientation of the tissue within the block.

When unequivocal / submucosal invasion cannot be established, the question of ‘early SCC’ arises. The major problem here is deciding whether or not frank invasion of the lamina propria is present. Difficulties increase on incisional biopsies showing proliferative, moderate or severe squamous epithelial dysplasia (see section 2.1). ‘Islands’ of epithelium within the lamina propria should be regarded as suspicious, but it is important to exclude whether they represent sectioned rete processes, especially if these are long and bulbous as in some reactive conditions, or odontogenic residues (Fig. 27), but see sections 2.6 and 2.7. Examination of serial sections and assessment of keratinocyctic atypia would be helpful. The integrity of the basement membrane could also be assessed in PAS-stained sections (Fig. 28). Caution should be exerted when there is subepithelial brisk and infiltrative inflammatory reaction (Fig. 17) as such reaction could effect disruption of the basement membrane and in turn compromise assessment.

When we decide early invasion is present, we categorise this as microinvasive SCC if tumour is confined to the papillary lamina propria as defined by the depth of the rete processes, and superficially invasive if the tumour remains confined to the reticular (deep) lamina propria and not yet involving the submucosal tissues mentioned above (Figs. 29, 30). (Note: the term superficially invasive cannot be applied to tumours centred on the alveolar processes of the jaws or gingival mucosa due to the absence of a submucosa.) While the need for measuring the reconstructed tumour thickness (section 2.3) in cases of superficially invasive SCCs
is obvious, such measurement may be redundant in cases of microinvasive lesions. It seems, however, justified when the affected epithelium is of an increased thickness.

If we are uncertain whether or not microinvasion has occurred in a background of severe dysplasia, proliferative or not, we report the histology as lacking unequivocal evidence of invasion or progressing to microinvasive SCC. In any case and pending on clinical judgement, such lesions could be treated similarly to microinvasive SCC since an incisional biopsy may not contain the most advanced part of the lesion. Alternatively, re-biopsy could be considered.

It is a matter of dispute whether sialadenotropism (spreading of severe squamous epithelial dysplasia / carcinoma *in-situ* along the lining of collecting salivary ducts) (Figs. 31, 32) should be regarded as invasion,^{29,30} which is similar to the prognostic uncertainty regarding the extension of malignant melanoma along cutaneous adnexae. That the spreading is usually confined within the ductal lining, breaching of the ductal basement membrane and penetration of adjacent stroma being exceptional, contributes to the difficulty. Given the uncertainty, we recommend that the depth of its extent should be included in the histopathology report. On routine histology, ducts affected by sialadenotropism are usually distinct from frank infiltration of surface origin because, pending on the plane of sectioning, of the rounded shape and or linear arrangement of the ducts section profiles and of the variable discernment of constituents of the ductal lining (Figs. 32-34). Immunohistochemical contributions await exploration. Secondary involvement of major salivary ducts by OSCC should not be confused with sialadenotropism (Fig. 35).

### 2.5 Vascular invasion

Care is needed to distinguish retraction artefact from intravascular embolisation. Practically, vascular invasion occurs in thin-walled vessels (Fig. 36), whereas involvement of muscular vessels is rare (Figs. 37, 38). In accordance with breast pathologists,^{31} we identify vascular invasion only when tumour-cell aggregates are within clear spaces that are completely lined by endothelial cells (Fig. 36). Occasionally, microthrombi associated with tumour-cell aggregates (Fig. 39) could draw the attention of the pathologist to vascular invasion. It is usually difficult, and of little or no importance, trying to identify the involved thin-walled vessel as capillary or lymphatic. Special techniques (eg. staining for elastin) are of little use and the term lymphovascular invasion is used to convey this uncertainty.^{31}

### 2.6 Bone involvement
There are two problems. Firstly, in deciding whether or not the full thickness cortical plate is destroyed by tumour and hence leading to pathological categorization pT4. It is important to remember that the superior, occlusal aspect of the edentulous alveolar process may show atrophy and defects due to physiological remodelling following extraction of teeth. It is necessary to judge the depth of bone loss due to tumour in relation to the surrounding bone contour. This method is applicable to bone lesions of any shape including horizontal, vertical and saucer-like (Fig. 36). The second problem relates to unequivocal pT4 cases when the pattern of involvement should be recorded for prognostic purposes. Two patterns, erosive and infiltrative (Figs. 40, 41; also see Fig. 3 in Woolgar and Triantafyllou), have been described, but in practice many cases show both. The region of bone showing the most advanced involvement, identified by radiography of the resection specimen prior to macroscopic dissection, should be histologically examined since the infiltrative pattern, if present, is more likely therein. As in other pathological assessments (section 2.3), the occurrence of the more unfavourable category is recorded. Tumour-bone interface is shown in Fig. 42.

In the dentate jaw it is important to assess extension via periodontal ligament since we have seen tumour entering cancellous bone and marrow spaces via this route (Figs. 43, 44).

Odontogenic vestiges found in marrow spaces of cancellous bone (Fig. 45), should not be misdiagnosed as tumour.

2.7 Pseudocarcinomatous hyperplasia and ‘benign perineural infiltration’

It is documented that reactive conditions may give rise to florid rete hyperplasia with frequent mitotic figures, and on masticatory mucosa, deep keratinisation with well-formed keratin pearls - so called ‘pseudocarcinomatous (pseudoepitheliomatous) hyperplasia’, and this could be misdiagnosed as well-differentiated SCC particularly in incisional biopsies. An awareness of such conditions (Table 2) is important and histopathological diagnostic algorithms have been provided by dermatopathologists.

Of the conditions shown in Table 2, we would like to draw attention to the pseudocarcinomatous hyperplasia associated with transepithelial elimination. While dermatopathologists are appreciative of this process, oral and ENT pathologists may be less well so. We have repeatedly observed transepithelial elimination of small sequestra and fragments of cementum in intra-oral biopsies (Figs. 46, 47) and Odell
and Morgan\textsuperscript{38} have illustrated other calcified material similarly eliminated. Florid pseudocarcinomatous hyperplasia associated with transepithelial elimination of sequestra could account for old reports of SCC arising in a background of chronic osteomyelitis with draining fistula\textsuperscript{39} and for the ‘worrying features’ noted in consultation requests we have received and concerning biopsies from fractured jaws. Awareness of transepithelial elimination and the lack of cellular atypia and destruction of healthy bone usually enable a confident diagnosis.

The histopathological diagnostic algorithms mentioned above are applicable in the mouth. Abenoza and Ackerman\textsuperscript{36} noted that hyperplastic adnexal lining contributes to cutaneous pseudocarcinomatous hyperplasia. Similarly involved collecting salivary ducts account for the depth of the proliferation in some oral pseudocarcinomatous hyperplasias. These are also lacking cellular atypia and tumour-induced stroma / desmoplasia (section 2.3), do not destroy host tissue and their epithelial arrangements are often elongated and angular contrasting with the often ‘rounded’, irregular tumour-cell aggregates of OSCC. The latter should not be, however, confused with lobular arrangements of atrophic, metaplastic salivary parenchyma, which characterise late stages of necrotising sialometaplasia (Fig. 48) (see also section 2.9).

As regards keratoacanthoma (KA), we endorse an origin from proliferating infundibular epithelium\textsuperscript{40} and intra-oral examples could represent misdiagnosed carcinoma \textit{cuniculatum} (Fig. 26). Similarly to mammary hyperplasias,\textsuperscript{41} ‘benign perineural infiltration’ has been reported in some KAs\textsuperscript{42} but this needs to be re-examined in the light of a possible continuum between KA and some well-differentiated SCCs.\textsuperscript{2}

Except for KA, other examples of ‘benign perineural infiltration', which could be misdiagnosed as OSCC, have been reported. This seems unjustly over-emphasised. The clinical setting is not right, most of these examples occurring in the capsule of odontogenic cysts (Fig. 49) and in apical granulomas,\textsuperscript{43-46} whereas the epithelial parenchyma of Chievitz’s organ is situated between small nerves rather than infiltrating them (Fig. 50).\textsuperscript{47}

### 2.8 Lesions with a papillary exophytic component, proliferative verrucous leukoplakia (PVL) and squamoproliferative lesions

The precise histological categorisation of mucosal lesions that include an exophytic growth component is a difficult and often encountered experience. Terms such as ‘papillary’ and ‘ verrucous’ are commonly used, but indiscriminately or rarely defined,
which results in confusion. There are no universally accepted definitions or
differential diagnostic criteria. We use the term papillary for lesions showing true
papillae, i.e. exophytic finger-like projections or mammillations with a fibrovascular
core covered by proliferated surface epithelium, keratotic or not. We reserve the term
‘verrucous’ for lesions showing a keratotic exophytic surface composed of sharp or
blunt epithelial projections with keratin-filled invaginations (plugging), but without
obvious fibrovascular cores.

The term ‘papillary dysplasia’ or ‘atypical papillary hyperplasia’ seems
appropriate for papillary lesions with dysplastic features, wherein invasion is not
detected. When a focus of invasion is detected in a papillary lesion, we use the term
‘papillary SCC’ (Fig. 51). Lesions with a ‘verrucous’ surface may be verrucous
carcinoma or show the conventional invasive growth pattern. We refer to the latter as
‘SCC with an exoendophytic growth pattern’.

The histological features of verrucous carcinoma, e.g. verrucous surface (as
defined above) and ‘elephant feet’-like downgrowth seeming to compress the
underlying connective tissue and typically showing minimal cytological atypia, are
widely known, but the diagnosis eventually depends on identifying the
characteristic peripheral buttressing or shouldering (Fig. 52). The latter may be
lacking in incisional biopsies and difficulties arise. Similar difficulties are experienced
in incisional or excisional biopsies of clinically extensive ‘white’ lesions, which
histologically show: 1) a ‘verrucous’ surface (as defined above); 2) increased
epithelial thickness, evidence of proliferation with often peripheral mild atypia; and 3)
a front advancing at more or less the same level and expanding, but confined to, the
lamina propria. The first step in such instances is to exclude the presence of foci of
conventional SCC with an obviously invasive growth pattern, which may be small and
or multiple. If, after thorough sampling of the whole specimen, we become convinced
that such foci are absent, we usually characterise the histology described above as a
stage in the life history of PVL. We find PVL, envisaged as a continuum of lesions
simultaneously occurring with or metachronously evolving into verrucous and / or
conventional SCC, as a useful concept and we do not believe that sequential
biopsies showing evidence of progression are required for its diagnosis. Also, we still
use the term if basal cell hyperchromatism and other atypia are evident. Having
reached a diagnosis of a PVL stage, it is essential that the pathologist alerts the
clinician to the progressive nature of the lesion and recommend complete excision or
close follow-up and re-biopsy.

It now becomes convenient to introduce the concept of oral
squamoproliferative lesion. We reserve this term for those occasions when incisional
biopsies (often of gingival lesions) show: 1) proliferation of oral epithelium, usually exoendophytic and resulting in variable epithelial thickness; 2) either papillary or verrucous exophytic component; 3) smoothly contoured, though irregular / asymmetrical and not pushing, endophytic component that advances at different levels of, but still confined to, a variably expanded lamina propria; 4) no evidence of frank invasion; and 5) variable dysplasia of deep keratinocytic layers or not. When the latter is present, the term dysplastic squamoproliferative lesion can be used. It could be argued that distinction from proliferative squamous epithelial dysplasia becomes blurred and we support the cautious use of squamoproliferative lesion with or without dysplasia, as a diagnosis. It is a last resort stratagem conveying that we are uncertain whether the lesion is malignant. The clinician has to be alerted to the need for mandatory follow-up as for PVL. Infection with candida is not uncommon and increases difficulties.

Finally, the distinction between squamous cell papilloma, viral warts and lesions such as palatal papillomatosis and papillary dysplasia / papillary SCC should be straightforward since the former group should not exhibit atypia. Superficial biopsies can be problematic especially if the lesion is infected with candida and shows reactive nuclear changes. Consideration of the clinical history and features, such as size, is also important in preventing misdiagnoses. Features suggestive of a viral effect, such as koilocytic change or hypergranulosis are not useful as they may be seen in benign, pre-malignant and malignant epithelial proliferations.

**2.9 Adenosquamous carcinoma**

This histological subtype, except for interesting theoretical considerations (eg. origin from basal cells of surface epithelium that are capable of divergent differentiation, expression of different phenotypes rather than simultaneous malignant transformation of surface and glandular epithelium or collision tumour), is treated separately because of possible limitations in definition and difficulties in recognition.

Two components, SCC (usually not well-differentiated) and adenocarcinoma, which remain distinct and separate is the usual requisite for the histological diagnosis of adenosquamous carcinoma. While the SCC component is superficial and may be *in situ* rather than invasive, the glandular component is usually within deeper parts of the tumour and shows formation of true luminal structures that should not misinterpreted as resulting from malignant acantholysis52 (see section 2.2). The structures, although usually small or collapsed and not cystic or rigid, are identified after diligent search and contain epithelial mucins demonstrable on AB pH 2.5-PAS
staining. Similarly to hybrid OSCCs, cervical node metastases of adenosquamous carcinoma (present in around 75% of cases) often consist wholly or predominantly of one component.

Such definition of adenosquamous carcinoma implies that the deep component resembles a non-specific adenocarcinoma. We have, however, observed occasional, otherwise conventional, squamous cell carcinomas wherein deep components express non-keratinising cell phenotypes and architectural patterns (e.g. cribriform) and/or features (e.g. comedo-necrosis, collagenous spherulosis) seen in types of salivary carcinoma (e.g. salivary duct carcinoma) (Figs. 53, 54) prompting the question whether the definition of adenosquamous carcinoma should be expanded or appropriately modified to include them.

The presence of epithelial mucins in the lumens of adenosquamous carcinoma suggests that clones of tumour cells are able to produce and release mucosubstances. Often, such clones are represented in occasional cells found as individual units or small collections either in the lining of luminal spaces or within solid cellular aggregates. Synthetic and secretory processes in such cells are revealed by AB pH 2.5-PAS staining, but they may not be advanced enough to result in typical mucous phenotypes recognisable on routine histological staining. On the other hand, tumours showing histology of a conventional, usually pG2, SCC may contain individual or grouped cells with easily identified mucous or goblet-like appearance (Fig. 55). In these instances the pathologist has to decide whether such cells are normal tissue elements trapped within the growing tumour or innate neoplastic constituents. When there are grouped mucous cells in a lobular architecture, it is likely that they correspond to preserved minor salivary glandular lobules invaded by SCC (Fig. 56). When the mucous cells appear to be neoplastic, the histological differential diagnosis should include adenosquamous carcinoma as well as high-grade mucoepidermoid carcinoma and SCC with mucous metaplasia. (The latter is poorly-described in the literature, but is theoretically possible and would be distinguished from high-grade mucoepidermoid carcinoma by assessing surface epithelial vs. subepithelial origin, centre of growth.) Detection of dysplastic oral surface epithelium and deep luminal spaces tip the scales in the direction of adenosquamous carcinoma, but these features could be absent in incisional biopsies. While reporting such biopsies, we note the possibilities and emphasise the academic rather than clinical significance of the uncertainty as these malignant lesions are behaving and treated similarly. In contrast to high-grade mucoepidermoid carcinoma and popular belief, we believe that the more common low- or intermediate-grade mucoepidermoid carcinomas are unlikely to be confused with
adenosquamous carcinoma. Their asymmetrically distributed cystic and solid aggregates comprising variable mixtures of cells showing mucous, clear, squamoid and simple phenotypes, and lack of keratin pearls are characteristic and instantly recognised.

Malignant squamoproliferative lesions with neoplastic mucous or goblet cells should not be confused with the so-called clear-cell SCC. This clear-cell subtype has also been recognised in skin\textsuperscript{53} and refers to conventional SCCs wherein swathes and small groups of neoplastic keratinocytes rather than individual units show ‘empty’, vacuolated cytoplasm (Fig. 57). The ‘empty’ keratinocytes share a ‘clear’, pale phenotype with mucous or goblet cells and could simulate a classic signet-ring appearance (Fig. 57b), but they do not contain mucosubstances. Possibly, the ‘clear-cell’ phenotype reflects a fixation artefact resulting in multiple small cytoplasmic ‘vacuoles’,\textsuperscript{53} which eventually coalesce to form a single large vacuole that pushes the nucleus against the plasmalemma (Fig. 53c).

The significance of preservation of a lobular architecture in distinguishing normal tissue from neoplastic elements has been noted above. This together with the lack of cellular atypia, are important features in recognising necrotising sialometaplasia (see section 2.7 and Fig. 48).

2.10 Carcinoma within the connective / submucosal tissues without an obvious surface mucosal origin

A biopsy may occasionally show an epithelial tumour within the lamina propria or submucosa that does not have an obvious origin from the overlying surface epithelium. If the tumour is a SCC, the probable explanation is that the biopsy is from an area of intact mucosa adjacent to a primary SCC and deeper sections may reveal surface origin or squamous epithelial dysplasia. If not, we usually comment in the report on the lack of an obvious surface origin and state that the appearances are consistent with an intra-oral primary arising at an adjacent site. In patients with recurrent SCC, submucosal or dermal nodules may be due to direct spread, non-contiguous extension or vascular spread from a deep-seated focus of tumour. Metastasis to a facial lymph node should also be considered. The diagnosis of lymphatic metastases to the skin of the neck from deep-seated cervical node metastases is usually straightforward, particularly when the clinical history is known.

If the features of an oral submucosal epithelial tumour nodule are not typical of SCC, the differential diagnosis includes salivary and odontogenic neoplasms and a metastatic deposit. An awareness of these possibilities and good knowledge of the
histological features of the full range of salivary and odontogenic neoplasms\(^3\) should prevent serious errors. Immunohistochemistry may be helpful in categorising suspected metastatic disease.

### 2.11 Changes in intra-oral skin flaps

Since the 1980s, free tissue transfer using vascularised skin-flaps such as the radial forearm flap has been a routine part of the management and rehabilitation of OSCC patients.\(^{54}\) At least 5% of patients go on to develop cancer at the junction of the skin-flap and native oral mucosa.\(^{26}\) Over the years, we have seen increasing numbers of biopsies from regions of skin-flap remote from its junction with mucosa and the diagnosis has included adnexal tumour, seborrheic keratosis, hyperkeratotic plaque, chronic hyperplastic candidiasis (CHC), CHC with squamous dysplasia, squamous dysplasia, papillary dysplasia, Bowen’s disease (carcinoma \textit{in-situ}) and SCC. Diagnosis of CHC can be difficult since its features in skin may be unfamiliar to oral pathologists used to seeing mucosal CHC. Generally, in cutaneous CHC, epidermal hyperplasia is associated with marked compact hyperorthokeratosis; subcorneal microabscesses are sparse and candidal hyphae lie haphazardly in large clumps within the superficial layers of keratinocytes.\(^{55}\) This ‘cutaneous reaction pattern’ is not seen in all cases; some biopsies show features more akin to mucosal CHC. Both patterns may be seen in the same biopsy and interpretation of the findings may be compounded by the presence of cellular and architectural atypia. Caution is needed when grading dysplasia in skin-flaps since the abnormal pattern of keratinisation is often striking and may lead to an erroneously high-grade categorisation if the pathologist is experienced in grading mucosal rather than cutaneous squamous dysplasia. Some patients have further biopsies after antifungal therapy and generally these show orthokeratosis and minimal, if any, dysplasia suggesting that the dysplasia was ‘reactive’. The biopsies diagnosed as Bowen’s disease showed features very similar to Bowen’s disease of skin of unexposed sites including spread down along pilosebaceous orifices and sharply demarcated margins. We have insufficient cases with prolonged follow-up to understand the aetiology and natural history of skin-flap lesions. However, a small number have progressed to invasive SCC, one showing the acantholytic subtype. Some, but not all, patients with dysplastic or carcinomatous lesions of the skin-flap have concomitant dysplasia of the oral mucosa and the aetiology and pathogenesis of the skin-flap lesions may differ in individual cases. Clearly, more studies showing the histological appearances of intra-oral skin flaps as they adapt to the oral environment (‘mucosalisation’)\(^{56}\) and
in disease are needed. When faced with a difficult case, it may be necessary to write a descriptive report explaining the findings and uncertainties and recommending mandatory follow-up with re-biopsy if there is ongoing concern.

3. Pathological assessment of the intra-oral resection specimen

Intra-oral resection specimens (Fig. 1) range from solely mucosa and underlying soft tissues through to complex en bloc resections of several anatomical areas or even organs such as a glossectomy en bloc with mandibulectomy and NDs. Surgical pathological anatomy texts such as Slootweg and de Groot\textsuperscript{57} provide basic instructions on handling specimens from the various anatomical areas together with diagrams showing recommended planes and directions for cutting the specimen. Here, we focus on procedures influencing pathological staging and prognosis, and mention practical tips that may be helpful.

3.1 Presentation of the specimen

Distortion of resection specimens can lead to poor orientation and incomplete or inaccurate identification of surgical margins. Some distortion is inevitable as different oral tissues shrink by different amounts during fixation of specimens.\textsuperscript{58,59} Thin specimens should be supported on card or pinned or sutured on to polystyrene or cork sheets and it is helpful if even large resection specimens including those containing bone are laid out and pinned in the correct anatomical position. The surgical staff should provide a simple line drawing or photograph showing the limits of the resection and marking, by ink or sutures, any areas of the margins, which are of concern or special interest. Care should be taken by the pathologist when inking the surgical margins - both peripheral and deep, bearing in mind that the deep aspect of large resections of the floor of mouth, may have been anatomically in contact with the tissues of nodal level I of the ND.\textsuperscript{60} Failure to recognise this anatomical relationship can lead to misinterpretation of the margin of clearance and confusion between the extent of direct spread of the primary tumour and extracapsular spread of metastatic tumour.

3.2 Slicing the specimen
The pathologist should avoid sampling errors due to inadequate slicing and poor block selection by following a routine protocol with attention to detail. It is essential to slice the whole specimen ensuring that each slice is about 4 mm thick. Generally, the slices should be laid in the cassette to show the same face. However, it is important that each slice is inspected with the naked eye. It is not uncommon for tumours, particularly of the tongue, to show narrow streaks or tiny satellite islands well ahead of the main front (see Fig. 5 in Woolgar and Triantafyllou²³) and such slices should be laid down to show these features. While cutting sections on the microtome, the pathology technicians should not discard early sections since narrow streaks / satellite islands can be easily cut through and it is advisable to save a ribbon of sections when something of special interest has been noted by the pathologist macroscopically. Whenever possible, large blocks that allow for large cross-sectional slices to be viewed intact should be made. However, complete slices of the specimen may be too large to fit into conventional cassettes (Fig. 23) and they need to be trimmed in a manner that allows reconstructions of the original slice and hence, accurate histological measurement of complete dimensions of the tumour (section 2.3), features of the advancing tumour front, and also the mucosa, submucosal / deep and bone margins. Care is also needed during labelling of the cassettes to ensure accurate reconstruction. We find the use of line drawings useful for this purpose.

3.3 Histological assessment

While histologically assessing the surface aspect of the tumour, in superficial tumours in particular, it is important to distinguish between regions of invasive carcinoma from adjacent carcinoma *in-situ*, if present, since only the former is included in the measurement of the surface diameter for UICC TNM staging classification.²⁴ In addition, it is essential that the complete area of the mucosal resection is carefully examined histologically, not just the surface of the *index* (first primary) tumour and its immediate periphery, since a multifocal origin cannot be excluded or there may be a synchronous smaller primary tumour (undiagnosed clinically) as seen in 9/11 of the resections with involved mucosal margins in our study of 301 consecutive resection specimens.²³

As regards the deep aspects of the tumour, it is essential that any streaks or satellite islands or tumour ahead of the main front, which may be involving nerves or vessels, are detected by systematic scanning of the whole specimen using a medium power objective so that these tumour foci are included in relevant measurements
(see section 2.3). Foci of lympho-mononuclear inflammatory cells ahead of the advancing front should prompt assessment of additional sections since they might reflect host reaction to a microsatellite (Fig. 25). However, the significance of such foci has not been investigated.

It is important to be aware of the more commonly encountered routes of invasion and tumour extension. For example, once tumour escapes from the muscle of the tongue, it may spread within the loose areolar tissues of the floor of mouth. In tumours of the anterior or lateral border of tongue, it is not uncommon to see tumour emboli within vessels running in the superficial or deep vascular plexuses (Fig. 25).

The status of the submucosal / deep and bone resection margin is recorded independently from the mucosal resection margin. In our study of surgical margins in OSCC resections, involved deep margins were more frequent in tumours of the maxillary alveolus (45%), retromolar region (38%) oropharynx (38%) and buccal mucosa (33%) than in tumours of the floor of mouth (19%), mandibular alveolus (17%) and oral tongue (11%). The buccal soft tissue margin overlying maxillary and mandibular resections can be particularly difficult to assess due to tight binding of soft tissue to bone resulting in difficulties in slicing the specimen and separating the soft tissues from bone without disrupting the integrity of advancing tumour front and surgical margin. Issues related to the assessment of bone involvement and the integrity of the cortical plates, have been discussed in section 2.6. When assessing the bone margins, particular attention should be paid to the base (deep margin) of mandibular rim resections and also periosteum at the anterior and posterior bone margins since together these accounted for over 50% of positive bone resection margins in our study. In the mandible, tumour involvement in relation to the inferior dental nerve should be assessed (Fig. 59). Spread along the nerve or within perineural spaces may be encountered in post-radiotherapy resections but in our experience, it is rarely seen in patients having primary surgery.

4. Pathological assessment of NDs

The terminology associated with NDs (Fig. 2) can be confusing. Multiple classifications are used, and as these are based mainly on clinical and surgical factors, they may be unfamiliar to the reporting pathologist. A clear appreciation of the expressions used at MDTs and on Pathology Request Forms is essential to avoid misunderstandings (Table 3).
4.1 Background knowledge

Good knowledge of the anatomy of the cervical lymphatic system, and, in particular, the boundaries, approximate number and variations in size and shape of nodes, and principle drainage basin and afferent routes of the six main nodal levels that may be removed at ND is essential. In addition, it is important to be aware of the other nodal groups, such as the facial, sublingual and lingual nodes, which are occasionally the site of metastases from intra-oral primaries. Also essential is an understanding of the life history of a metastasis within an individual lymph node from embolus to extracapsular spread (Fig. 60).

Misunderstandings and pitfalls related to the incorrect use of histopathological terminology can be avoided by thorough knowledge and strict application of the commonly used expressions (Table 4).

Guidelines and protocols for specimen presentation and macroscopic assessment, harvesting and trimming of lymph nodes, summarised in Table 5, should be followed with care, audited and modified as necessary.

4.2 Special considerations at anatomical level I

The submandibular salivary gland, removed as part of level I, should be assessed. In the non-irradiated patient, the glandular parenchyma is rarely involved by direct spread from nodal metastases, and hence, processing a single representative slice is usually sufficient. Immunocompromised and irradiated patients may show widespread glandular involvement via direct extension from involved submandibular nodes and multiple slices may be necessary. In addition, the submandibular salivary gland can also be involved by direct spread of a large floor-of-mouth tumour. In this case, the macroscopic assessment and dissection can be difficult and the precise origin of the submandibular mass may remain uncertain. Problems in dissecting the mass are compounded when the mass is firmly fixed to the body of mandible. In this situation, we leave part of the tumour mass attached to the mandible and examine them en bloc following decalcification.

4.3 Harvesting and trimming of lymph nodes

During the macroscopic assessment of the fixed dissection, if enlarged nodes are visible on or bulging out of the surface of the specimen, the area should be inked to facilitate assessment of the surgical margins. Increasing the fixation time beyond 24
hours makes nodes more palpable. While lymph nodes are often perceived as ‘brown islands in a yellow sea’, small nodes particularly at levels IIB and V may resemble adipose tissue or loose connective tissue in colour. Although these nodes are palpable due to their firm consistency, it is advisable to process any uncertain material. Larger nodes (around 10 mm or more) should be bisected or sliced through the hilum. If there is obvious metastatic tumour, slicing and block selection must be designed to show the extent of any ECS. Some involved nodes / nodal masses may be cystic (see Fig. 10 in Woolgar and Triantafyllou\(^{62}\)) and it is advisable to measure the maximum profile diameter of the node prior to cutting and collapse of the cyst lumen. If, on slicing, the node appears negative, all slices should be processed. Reslicing the bisected node at 90 degrees to create multiple hemispherical segments permits assessment of greater areas of the sub-capsular sinus - the likely site of ITCs and micrometastases. Salvage NDs are often distorted by fibrosis from previous surgery and or radiotherapy. Tumour and any residual nodes may not be macroscopically distinguishable from scar tissue and multiple slices of all doubtful tissues should be processed.

4.4 Histological assessment of nodal metastases and extracapsular spread

In the histological assessment of metastatic disease, stromal reaction (Fig. 20) is a useful feature to aid distinction between early metastasis and an embolus impacted in an intranodal thin-walled vessel. The histological profile diameter of tumour in each positive node needs to be determined with reasonable accuracy since it influences the pathological stage. When multiple foci of tumour are detected in a single node, the profile diameter of the deposit corresponds to that joining the most extremely opposite foci (see Fig. 12 in Woolgar and Triantafyllou\(^{62}\)). This may be difficult when the tumour is arranged as ITCs or tiny islands, but measurements could be easier on sections stained for cytokeratin immunohistochemistry (Figs. 64, 65).

Accurate detection of the presence and extent of ECS is important in post-operative management and prognosis prediction.\(^{64,65}\) The extent of unequivocal ECS can be recorded by reference to the perinodal tissues and structures involved or by estimating the distance in mm from a reconstruction of the original position of the node capsule.\(^{62}\) ECS may be focal - involving only the hilum, for example - and the diagnosis of early ECS is often challenging. Subcapsular and peripherally located tumour may abut onto the node capsule and may be accompanied by desmoplasia. When this suspicious feature is seen in a single node and there is no obvious ECS elsewhere in the ND, then additional step and serial sections should be examined. If
these do not reveal unequivocal ECS, then it is probably safe to regard this as tumour confined to the node. If there is bulging of the node with a microscopic prominence of tumour or tumour stroma (see Fig. 17 in Woolgar and Triantafyllou), we record this as ECS confined to the immediate pericapsular area. Emboli of tumour cells or ITC within the perinodal thin-walled vessels (see Fig. 20 in Woolgar and Triantafyllou) or within the capsular sinuses do not constitute ECS but they should be noted since they have additional prognostic value.

4.5 Soft-tissue tumour deposits

Tumour nodules in the connective tissue of a lymph drainage area without histological evidence of residual lymph node are not uncommon. They may be detectable at the macroscopic dissection stage, but more often are only evident on histological assessment. These should be classified in the pN category if the nodule has the form and smooth outline of a lymph node, but regarded as discontinuous extensions or resulting from invasion of vascular walls if the nodule has an irregular outline. In such cases, other obvious nodes are likely to be involved by tumour and the pN status would have been already established.

The number of positive nodes may be uncertain due to matting. However, a rough estimate of the number of nodes contributing to a matted mass is often possible and hence, conferring pN2b status. Failure to recognise that more than one node is contributing to a large metastatic deposit would probably not be a significant lapse since the likely stage would be pN2a/pN3 on account of the measured size.

4.6 Procedural suggestions

Inaccuracies in histological detection are unavoidable but should be reduced to a minimum by a meticulous routine, alertness and concentration. It is helpful to assess the microscope slide initially with the naked eye to see the number, size and location of nodal profiles, and to record any tumour deposits. Each slide should then be scanned at low power (Figs. 66, 67a). Particular attention should be paid to the nodal sinuses examining each nodal profile with medium power objectives (Fig. 67b). Suspicious areas should be examined with a high power objective to confirm epithelial elements (Figs. 67c, d). It is helpful to adopt the system of marking the slide label with a red dot when nodes or profiles are assessed as positive and adding an arrow to indicate presence of ECS. Such a simple system saves time when writing
the report and presenting the findings in a table\textsuperscript{62} and also, facilitates retrieval of positive nodes for MDTs or teaching sessions, \textit{etc.}

Errors due to histological misinterpretation can be minimised by experience and good knowledge of potential pitfalls. The more common potential errors and tips on how to avoid them are shown in Table 6.

\section*{5. Pathological contributions to the prognosis of OSCC}

Histopathologically assessed features of the surgical resection specimen continue to provide information that is central to determining the post-operative treatment needs and prognosis for an individual patient. The features are briefly revisited here. For details, see Woolgar.\textsuperscript{66}

\subsection*{5.1 Prognostic features related to the primary tumour}

\subsection*{5.1.1 Site}

The reduction in 5 year survival for more posteriorly located tumours - oropharyngeal compared to oral cavity - had been recognised for many years\textsuperscript{67} and is probably due to the association between tumour site and occurrence of cervical node metastasis; pTN stage at presentation; histological grade and features of the advancing tumour front such as pattern of invasion, and peri- or endoneural and lymphovascular invasion; the surgeon’s ability to achieve clear resection margins and the occurrence of metachronous primary tumour.\textsuperscript{66} Within the oral cavity itself, the affect of site is less certain. It was not a significant independent factor in a recent (2008) study\textsuperscript{68} involving 489 intra-oral cancer patients treated by primary surgery and it may be that in some previous studies, site was acting as a surrogate for more important factors.

\subsection*{5.1.2 Size}

The size of the tumour affects both the choice and outcome of treatment. In the aforementioned 2008 study,\textsuperscript{68} there were significant differences in overall survival in relation to clinical T stage and pathological tumour diameter and pT stage in two patient groups: surgery with and surgery without neck dissection. Although tumour diameter is used in UICC pT staging,\textsuperscript{24} tumour thickness is now recognised as a more accurate histological predictor of occult nodal metastasis, local recurrence and
survival. The correlation between tumour diameter and thickness tends to be poor. The ‘critical’ thickness is highly site dependent, but 4 mm is a useful average for OSCC with thicker tumours having a fourfold increased risk of nodal metastasis than thinner tumours. In predicting survival, the reconstructed thickness measurement, as defined in section 2.3, is robust. For example, in a 1999 study the tumours were from diverse sites within the mouth and oropharynx, yet the mean thickness in patients dying of or with cancer was twice that of survivors or patients dying free of cancer.

5.1.3 Histological grade of conventional squamous cell carcinoma

Generally, large studies report a correlation between WHO histological grade (see section 2.3) and survival and in the 2008 study, there was a significant difference in disease-specific and overall survival. The 5 year disease-specific survival was 89%, 68% and 45% for pG1, pG2 and pG3 tumours, respectively (p<0.0001). However, WHO grade alone shows poor correlation with outcome in an individual patient possibly because the system is poorly discriminating, most OSCCs being graded as pG2 (see section 2.3). The invasive front, multifactorial histological malignancy grading system, although open to similar criticisms (see section 2.3), it is reportedly more useful in predicting nodal metastasis, local recurrence and survival. The pattern of invasion which reflects cellular cohesion (see section 2.3) is the single most important feature and it has predictive value in the clinical setting. In addition, the pattern of invasion reflects in vitro markers of malignancy such as loss of contact inhibition, and tumour cell motility.

5.1.4 Lymphovascular and peri-/endoneural invasion

Both these factors show a significant association with tumour size, invasive front, multifactorial histological malignancy grading, and outcome in terms of nodal metastasis, status of resection margins, local recurrence and survival (Woolgar, 2006). In the 2008 study, peri- or endoneural invasion was an independent predictor of all causes survival in Cox regression modelling.

5.1.5 Bone invasion

The UICC TNM staging classification categorises involvement of bone with penetration of the mandibular and maxillary cortical plate into cancellous bone and
marrow spaces as T4, but it is uncertain whether the consequent stage IVA status is justified except in cases with an infiltrative (as opposed to erosive) pattern of bone involvement.

5.1.6 Sialadenotropism

It has been reported that sialadenotropism is associated with increased local recurrence and further primary tumours, but see section 2.7.

5.1.7 Involvement of overlying skin

This is a grave prognostic sign. For example, involvement by direct spread and lymphatic spread are associated with median survival of only seven and three months, respectively.

5.1.8 Histological subtypes

The prognosis of verrucous carcinoma and carcinoma cuniculatum is generally good since nodal metastases do not occur while adenosquamous carcinoma and basaloid squamous cell carcinoma are regarded of poor prognosis due to early regional and distant metastases, but see relevant comments in section 2.2. Assessment of the prognostic value of other subtypes is limited by inconsistent recognition and documentation.

5.1.9 Histological status of the resection margins

This is a robust prognosticator. In the 2008 study, in Cox regression modelling, both margins and pN status were independent predictors of mortality at p<0.001. The relative risk of death ratio was 2.2 [1.4-3.5 confidence interval (CI)] for close and 3.4 (2.1-5.6 CI) for involved margins in comparison to clear margins.

5.2 Prognostic features related to the cervical (regional) lymph nodes

The prognostic importance of the presence and extent of cervical lymph node metastasis has been recognised for many decades and several independent authorities have reported an association between outcome (in terms of regional recurrence and, or survival) and the features listed in Table 7. There is no general
agreement on which features are the best prognosticators and factors influencing the stringency of the pathological assessment probably explain, at least in part, the lack of consensus. The importance of pN status as an independent predictor of mortality in the 2008 study\textsuperscript{68} is clear. The relative risk of death was 2.2 (1.3-3.8 CI) for pN1 and 4.8 (3.1-7.5 CI) for pN2-3 compared to pN0. The prognostic significance for micrometastases and ITCs is not known for OSCC, but studies of sentinel nodes\textsuperscript{74} may provide an insight.

5.3 Additional prognostic features

Between 5-25\% of OSCC patients have clinical evidence of distant (not locoregional) metastases within two years of the initial diagnosis.\textsuperscript{66} Pathological TNM stage pN2 or pN3, especially if ECS is present, are most at risk and 90\% are dead within two years.\textsuperscript{64}

Local relapse due to a true recurrence occurs earlier and has a worse prognosis than relapse due to a metachronous primary tumour.\textsuperscript{26} In addition, relapse in the neck due to persistence or recurrence in the operated or irradiated field has a worse prognosis than relapse due to growth of occult metastases outside the original field of treatment or in the contra-lateral neck.\textsuperscript{26}

Both epithelial dysplasia peripheral to the primary tumour and an \textit{index} tumour of multifocal origin (multiple separate foci of invasion within a larger region of epithelial dysplasia) are associated with an increased risk of serial primary tumours. Although the metachronous tumours are usually diagnosed when small, together with systemic metastases, they account for most of the disease-specific deaths occurring after 24 months.\textsuperscript{26}

6. Epilogue

This article has addressed issues facing the diagnostic histopathologist during routine assessment of surgical material from OSCC patients. It is hoped that the practical approach taken, has highlighted commonly encountered questions and experiences, identified pitfalls and, when possible, offered tips and guidance. It is important to remember that the pathological assessment and generation of the final report involves staff from multiple disciplines. Detailed consideration of potential problems due to technical, secretarial and clerical errors is beyond the scope of the present article, save to point out that the pathologist is ultimately responsible for
establishing, overseeing and auditing laboratory standard operating procedures. The importance of close co-operation with clinicians is obvious.

References


15. Broders AC. Squamous-cell epithelioma of the lip. *JADA* 1920; **74**:656-64.


Table 1  An index of the topics discussed as common questions, experiences and problems faced by the pathologist diagnosing and assessing incisional biopsies and surgical resections of primary OSCCs

<table>
<thead>
<tr>
<th>Topic</th>
<th>Question, experience or problem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proliferative squamous epithelial dysplasia</td>
<td>Definition</td>
</tr>
<tr>
<td>Histological subtypes of OSCC</td>
<td>Conventional OSCC vs. subtype 'Pure' vs. hybrid tumours ‘Suspicious spindle cells’ Changes due to previous surgery or radiotherapy</td>
</tr>
<tr>
<td>Grading systems of conventional OSCC, growth patterns and measurements</td>
<td>Definitions Assessment</td>
</tr>
<tr>
<td>Deep (unequivocal) vs. superficial (early / minimal) invasion</td>
<td>Definitions Assessment Microinvasive vs. superficially invasive SCC Carcinoma cuniculatum Sialadenotropism</td>
</tr>
<tr>
<td>Vascular invasion</td>
<td>Assessment</td>
</tr>
<tr>
<td>Bone involvement</td>
<td>Assessment Criterion for pT4 status Erosive vs. infiltrative pattern Spread via the periodontal ligament Benign odontogenic vestiges</td>
</tr>
<tr>
<td>Pseudocarcinomatous hyperplasia and 'benign' perineural infiltration</td>
<td>OSCC vs. pseudocarcinomatous hyperplasia vs. transepithelial elimination vs. sialometaplasia Keratoacanthoma vs. carcinoma cuniculatum Neuroepithelial complexes</td>
</tr>
<tr>
<td>Lesions with a papillary exophytic component , proliferative verrucous leukoplakia and squamoproliferative lesions</td>
<td>Definitions including those of papillary and verrucous Verrucous vs. papillary vs. exoendophytic conventional SCC</td>
</tr>
<tr>
<td>Adenosquamous carcinoma</td>
<td>Assessment of ‘glandular’ elements Clear-cell SCC Sialadenotropism vs. hyperplastic salivary ducts vs. sialometaplasia</td>
</tr>
<tr>
<td>Carcinoma within the connective / submucosal tissues without an obvious surface mucosal origin</td>
<td>Assessment</td>
</tr>
</tbody>
</table>
Table 2 Conditions that may be accompanied by pseudocarcinomatous hyperplasia

1. Granular cell tumour
2. Necrotising sialometaplasia
3. Chronic inflammation and mild chronic trauma
4. Denture-induced fibroepithelial hyperplasia
5. Palatal papillary hyperplasia
6. Hypertrophic lichen planus
7. Chronic discoid lupus erythematosus
8. Chronic hyperplastic candidiasis
9. Blastomycosis
10. Keratoacanthoma
11. Wegener’s granulomatosis
12. Transepithelial elimination of degenerate material

Table 3 Terminology used to describe neck dissections (for details see Woolgar and Triantafyllou, 2007)

<table>
<thead>
<tr>
<th>Basis of classification</th>
<th>Categories</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indication</td>
<td>Therapeutic; elective; salvage</td>
</tr>
<tr>
<td>Timing</td>
<td>Simultaneous with resection of primary tumour; delayed</td>
</tr>
<tr>
<td>Surgical relationship to primary tumour resection</td>
<td>In continuity; discontinuous</td>
</tr>
<tr>
<td>Laterality</td>
<td>Unilateral; bilateral; ipsilateral; contra-lateral</td>
</tr>
<tr>
<td>Extent</td>
<td>Radical (classical); extended radical; function-preserving; comprehensive (modified radical, Bocca); selective; supra-omohyoid; anterolateral</td>
</tr>
</tbody>
</table>
Table 4 Definition of terms used in pathological staging of NDs

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated tumour cells (ITCs) (Figs. 61-63)</td>
<td>In any single node, collections of tumour cells totalling not more than 0.2 mm in greatest dimension (seen in HE-stained or on immunohistochemistry); they could be microemboli in perinodal vessels or in nodal sinuses or stroma</td>
</tr>
<tr>
<td>Micrometastasis</td>
<td>In any single node, tumour deposits confined within the nodal capsule, measuring between 0.2 and 2 mm and usually showing evidence of successful settling and growth (mitotic activity, stromal reaction)</td>
</tr>
<tr>
<td>Conventional metastasis</td>
<td>Profile diameter of the tumour deposit(s) exceeds 2 mm in total</td>
</tr>
<tr>
<td>Occult (covert) metastasis</td>
<td>Not suspected clinically and maybe ITCs, micrometastasis or conventional metastasis</td>
</tr>
<tr>
<td>‘Overflow’ pattern</td>
<td>In most cases, metastatic tumour develops initially in one or more lymph nodes draining the lymphatic basin of the primary tumour and then progresses in an ‘overflow’ fashion to produce an ‘inverted-cone’ shape</td>
</tr>
<tr>
<td>Skip metastases</td>
<td>Metastases at non-contiguous nodal levels such as levels I and IV</td>
</tr>
<tr>
<td>‘Peppering’</td>
<td>ITCs or micrometastases in multiple nodes in the absence of a conventional metastasis</td>
</tr>
<tr>
<td>Extracapsular spread (ECS)</td>
<td>Tumour infiltrating beyond the node capsule</td>
</tr>
</tbody>
</table>
Table 5 Guidelines for the pathological assessment of NDs, potential pitfalls and possible solutions

In theatre after operation:

1. Label the centre of each anatomical nodal group with tags or sutures and pin or suture specimen onto a polystyrene or cork board in correct anatomical position avoiding over-tight sutures (risk of poor fixation and tearing of tissues); alternatively, divide ND into its component anatomical levels (taking care not to disruption the integrity of the primary tumour resection) and place each level in a separate container
2. Check the specimen is fully immersed in fixative (10% buffered formalin)
3. Submit additional nodal groups in separate labelled container
4. Give complete accurate details on the Pathology Request Form using waterproof ink (patient details, type of primary tumour resection and neck dissection, key to tags, and a line diagram or photograph)
5. Label specimen container with matching patient details

In laboratory:

1. Search the soft tissues of the fixed specimen and identify all lymph nodes >3-4 mm by observation and palpation
2. Harvest the nodes (with their adjacent fibro-adipose tissue attached) from within each anatomical level and block out in labelled cassettes, slicing larger nodes; position of nodes could be indicated on line diagram or photo; alternatively, nodes could be harvested from all anatomical levels prior to blocking out, but take extra care over preserving nodes in separate groups corresponding to the levels
3. Process by standard histological methods, and stain with HE
4. Histological assessment of a single HE-stained section from each block
5. Step / serial sections and / or immunohistochemistry in selected cases (micrometastasis versus conventional metastasis; extent of ECS; to investigate suspicious areas, etc)
**Table 6** Potential pitfalls in histological interpretation and possible solutions (for illustrations, unless otherwise noted. see Woolgar and Triantafyllou\textsuperscript{62})

<table>
<thead>
<tr>
<th>Pitfall</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive changes versus metastasis</td>
<td>Sinus macrophages are non-cohesive, pale stained, no mitotic figures; eosinophils may be associated with tumour deposits (Figs. 67d, 68a, b, Fig. 69a, b); immunohistochemistry for cytokeratins rarely required (Figs 68c, 69c)</td>
</tr>
<tr>
<td>Sinus histiocytosis</td>
<td></td>
</tr>
<tr>
<td>Vascular transformation of sinuses</td>
<td>Florid early fibrosis and newly formed thin-walled vessels; care is needed to distinguish from neo-vascularisation evoked by a tumour deposit</td>
</tr>
<tr>
<td>Changes in germinal centres</td>
<td>Immunohistochemistry for cytokeratins</td>
</tr>
<tr>
<td>Hyaline change within lymphoid parenchyma</td>
<td>Not attributable to amyloid or scirrrous stroma of tumour deposit</td>
</tr>
<tr>
<td>Granulomatous reactions:</td>
<td>Immunohistochemistry for cytokeratins and macrophage markers plus clinical history, etc</td>
</tr>
<tr>
<td>sarcoidal / immunological reaction to</td>
<td></td>
</tr>
<tr>
<td>drained tumour products; foreign-body</td>
<td></td>
</tr>
<tr>
<td>granulomata to small metastasis and /</td>
<td></td>
</tr>
<tr>
<td>keratin deposit (eg. post-radiotherapy);</td>
<td></td>
</tr>
<tr>
<td>concurrent granulomatous disease, eg.</td>
<td></td>
</tr>
<tr>
<td>tuberculosis</td>
<td></td>
</tr>
<tr>
<td>Benign cellular inclusions</td>
<td>Uni- / bilayered duct-like structures lined by simple or oncocytoid epithelium</td>
</tr>
<tr>
<td>Salivary inclusions</td>
<td></td>
</tr>
<tr>
<td>Thyroid follicles</td>
<td>More likely at levels III / IV</td>
</tr>
<tr>
<td>Naevus cell nests</td>
<td>Immunohistochemistry</td>
</tr>
<tr>
<td>Flushing of nodal sinuses at multiple</td>
<td>May be mistaken as sinus histiocytosis; immunohistochemistry for cytokeratins; seen in around 1% of positive NDs\textsuperscript{63}</td>
</tr>
<tr>
<td>anatomical levels with malignant epithelial cells versus sinus histiocytosis</td>
<td></td>
</tr>
<tr>
<td>Cystic metastases versus branchial cyst and primary branchial carcinoma</td>
<td>Age &lt; or &gt; 40 yrs; process complete cyst; examine step serial sections for</td>
</tr>
</tbody>
</table>

cytological atypia / obvious SCC; clinicopathological correlation

Failure to recognise a subtype of SCC
Confusion between acantholytic SCC and adenocarcinoma
Confusion between spindle cell SCC and other spindle cell neoplasms or stromal reactions
Hybrid SCC showing different cellular phenotypes
Concurrent metastasis and leukaemia / lymphoma (double / dual pathology’)

Awareness and AB pH 2.5-PAS
Awareness and immunohistochemistry
Awareness that metastasis may consist of a single cellular phenotype
Awareness; patients with leukaemia / lymphoma often show multiple metastases in multiple levels; effacement of nodal architecture / loss of follicles by lymphoma; immunohistochemistry (Fig. 70)

Table 7 Prognostic features related to the regional lymph nodes (for details, see Woolgar, 2006)

1. Metastatic status - nodal metastasis present versus absent
2. Laterality of positive nodes
3. Number of positive nodes
4. Size of metastatic deposit(s)
5. Anatomical level of involvement
6. Extracapsular spread
7. Embolisation / permeation of perinodal lymphatics
8. pN stage
Legends

Plate 1
Note: Unless otherwise specified, the photomicrographs in this article are from sections of routinely processed tissue, which were stained with haematoxylin and eosin (HE); it was not deemed necessary to give objective magnifications. Figure 1 Resection of the anterior maxilla for an ulcerated SCC centred on the anterior edentulous alveolar process and extending to hard palate and vestibule. An ulcerated, satellite nodule is seen (arrow). Figure 2 Resection of anterior tongue (asterisk), floor of mouth (not seen) and mandible (arrow), which is in continuity with bilateral neck dissections. Figure 3 Acantholytic OSCC. Section profiles of, at least, five pseudolumens are seen. The central, larger and somewhat collapsed pseudolumen has an irregular contour and contains ‘acantholytic’ cells. Some of the latter are rounded with deeply-staining nuclei, which suggests apoptosis. Whether apoptosis results in or is secondary to malignant acantholysis is speculative. Note the single (‘basal’) layer of malignant keratinocytes (arrowhead), which partly surrounds the pseudolumen and faces the stroma. Figure 4 Immunohistochemistry for MNF116 decorates a small collection of non-cohesive malignant keratinocytes at the advancing front of an OSCC. Although non-cohesive, the cells appear ‘grouped’ and not dispersed (compare with Fig. 15). This was an isolated finding suggesting a focally expressed loss of cohesion; alternatively the collection could reflect an early stage in the clonal expansion of a non-cohesive cell. Immunohistochemical double staining for cytokeratins and for cell-cycle markers could be useful in exploring these possibilities. Figure 5 From an incisional biopsy diagnosed as basaloid OSCC. Except for easily identified palisading, the peripheral tumour cells are reversely polarised (away from stroma nuclei) and show subnuclear ‘vacuolation’ (arrowhead); this emphasises similarities to ameloblastoma. Assessment of the excisional specimen showed that the bulk of the tumour consisted of conventional SCC. Figure 6 Recurrent OSCC showing mixed squamous (large, polygonal or spindled with abundant eosinophilic cytoplasm and pleomorphic nuclei) and basaloid (small, haematoxyphilic with indistinct borders and bland nuclei) phenotypes (a); the phenotypes are further distinguished by immunohistochemistry for AE1/AE3, which selectively stains squamous cells (b).

Plate 2
Figure 7 Typical spindle cell carcinoma. The arrows indicate two angular tumour cells in mitosis (a); immunostaining for SMA showed myofibroblastic reaction in other regions of the tumour (b). The proliferated myofibroblasts are selectively stained and easily distinguished from the unstained tumour cells (T); the arrow indicates an enlarged ‘epithelioid’ tumour cell. Figure 8 Myofibroblasts in the stroma of a conventional SCC. Note their abundant cytoplasm and ‘rigid’ appearances (arrows); the arrowhead indicates an islet of malignant keratinocytes. Figure 9 Nasopharyngeal-type phenotypes in an OSCC. Figure 10 An ulcerated endophytic transitional-type carcinoma at the lateral border of the tongue (a); that the tumour was
associated with lymphoid aggregates (not seen on this magnification) suggests that it arose in the lingual tonsil; typical ribbons (b) and ‘vertical’ orientation of peripheral tumour cells (c). **Figure 11** An ulcerated, endophytic, hybrid, squamous (S) and transitional-type (T) carcinoma (a). Note the markedly asymmetrical silhouette of the tumour. The area enclosed in rectangle is magnified in (b) to show eosinophilic (lower part of the photomicrograph) and haematoxyphilic (upper part of the photomicrograph) components. **Figure 12** Special staining (Masson-Fontana) shows dendritic melanocytes, one of which is indicated by the arrow, and variable hyperpigmentation of malignant keratinocytes in a pigmented OSCC.

**Plate 3**

**Figure 13** Brisk non-infiltrative inflammatory reaction in OSCC. Note the islets of malignant keratinocytes at the advancing front (type-III pattern of invasion); one islet is indicated by the arrow; nuclear aberrations are seen. **Figure 14** The arrows indicate malignant keratinocytes that have lost cohesion at the ‘tip’ of an infiltrating column, possibly an ‘early’ type-IV pattern of invasion. In contrast with Fig. 4, this was not an isolated finding. **Figure 15** Advanced type-IV pattern of invasion. There is asymmetrical, widespread distribution of malignant keratinocytes that are infiltrating as individual units; two of them are indicated by arrows. (Compare with Fig. 4.) Inflammatory reaction is patchy. **Figure 16** Individual, non-cohesive malignant keratinocytes and variably dyscohesive cords and islets are appreciated on immunohistochemistry for MNF116. **Figure 17** Brisk and infiltrative inflammatory reaction in OSCC; many tumour cells underwent apoptosis and appear as glassy, rounded, eosinophilic (‘colloid’) bodies. **Figure 18** Brisk inflammatory reaction in OSCC, which is enriched with eosinophils. **Figure 19** Typical desmoplasic stroma (S) in OSCC. Note the absence of inflammatory cells and the sheathing of tumour cords and islets by stromal cells; this suggests that the latter are newly-formed and not pre-existing.20 **Figure 20** The inset shows section profiles of a cervical lymph node, one of which shows cystic SCC metastasis with induced stroma. Viable tumour and stroma project into the ‘cyst lumen’ in a drumstick-like configuration. This is better appreciated on higher magnification. The pale / ‘oedematous’ appearance of the stroma (S) is attributable to accumulation of glycosaminoglycans.

**Plate 4**

**Figure 21** Endo- (arrow) and perineural (arrowheads) invasion in OSCC. **Figure 22** Section from a frontal slice cut through the centre of an ulcerated endophytic SCC on the lateral border of the tongue; s, t and d indicate the supero-inferior surface diameter, reconstructed thickness and distance of the tumour from the deep excision margin respectively. The inferior mucosal (floor-of-mouth) margin appears involved (< 1.0 mm) (arrowhead). The superior mucosal (dorsal lingual) margin is free from tumour. **Figure 23** Cut surface of a markedly asymmetrical, ulcerated, solid, endophytic OSCC with satellite coin-shaped nodules. The maximum diameter of the tumour, which corresponds to the line segment, should be recorded prior to further slicing since its reconstruction from the histological sections of the slices would
be an approximate. **Figure 24** Variably sized, rounded islands consisting of well-differentiated stratified squamous epithelium with variable patterns of central keratinisation (I₁–I₆), are seen below an expanded and fibrotic lamina propria (asterisk) and surround salivary glandular elements (G). This qualifies as unequivocal invasion. Further support for the tumoral nature of the islands is given by their larger size and wider distribution than salivary lobules (compare with Fig. 48) and by the retraction artefact (arrow) seen between the island I₄ and adjacent stroma. The well differentiated epithelium of I₁–I₆ also excludes sialadenotropism (see Fig. 33). The surface oral epithelium (upper left part of the photomicrograph) has proliferated.

**Figure 25** There is unequivocal invasion (arrowhead) deeply in the submucosal fat below severely dysplastic oral epithelium (E). Although the invasive elements, shown at higher magnification in the inset, could be missed on casual inspection because of their small size, the lymphoid infiltrate associated with them should draw the attention of the pathologist.

**Figure 26** Step-section profiles of intra-oral carcinoma *cuniculatum*. The profile indicated by the arrow appears crateriform / cup-shaped and ‘pushing’ the submucosal skeletal muscle. However, invasion of the latter was detected in other profiles. **Figure 27** Biopsy from the alveolar mucosa. Three rounded epithelial islets of ‘clear’ / pale appearance, presumably odontogenic, are seen in the expanded and fibrotic lamina propria. Amalgam tattooing is discernible in the lower right part of the picture. **Figure 28** A focus of microinvasion (arrowhead) as appreciated in a section stained with PAS-H. It is unlikely that the absence of basement membrane reflects tangential sectioning.

Plate 5

**Figure 29** Superficially invasive OSCC. The advancing front of the tumour (arrowhead) is above the submucosal skeletal muscle fibres (M). Note the hyperaemic capillary-sized vessels, a suggestion of neovascularisation, around the front. **Figure 30** In comparison to Fig. 29, this superficially invasive OSCC penetrates deeper, but it is still confined to an expanded lamina propria and the musculature (M) is not involved. **Figure 31** Step-section profiles of salivary ducts (arrows) affected by sialadenotropism. **Figure 32** Severe squamous epithelial dysplasia / carcinoma *in-situ* spreads from surface oral epithelium in the upper left part of the photomicrograph to involve the medial part of a collecting salivary duct. **Figure 33** Sialadenotropism has resulted in expansion of a transversely sectioned collecting duct (D₅). Possibly the markedly thickened, dysplastic lining of D₅ effected partial obstruction and adjacent distal ductal segments (D₁, D₂), which lead from glandular lobules (G₁, G₂), became thus ectatic. Compare the rounded profile of D₅ with the irregularly dilated D₁ and D₂. Inflammatory reaction (asterisk) partly surrounds D₅, but invasion is not seen. **Figure 34** Transversely sectioned collecting salivary duct partly affected by sialadenotropism. The dysplastic region is thickened and bulges into the lumen (L), but it is still covered by normal cylindrical adluminal cells. Note the eosinophilic cytoplasm of the latter. **Figure 35** SCC involves Wharton’s duct and becomes exposed to the lumen. **Figure 36** Section profiles
(arrowheads) of an apparently tortuous, subepithelial lymphatic vessel are permeated by OSCC. The arrow indicates the possible site where tumour penetrates the vessel.

Plate 6

**Figure 37** Tumour embolus (arrow) in a large arteriole subadjacent to the advancing front of an OSCC. **Figure 38** Non-cohesive OSCC (T) destroys the wall of a muscular vessel. **Figure 39** A slender column of largely cohesive malignant keratinocytes invades a thin-walled vessel. The invasion could be missed on casual inspection save for the fibrinous clot (arrow), which has formed on the ‘tip’ of the penetrating column. **Figure 40** An exoendophytic SCC (T) of the retromolar region has caused erosion of the underlying cortex and exposure of cancellous bone. The margins of the erosion are indicated by the arrows. **Figure 41** Infiltration of jaw cortex by SCC. Note the partial destruction of a Haversian system (H). **Figure 42** SCC-bone interface. Tumour elements (arrowheads) at a distance from bone evoke osteoclastic resorption, one osteoclast being indicated by the arrow (a); the osteoclastic resorption has subsided and tumour elements (arrowheads) are close to the bone surface (b). **Figure 43** A SCC (T) of the alveolar process penetrates the periodontal ligament (arrow) and focally destroys a tooth (arrowhead).

Plate 7

**Figure 44** SCC (T) that begins to infiltrate the periodontal ligament between alveolar bone (B) and cementum (C). **Figure 45** Two ovoid islets of odontogenic epithelium are present in the fibrous stroma filling inter-trabecular spaces of cancellous bone. **Figure 46** Transepithelial elimination of a small sequestrum (arrow). **Figure 47** Downward growth of an epithelial column (asterisk) is seen at the margin of an ulceration (U), which is involved with elimination of a fragment of cementum (area enclosed in the rectangle) (a); the area is magnified to show the bland features of the proliferated epithelium (b). **Figure 48** Necrotising sialometaplasia. Ulceration (U), presumably deep, exposes a collection of solid, mitotically inactive, squamoid islets (arrow), presumably submucosal. Although lumens are not seen, the islets are not dispersed, being grouped in a ‘lobular’ arrangement. This assists in their confident identification as metaplastic salivary parenchyma. Compare with Fig. 24. **Figure 49** Two benign neuro-epithelial composites (arrows) in the capsule of a dentigerous cyst. The lumen and lining of the cyst are not shown here. **Figure 50** Fibro-fatty tissue contains the transversely sectioned profiles of small nerve fascicles (arrow) and Chievitz’s organ (right part of the photomicrograph). The epithelial parenchyma (P), cellular stroma (S) and capsule (C) of the latter can be seen. Note that the nerve fascicles are situated outside the organ. The stroma of the organ contains myelinated fibres, not seen at this magnification, but these remain as individual units and are not arranged as fascicles. The appearances do not resemble those seen in Fig. 21.
Figure 51 Section profiles of papillary squamous cell carcinoma. The exophytic part of the tumour shows asymmetrically distributed fronds; the endophytic part is irregular and penetrates at different levels (arrows). Compare with Fig. 52. Figure 52 Section profiles of verrucous carcinoma. In comparison with Fig. 51, the tumour advances at more or less the same level. This is highlighted by the banded and parallel to the surface inflammatory reaction (arrowhead) (a); shouldering is present (arrows) (b). Figure 53 While elements of conventional SCC are present in the left half of the photomicrograph, cribriform arrangements are seen in the right, above a collecting salivary duct (a); elsewhere, central comedo-necrosis (C) affects rounded islands of non-keratinising carcinomatous cells (b). Figure 54 The superficial part of a carcinoma arising from dysplastic oral surface epithelium (E) shows bands of malignant keratinocytes associated with hyaline material (arrow) (a); the deep part of the carcinoma shows rounded non-keratinising islands with hyaline droplets (arrow), which resemble those seen in adenoid cystic carcinoma (b).

Plate 9

Figure 55 Areas in an otherwise conventional OSCC, show small lumens (L) and mucus-producing cells with characteristic haematoxyphilic cytoplasm (arrows), which occur as collections or isolated (a, b). Special staining (PAS-H) decorates the mucous cells and shows neutral mucosubstances in lumens (indicated by arrow and L) (c, d, e). Note the differences in mucosubstance content between the cells surrounding the lumens in (d) and (e). Figure 56 Minor salivary gland overrun by SCC. Paley-stained mucous tubulo-acini and a small duct (arrow) are easily identified. Figure 57 Large ovoid islands of clear-cell SCC are impinging on oral surface epithelium (a); unilocular cytoplasmic ‘vacuoles’ and ‘signet-ring’ phenotypes (arrow) are seen (b); cells with multilocular cytoplasm, one of which is indicated by the arrowhead (c).

Plate 10

Figure 58 The inset shows section of a slice cut from a resection for SCC (T) of the lateral border of tongue. The area enclosed in the rectangle has been magnified to show invasion (arrowhead) ahead of the main tumour, probably an embolus in the deep vascular plexus. The arrows indicate ectatic thin-walled elements of the superficial vascular plexus. Figure 59 Section of a decalcified mandibular slice shows transverse profile of the inferior dental nerve. One of the constituent fascicles (arrow) shows endo- and perineural invasion, magnified in the inset. SCC is present in the upper left corner. Figure 60 OSCC emboli in capsular lymphatics of a cervical lymph node. Established metastasis in the nodal parenchyma is seen in the upper part of the photomicrograph. The arrow indicates valves of a lymphatic. Figure 61 and Figure 62 show ITCs (arrows) in cervical lymph nodes. The ITCs are larger than the adjacent small lymphocytes, show a somewhat irregular / angular nuclear contour, and have eosinophilic nucleoli and cytoplasm. By contrast immunoblasts are usually rounded and haematoxyphilic. Figure 63 ITC (arrow) in cervical lymph node, detected on 34BE12.
immunostaining. Diffusion of the immunoreaction product to adjacent lymphocytes has occurred. **Figure 64** Immunostaining for MNF116 highlights non-cohesive malignant keratinocytes in a cervical lymph node, which could be difficult to detect on routine histology. **Figure 65** Immunostaining for cytokeratins, here 34BE12, facilitates measuring the maximum diameter (line segment) of dyscohesive OSCC cervical metastases.

Plate 11

**Figure 66** Section profiles of bisected cervical lymph node with instantly recognised metastasis (arrows). The nodal halves had not been laid in the cassette to show the same face - hence, the different appearance of the metastasis profiles. The tumour deposits consist of heavily keratinised SCC and their maximum diameter can be easily measured. H indicates the hilum of the node. **Figure 67** In contrast to Fig. 66, the section profiles of this node do not show obvious metastases at scanning magnification (a); the nodal sinuses are, however, expanded and filled with 'compact' eosinophilic cellular masses (b); this together with the keratin pearl (arrow) should alert the pathologist. Examination with a high-power objective of other 'suspicious' regions, for example the area enclosed in the rectangle, confirms SCC. The cytology of the larger malignant keratinocytes (arrowheads), as described in Figs 61 and 62, and the association with eosinophils (arrows) are characteristic (c, d). **Figure 68 and Figure 69** Features of sinus histiocytosis (Fig. 68) and metastasis of non-keratinising SCC (Fig. 69) are shown at the same magnification for comparison. The irregularly contoured, 'loose' and heterogeneous / mixed with lymphoid cells histiocytic infiltrate (Fig. 68a) contrasts with the more 'compact' and homogeneous tumour deposit (T) (Fig. 69a).

Plate 12

**Figure 68 and Figure 69** (continued) The differences in looseness and homogeneity between sinus histiocytosis (Fig. 68b) and metastasis (Fig. 69b) are better appreciated on higher magnification; the arrows indicate a cleaved nucleus in the reactive condition and a mitosis in the metastasis. Immunohistochemistry for the CD68 antigen decorates macrophages in the reactive condition (Fig. 68c) and metastasis (Fig. 69c); while macrophages in the loose microenvironment of sinus histiocytosis appear plump and rounded, those squeezing against the unstained tumour cells (T) appear elongated. **Figure 70** Example of dual pathology. The asterisk indicates a subcapsular deposit of non-keratinising OSCC in a cervical lymph node (a); sections profiles of the node containing the deposit do not show obvious follicles (b), which should prompt a closer examination of the lymphoid parenchyma itself. (For comparison with Fig. 70b, Fig. 70c shows a merely reactive lymph node with obvious follicles.) On higher magnification, effacement of nodal architecture by chronic lymphocytic leukaemia / small lymphocytic lymphoma is seen (d). This was confirmed by immunohistochemistry (e). Metastases of SCC were found in multiple cervical nodes (levels II, III and IV) of this patient because of the compromised immune system.