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REVIEW ARTICLE

Prognostic biological features in neck dissection specimens

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Abstract The superior prognostic value offered by routine histopathological staging of neck dissections, as compared to clinical staging using palpation and modern imaging techniques, is well established in the literature concerning the management of squamous cell carcinoma of the head and neck. In this review, we discuss the definitions and criteria used in standardised routine histopathological reporting and explore additional potential nodal prognostic features. In addition, we critically appraise the value of immunohistochemistry, histochemistry, molecular and other non-morphological techniques and suggest tumour and host features that merit further investigations.

Keywords Head and neck cancer · Histopathology · Metastasis · Molecular analysis · Prognosis

Introduction

The classic and modified forms of neck dissection (ND) are performed for the diagnostic staging and control of metastatic disease, arising mainly from cancers of surface mucosae of the upper aerodigestive tract (oral, oropharyngeal, nasopharyngeal, laryngeal mucosae and sinonasal Schneiderian membrane) and epidermis of the scalp, face and neck. The cancers are usually squamous cell carcinoma (SCC). Less frequent types include adenocarcinomas of the major and minor salivary and sinonasal glands; and malignant melanoma and neuroendocrine carcinoma of skin and mucosal origin.

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Since the early days of modern head and neck surgery and diagnostic pathology, the tissues obtained during NDs have been submitted for histological examination to identify cervical lymph node metastases, a long established indicator of adverse prognosis. Originally the examination had been centred on the few larger nodes thought likely to be involved by metastatic disease. Incomplete as that limited sampling may now appear, it had been regarded as superior to pre-operative clinical examination and imaging assessment of the neck, and supported the need for a pathological TNM staging system. The rise, establishment and expansion of head and neck cancer specialists called for a more detailed and standardised pathological assessment and reporting. Although still based on routine, gross pathological and histological assessment, the resulting refined schemes provided valuable information on biological features related to cervical lymph node metastases in SCC and other head and neck malignancies, which influence prognosis. Although these features secure the status of the p"N" component of the current UICC (Union for International Cancer Control) and AJCC (American Joint Committee on Cancer) pTNM staging system, the additional information on their relative prognostic significance challenges the rather oversimplified UICC pathological staging criteria [1].

Advances in histochemistry, immunohistochemistry and molecular techniques and the establishment of tissue banks have enabled further investigation of cervical lymph node tissues. While some of the newer technologies confirm or expand inferences drawn with the use of routine histology, the clinical value of others remains to be assessed independently of their academic merit.

In this review, we summarise the prognostic importance of traditional and emerging biological features in NDs, with particular attention to the implications for local, regional and systemic relapse, disease-free (DFS), disease-specific (DSS) and overall survival (OS). We also provide recommendations for enhancing the prognostic value of current pathological N staging. Some illustrations are included and others may be accessed in the cited references.

Natural history of metastasis as perceived by routine histology

The embolic nature of lymphatic metastases is well established and borne out by histological studies on routine NDs [2–6] (Fig. 1). Tumour cells at the primary site gain access to lymphatic vessels and are carried within the lymph to the local lymph nodes. The first lymph nodes which are accessed from any given primary site are often referred to as the first echelon or sentinel nodes. Emboli enter via the afferent lymphatics and traverse the capsular sinus to reach the nodal parenchyma. Depending on successful angiogenesis and the formation of desmoplastic stroma (desmoplasia), the tumour embolus may anchor to the lymphoid parenchyma, grow and pass through the stages recognised as so-called isolated tumour cells (ITCs), micrometastasis and conventional metastasis [1, 7–9] (Figs. 2, 3, 4). The metastatic deposits expand and infiltrate the nodal architecture and eventually spread by two main routes: (1) to the extracapsular tissues; and (2) by shedding further emboli which pass via the efferent lymphatics to a lymph node at the next, anatomically-lower level of drainage. This overflow pattern of progression results in the "inverted cone lesion" of advanced metastasis seen in the majority of oral and oropharyngeal SCCs [2, 3].

The anatomical levels of the cervical lymphatic system are well established [10]. Levels I, II and III are at highest risk for metastasis from cancer of the oral cavity, whereas levels II, III and IV are at highest risk for metastasis from carcinomas of the oropharynx, hypopharynx and larynx [11, 12]. Initial embolic involvement of multiple lymphatic routes (including fast tracks to nodes at levels III and IV, low in the neck), related to the site and size of the primary tumour, appears to explain the occurrence of sentinel nodes at multiple anatomical levels, "skip metastases" and "peppering", and bilateral and contralateral metastases [2, 3]. The latter three patterns are more common in oropharyngeal (especially base of tongue), oral tongue and midline tumours [2, 3, 11, 13].

Biological features and events in the natural history of metastasis of prognostic significance

We have categorised the various nodal features on the basis of the availability and clinical applicability of the techniques used.

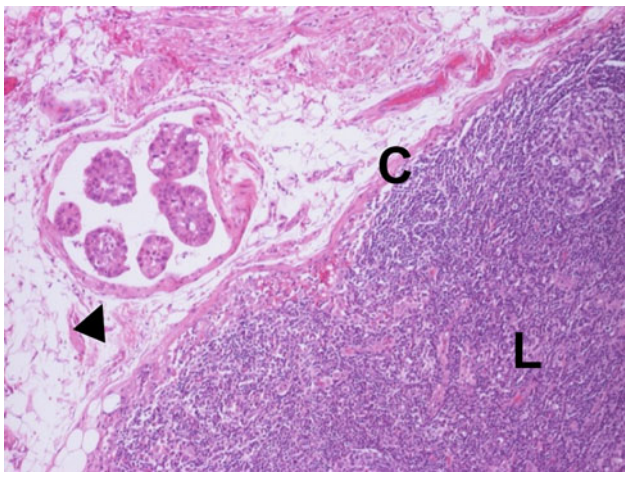

Fig. 1 Emboli of high-grade mucoepidermoid carcinoma in a perinodal lymphatic (*arrowhead*). *C* nodal capsule, *L* nodal parenchyma (haematoxylin and eosin, objective magnification ×4)

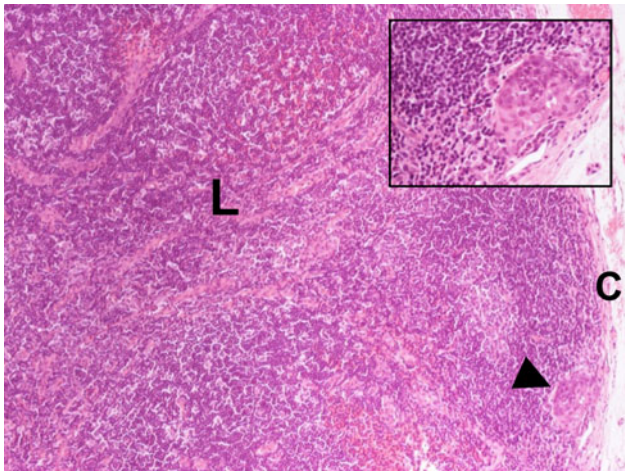

Fig. 2 A subcapsular, metastatic deposit of oral SCC, measuring <0.2 mm in profile diametre and qualifying as ITCs (*arrowhead*). The nodal area is intentionally shown at low magnification so that the relative proportions of deposit and nodal parenchyma (*L*) are appreciated. *C* nodal capsule (haematoxylin and eosin, objective magnification ×4). The ITCs are magnified in the inset; their eosinophilia allows distinction from adjacent haematoxyphilic lymphocytes (objective magnification ×20)

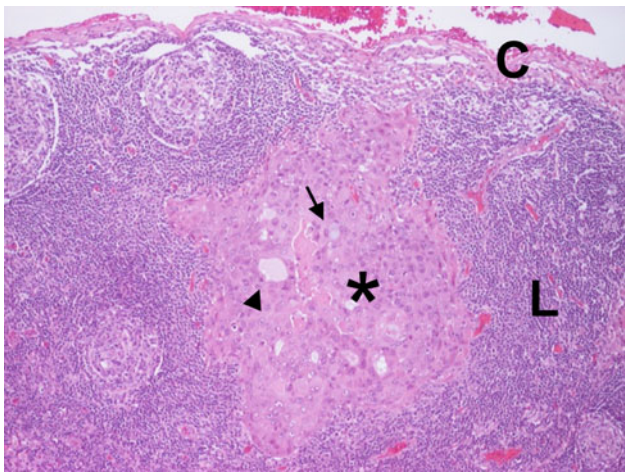

Fig. 3 A small, nodal metastasis of high-grade, mucoepidermoid carcinoma (*asterisk*). The metastatic deposit is largely squamoid, but occasional mucous cells (*arrow*) and lumens (*arrowhead*) are present. Desmoplasia is not seen. Compare with Fig. 4. *C* nodal capsule, *L* nodal parenchyma (haematoxylin and eosin, objective magnification ×4)

Features assessed by conventional and widely available means (routine histology)

Features of established prognostic significance

These have been reviewed, particularly in relation to oral SCC [14–16] and are summarised in Table 1. Many of the features are surrogate markers for an assessment of the volume of metastatic disease. It seems logical to assume

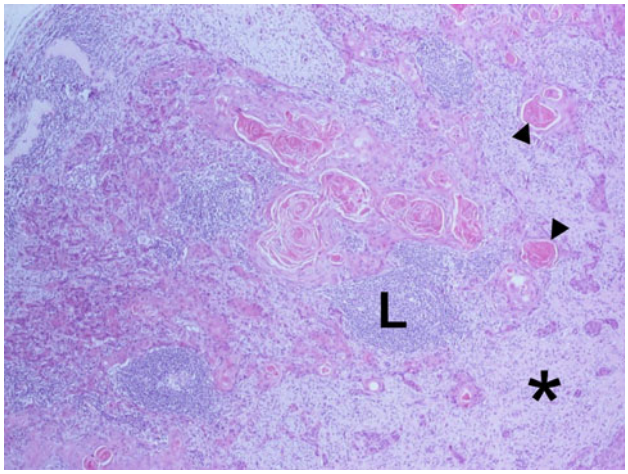

Fig. 4 Conventional nodal metastasis of oral SCC showing florid desmoplasia (*asterisk*). The desmoplasia is rich in glycosaminoglycans and appears haematoxyphilic, which allows distinction from the eosinophilic tumour. The *arrowheads* indicate keratin pearls. *L* residual nodal parenchyma (haematoxylin and eosin, objective magnification ×2)

that determining the latter would be a powerful tool, eliminating the need for assessing individual variables, especially if the location and cellular cohesion of metastatic tumour could be factored in. Currently, this seems difficult to achieve. A combined histopathological and morphometric approach to determine volume fractions of metastasis may be considered, but it would be time consuming and hence, seems unrealistic.

Given the difficulties in determining the absolute volume and detailed 3-dimensional imaging of metastatic disease, the features listed above should be assessed as regards their relative value in predicting regional recurrence and survival, so that front runners of prognostic significance in the clinical setting are identified. Unfortunately, relevant studies have presented contradictory and discordant findings. The lack of agreement may be due to factors influencing the accuracy of the pathological assessment (such as lack of standardised protocols and universally accepted definitions and categorisation criteria; multiple pathologists with variable experience and expertise; and sampling errors); and factors related to the cohort under study (criteria for entry such as inclusion of previously irradiated patients, differing proportions of therapeutic and elective or opportune NDs, different protocols for post-operative adjuvant therapy, and different follow up regimes and outcome measures). Inter-observer variation in classification is significant with studies indicating poor agreement between pathologists even when simply assessing extracapsular spread (ECS) versus no ECS [17]. Of the factors influencing the individual pathological assessment, classification of metastases, histological recognition of early, microscopical ECS and interpretation of

Table 1 ND prognosticators of oral SCC

1. Metastatic status (nodal metastasis present *versus* absent)
2. Laterality of positive nodes
3. Number of positive nodes
4. Percentage of positive nodes (ratio of positive nodes: total number of assessed nodes)
5. Number of positive anatomical levels
6. Highest positive anatomical level
7. Size of largest metastatic deposit
8. Matted nodes
9. Presence and extent of spread outside the nodal capsule (extracapsular spread)
10. Soft tissue tumour deposits
11. pN stage

soft tissue deposits have ignited controversy [18–23]. In an attempt to overcome the difficulties, we have suggested definitions for each of these along with recommended actions [7–9], which are summarised in Table 2. Interrelated features, for instance the number of positive nodes and pN stage or matted nodes and soft tissue tumour deposits [7–9, 24] may also affect analysis.

Only a few, though independent, studies have used multiple regression analysis to assess the relative prognostic value of individual features.

In oral SCC, ECS has emerged as the single most important feature in predicting DSS for the sizeable fraction of patients presenting with nodal metastases [25–28]. The association of ECS and distant metastases has also been independently highlighted [26, 27]. Woolgar et al. [29] found that ECS was the best prognosticator in the stepwise regression model of Cox—it was even more important than involved margins at the primary site. The Kaplan–Meier survival curves showed that patients with macroscopical (i.e. grossly seen) ECS tended to die within the first year following surgery, while patients with only microscopical (i.e. histologically detected) ECS tended to

Table 2 Definitions of terms used to describe cervical metastatic disease

Term	Definition	Action/notes	UICC TNM categorisation
Isolated tumour cells (ITCs)	In any single node (parenchyma, sinuses or capsular/perinodal vessels), collections of tumour cells totalling not more than 0.2 mm or 200 cells in greatest dimension	Detected by morphological (routine histology or immunohistochemistry) or non-morphological techniques (flow cytometry, DNA analysis)	pN0(i+) pN0(mol+)
Micrometastasis	In any single node, single or multiple tumour deposit(s) confined within the lymph node, totalling between 0.2 mm and 2.0 mm in greatest dimension	May show evidence of growth (mitotic activity, desmoplasia). Often detected by routine histology	pN1(mi), pN2b(mi), pN2c(mi)
Conventional metastasis	In any single node, total profile diameter of tumour deposit(s) exceeds 2 mm	Measure across total diameter of poorly cohesive tumour cells	pN1, pN2a, pN2b, pN2c, pN3
Occult (covert) metastasis	Any metastatic deposit not suspected clinically	May be ITCs, micrometastasis or conventional metastasis	
Established microscopic ECS (microECS)	Tumour breaching the nodal capsule/invading perinodal fat, which is only detected on histological assessment	May be focal (e.g. at hilum). Look for bulging or hillock. If nodal capsule is absent, assess/ reconstruct nodal contour	
Early microECS	Tumour confined to the lymph node, but desmoplasia alone extends to the immediate pericapsular area		
Macroscopic ECS (macroECS)	Tumour spreading beyond node capsule evident on macroscopic assessment/dissection	Record tissues involved. May be associated with fusion of adjacent nodes or fixation to perinodal structures	
Soft tissue deposits	Tumour deposits within adipose tissue or muscle in area of lymphatic drainage without any obvious residual node structure	Assumed to be replaced lymph nodes or deposits arising in emboli—hence, include in nodal count	
Overflow pattern	Metastatic tumour is most advanced in first echelon nodes with embolic spread trickling to lower anatomical levels to create an inverted cone topography		
Skipping	Metastases at non-contiguous nodal levels		
Peppering	ITCs/micrometastases in nodes at multiple anatomical levels in absence of conventional metastasis		
Fast-tracking	>Metastasis via a route draining directly to level III/IV		

die during the second year so that the survival probability was similar by 3 years [29]. In a later study in which patients with ECS received higher and more timely doses of post-operative radiotherapy [30], there was a significant difference in the 5 year OS for patients with microscopical and macroscopical ECS (31 and 19 %, respectively). More recently, ECS has also been established as indication for post-operative chemoradiotherapy [31, 32].

The biology of ECS is, however, poorly understood. As ECS can be a feature of variably sized oral SCCs [29, 30], the event is not solely attributable to time. It is clear that other factors intrinsic to the tumour itself, its microenvironment and host reactions should be considered as well.

The concept of ECS as a powerful adverse prognosticator per se, may require further refinement. For instance, in assessing the "critical" extent of only microscopical ECS, the measurements in mm may be made perpendicular from the nodal outline or capsule to the most distant, perinodal infiltrative margin of the tumour. Support for this is given by the differences in the 5-year OS noted above together with the suggestion that the degree of ECS influences the effectiveness of adjuvant radiotherapy in preventing regional recurrence in the neck [30]. Of the other nodal prognostic factors, laterality and highest anatomical levels of involvement have emerged as front runners [30].

In contrast to the findings in oral SCC, the prognostic impact of ECS appears limited in oropharyngeal SCC. There have been few studies of patients with oropharyngeal tumours of known human papillomavirus (HPV) status. From a recent study [24] in which 89 % of the 101 primary oropharyngeal tumours were p16 positive, it emerged that a poorer survival (DFS, DSS, OS) was associated with soft tissue metastatic deposits but not with ECS of any extent if nodal tissue or architecture was still discernible. Negative p16 status and higher T-stage also correlated with poorer survival but patient age, gender, largest lymph node size, N-stage, chemotherapy treatment and resection margin status showed no significant correlation with survival on univariate survival analysis. The soft tissue metastatic deposits were associated with higher T stage and on multivariate analysis, were not an independent predictor of poor survival. Interestingly, the extent (grade) of ECS was independent of the size of the largest metastasis and the tumour p16 status suggesting, as in oral SCC, that multiple patient and tumour factors account for the extent of ECS. In a later study of only HPV-related p16-positive oropharyngeal carcinomas [33], the same investigators found that the strongest prognosticators of outcome (DSS, DFS and OS) were high T-stage and positive margin status. In contrast to the findings in oral SCC [29, 30], the oropharyngeal study of p16-positive tumours [33] found that ECS had weak or no prognostic impact in patients receiving post-operative radiotherapy, and that distant metastasis was more common than locoregional recurrence.

The prognostic importance of strictly defined and graded ECS and other nodal features in p16-negative oropharyngeal SCC patients is uncertain. However, given the current state of knowledge regarding oral SCC, it seems likely that ECS should still hold prognostic significance in such patients.

Features of unknown prognostic significance

These are summarised in Table 3.

In the UICC TNM Classification of Malignant Tumours [1] and in the pathological TNM classification, the definitions of micrometastasis and ITCs (Fig. 2) are based on their size and given in Table 2. The presence of a micrometastasis qualifies as a positive node and is indicated by the addition of the suffix "mi" (for instance, pN1(mi), pN2b(mi) or pN2c(mi)) [1]. In contrast, nodes containing only ITCs (profile diameter <0.2 mm or fewer than 200 cells) are categorised as pN0 [1]. Histological studies of opportune and elective NDs show micrometastases and/or small conventional metastases in up to 30 % of dissections, exceeding the rate of conversion to clinically positive necks in oral and oropharyngeal SCC patients managed by a "wait and see" policy, and suggesting that many micrometastases do not progress [34]. This supports the traditional view that micrometastasis or ITCs do not affect prognosis in oral or oropharyngeal cancers. Evidence to the contrary has emerged, however, from a recent study of sentinel lymph nodes in patients with early stage oral and oropharyngeal SCC [35]. This study reported statistically significant differences in DSS and/or OS between patients with a negative sentinel node and those whose sentinel nodes contained micrometastases or ITCs [32]. It is notable that micrometastasis or ITCs are of established prognostic significance in gynaecological, mammary, gastric, colorectal and oesophageal cancers [36–40].

Table 3 Features of unknown prognostic significance in ND specimens assessed by routine histology

1. Micrometastasis and ITCs
2. Perinodal emboli
3. Cystic metastasis in HPV/p16 negative tumours
4. Invasion pattern of extranodal spread
5. Hybrid phenotypes, dedifferentiation/high-grade transformation of metastatic deposit(s)
6. Desmoplasia
7. Collagenous stroma
8. Nodal capsular alterations
9. Reactions in non-metastatic lymph nodes

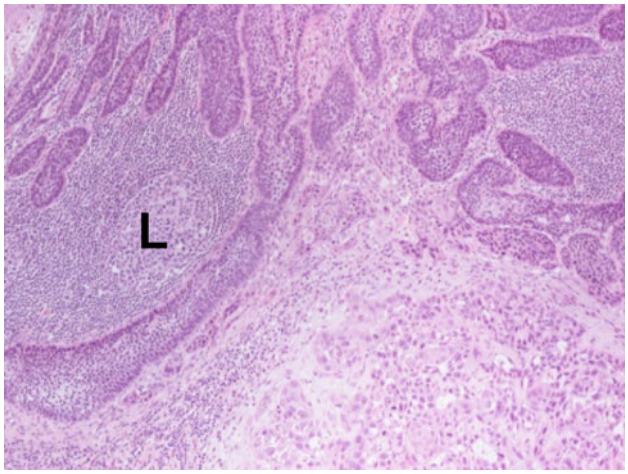

Fig. 5 Conventional nodal metastasis of oral SCC showing basaloid (*upper half of the picture*) and squamoid (*lower right of the picture*) cell phenotypes. *L* residual nodal parenchyma (haematoxylin and eosin, objective magnification ×4)

Perinodal lymphatic emboli (Fig. 1) seem easier to detect than their counterparts around the primary tumour.

It is important to distinguish between: (1) pseudocystic metastases resulting from central liquefactive necrosis or degeneration of an often heavily keratinised metastatic SCC in an otherwise solid node; and (2) true, cystic metastases characterised by a fluid-containing cavity lined by dysplastic/carcinomatous epithelium, often papillary, and supported by peripheral, lymphoid parenchyma. The occurrence of true, cystic metastases in cervical lymph nodes is established [41–44]. Interest in these initially focused on their distinction from branchial cleft (branchiogenic) carcinoma [45–48] and their significance as the presenting feature of an occult oropharyngeal or, less commonly, nasopharyngeal primary carcinoma [49]; and, more recently, on their significance as indicator of a distinct subset of oropharyngeal cancer characterised by particular origin and association with HPV [50, 51]. True, cystic, cervical metastases seem associated with carcinomas arising in Waldeyer's ring [52]. Regauer et al. [53] reported that while cytokeratin (CK) 7 is expressed in the primary and cystic metastatic deposits, it is absent from the normal lymphoepithelium covering Waldeyer's ring. On these grounds and endorsing CK7 as a marker of ductal differentiation, they suggested that the true, cystic metastases are attributable to carcinomas arising from excretory ducts of adjacent minor salivary glands with only limited surface or crypt involvement [53]. Except for interesting histogenetic considerations, these tumours show distinctive clinical features including lack of the usual tobacco and/or alcohol risk factors; occurrence in a younger age group; and small and often occult and slow-growing primary lesion [52]. The favourable prognosis of cystically metastasising carcinomas of Waldeyer's ring compared to oral or nasopharyngeal lesions with solid or pseudocystic, as defined above, metastases, originally ascribed to the intimate relationship of lymphocytes with crypt epithelium [53], is likely explained by their association with HPV-16/18 [50, 51]. The favourable response of HPV-associated carcinomas to organ-sparing treatment modalities and consequent improved prognosis is established [50, 54]. As the primary tumour associated with true, cystic metastasis shows non-keratinising morphology with a basaloid cell phenotype [50], HPV testing (screening by immunohistochemistry for p16 followed by in situ hybridisation for HPV) would be required for differentiation from true basaloid SCC, a specific histological variant that, when not arising in the oropharynx and associated with HPV, is clinically aggressive with a high rate of distant metastasis [51, 55–57].

Prognostic significance, if any, of the invasion pattern (cohesive versus dyscohesive/non-cohesive) at the advancing front of ECS has not been considered.

Hybrid phenotypes of metastatic oral and oropharyngeal SCC are known (Fig. 5) and dedifferentiation or high-grade transformation can be seen in metastases of salivary adenocarcinomas [8]. Assessing possible significance of these events is difficult in ND specimens because of undetermined or rare incidence.

That formation of desmoplastic stroma seems a requisite for the successful anchorage and growth of the metastatic deposit within the nodal parenchyma has been mentioned above. Desmoplasia usually comprises various mixtures of myofibroblasts and extracellular-matrix glycosaminoglycans (GAGs) [8, 9]. The eosinophilic cytoplasm of spindled myofibroblasts and the haematoxyphilic, myxoid appearance of accumulated GAGs conveniently enable identification of desmoplasia by routine histology (Fig. 4). As the presence of myofibroblasts in or absence of particular GAGs from the stroma of primary oral SCC are adverse prognosticators [58–62], it seems logical to assume that features of desmoplasia in nodal metastases are similarly significant and currently investigated. Little or no attention has been paid to desmoplasia in nodal metastases of other head and neck malignancies. Differences in incidence and ratios of desmoplastic components cannot be excluded. In our experience, metastases of salivary adenocarcinomas are not usually associated with desmoplasia (Figs. 3, 6), whereas metastases of neuroendocrine carcinomas may show desmoplasia rich in myofibroblasts [8]. Caution should be exerted because of limitations in the material available. These tumours are less common than oral SCC and their surgical resection is not usually accompanied by ND. The various histological types of salivary adenocarcinomas add to the difficulties, and require further assessment.

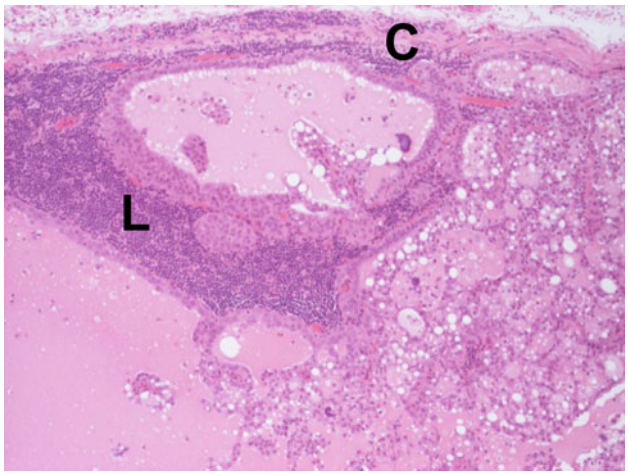

Fig. 6 Conventional nodal metastasis of acinic cell carcinoma. Desmoplasia is not seen. Compare with Fig. 4. *C* nodal capsule, *L* residual nodal parenchyma (haematoxylin and eosin, objective magnification ×4)

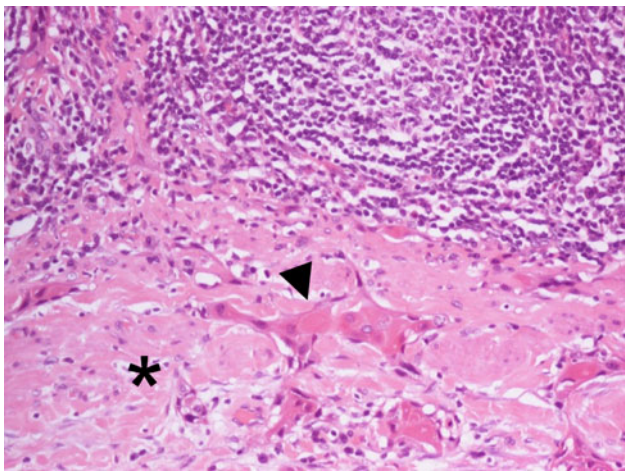

Fig. 7 Metastatic deposits of oral SCC (*arrowhead*) in collagenous stroma (*asterisk*). Nodal parenchyma is present at the *upper* half of the picture (haematoxylin and eosin, objective magnification ×10)

Very occasionally the metastatic deposit is set in dense collagenous stroma (Fig. 7). Whether this reflects a variant of desmoplasia or a microenvironment adversely affecting the growth of the deposit is unknown.

Alterations of the capsule in metastatic nodes are not rare and appear as focal or widespread thickening. A relationship to early ECS and a host response to the growing tumour are possible explanations. This is of academic as well as clinical interest, because focal capsular thickening may be difficult to distinguish from early ECS (Table 2). A recent investigation did not report a difference in clinical outcome between metastatic nodes with or without thickened capsule [24].

The local reactions in non-metastatic lymph nodes in positive and negative NDs, include sinus histiocytosis, cortical and/or paracortical hyperplasia, parenchymal depletion, vascular transformation, fibrosis, hyalinisation and sarcoidal/foreign-body granulomata (Fig. 8), and on occasions, may cause diagnostic difficulty. Possible pitfalls have been previously discussed [7, 8, 63]. The prognostic value of the different patterns, if any, is uncertain [64]. The generalised florid follicular or paracortical hyperplasia, more often associated with a large primary tumour in a younger patient, may reflect a host response to the tumour, reaction to recent biopsy or other investigative procedures, or a coincidental bacterial or viral infection [65]. Data on nodal parenchymal and vascular alterations following irradiation is lacking and it is unclear whether they account for the altered pattern of metastatic spread seen in salvage NDs [66].

Features assessed by specialised, though often available, means (immunohistochemistry, histochemistry)

Diagnostic applications

Although routine application of immunohistochemistry in detection of small cervical metastases appears attractive [67, 68], given the numbers of NDs, multiple nodes processed and the required multiple antibodies, it would present enormous logistical problems and cannot be accepted as a routine procedure in the examination of all specimens. In our opinion, endorsement of standardised neck reporting protocols [7, 8] provides the experience dedicated pathologists need to confidently detect small

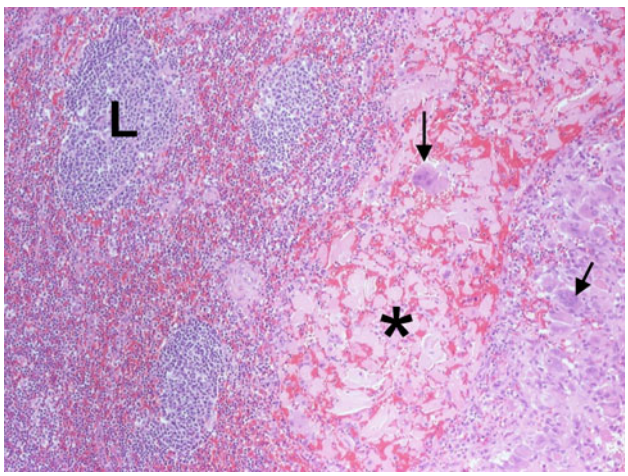

Fig. 8 Florid foreign-body granulomatous response to metastatic oral SCC, resulting in formation of eosinophilic, keratinous debris (*asterisk*). The *arrows* indicate multinuclear histiocytes. *L* nodal parenchyma (haematoxylin and eosin, objective magnification ×4)

conventional metastases or micrometastases on routine histology alone. We have previously suggested morphological criteria to assist assessment in the case of SCC, the most common head and neck malignancy [7, 8, 63]. This is straightforward when keratinisation and/or desmoplasia are present; in their absence, subtle changes in colour and nodal architecture, and appreciation of intercellular cohesion, mitotic activity and admixture with eosinophils would alert the primed pathologist to the presence of covert metastatic deposits [7, 8, 63]. Even ITCs, when they appear as tiny collections and not as individual units (Fig. 2), can be routinely detected without resorting to immunohistochemistry. In our practice, the application of immunohistochemistry in the pathological assessment of NDs is therefore very selective/occasional only and aims to confirm an already suspected metastasis rather than prospectively detect it. In the case of SCC, a panel of pan-CK antibodies, for instance AE1/AE3, MNF116 and 34βE12, would serve that purpose.

Immunohistochemistry would be helpful in: (1) assessing sentinel nodes; and (2) detecting ITCs occurring as individual cells, nodes containing such cells being categorised as pN0 followed by the suffix (i+) [1]. While the value of the former seems justified, particularly in institutions where sentinel node-based strategies are routinely applied, endorsement of the latter seems difficult because of logistical problems and controversy regarding the prognostic significance of ITCs as discussed above.

The application of histochemistry in the pathological assessment of NDs seems confined to the detection of mucin in metastatic deposits, the AB (Alcian Blue) pH 2.5-PAS (periodic acid-Schiff) procedure being most useful.

Investigative considerations

Although these would be invaluable, in general, research has tended to focus on the metastatic risk by studying features of the primary tumour rather than the metastatic deposit. Nevertheless, decreased expression of epithelial cell adhesion molecule (Ep-CAM), nm23 and, possibly, CD44 has been reported in cervical metastases of SCC in comparison with the primary tumours [69, 70].

Table 4 proposes relevant immunohistochemical investigations that seem worthy of pursuing and may provide evidence of variably aggressive subclones in the metastatic deposit.

Histochemistry would be of value in assessing possible differences in mucosubstances between primary and metastatic salivary adenocarcinomas.

The use of electron microscopy in the investigation of cervical, nodal metastasis is limited [71].

Table 4 Proposed immunohistochemical investigations of cervical metastatic deposits

Assessment of the metastatic deposit itself
1. Cytoskeleton (e.g. CKs)
2. Intercellular cohesion (e.g. cell adhesion molecules/CAMs)
3. Cell cycle/kinetics (e.g. Ki67, p53)
4. p16 screening prior to HPV detection
Assessment of nodal desmoplastic components
Assessment of deposit/desmoplastic stroma interactions
1. Basement membrane
2. Integrins
Assessment of nodal parenchymal reactions/subpopulations of defence cells
Assessment of nodal angiogenesis (growth factors/receptors)

Features assessed by highly specialised means of rather limited availability (molecular and other non-morphological technologies)

The trends are similar to those discussed above. The high throughput molecular techniques such as microarrays and mass spectrometry, "omics" technologies, FISH and reverse transcriptase (RT)-PCR, have little application in routine histopathological assessment of ND specimens [72, 73], with the exception of seeking HPV in nodal metastases from unknown primary or oro- and naso-pharyngeal carcinomas (see above) and in detecting ITCs or micrometastases. Nodes containing ITCs detected by non-morphological techniques are categorised as pN0(mol+) [1]. RNA-based markers have been found useful in detecting micrometastases and include CK20 mRNA expression [74] and RT-PCR for CKs 14, 19 and 20 [75, 76].

In contrast, molecular techniques are now widely used in research. Again, this tends to focus on the metastatic risk by studying features of the primary tumour (see above) rather than prognostic factors related to established nodal metastatic deposits. Genetic or gene expression signatures—sets of predictive genes—studied by microarrays or immunohistochemical signatures studied by tissue microarrays (TMAs) may be more valuable in predicting the metastatic risk than single markers [77–79]. The potential of microRNAs, a class of short non-coding RNAs that down-regulate gene expression, as diagnostic and prognostic tools is as yet uncertain [80]. One-step nucleic acid amplification (OSNA) has been used intraoperatively to detect CK19 mRNA expression in nodes [81, 82]. Another approach is determination of p53 mutations in nodes using mutant allele specific amplification (MASA) [83]. Cancer cells in blood and bone marrow and lymph nodes have been detected by RT-PCR for E48 (Ly-6D) antigen, selectively expressed in head and neck SCC [84, 85]. TCR

repertoire analysis in metastatic, cervical, lymph nodes has also been undertaken [86].

Support for the potential value of molecular analysis of metastatic nodal tissue is given by the finding of different methylation patterns between primary and metastatic head and neck SCC [87]. Similar investigations of salivary tumours or subsets of SCC arising at particular sites (i.e. oropharyngeal, oral or nasopharyngeal) are desirable. Molecular analysis of viable tumour in post-irradiated lymph nodes (i.e. following primary therapy for oral SCC) and recurrence in the neck to define markers of resistance and novel mutations, which may be targeted for therapy, also appears of interest.

So far routine clinical application for the relatively costly and often time consuming molecular techniques remains a challenge. They still compare unfavourably with the traditional pathological assessment. In addition to cost and logistical considerations, a potential problem in using molecular techniques to identify early metastatic disease is the occurrence of false-positive results due to the stability of tumour DNA [73].

However, traditional pathologic assessment requires a ND to be performed, and ND is part of the treatment in only a selection of patients with head and neck SCC and still is an invasive procedure with associated morbidity. Novel, non-invasive technologies would be therefore welcome in clinical practice. Genetic signatures, although promising, have been insufficiently strong to be valuable for decision-making in an individual patient and seem to offer no benefits over traditional histological prognosticators up to date [73]. Nevertheless, molecular technology is rapidly advancing and shall hopefully come nearer to clinical implementation [88].

Correlations with preoperative assessment

Although this review focuses on assessing cervical tissues post-operatively obtained, it was felt that a brief comparison between such analysis and various types of pre-operative assessment of the neck would be useful.

Advanced metastatic disease with cystically degenerate/matted nodes is generally obvious on clinical palpation of the neck except following previous treatment by surgery and/or (chemo-) radiotherapy when it may be indistinguishable from extensive fibrosis [66]. Even in the untreated neck, however, palpation and routine imaging underestimate the extent of metastatic disease in terms of anatomical level of involvement, number of involved nodes, and presence and extent of ECS [30]. The criteria for diagnosis of established metastases by MRI and CT seem established [85], but the detection of small metastatic deposits and early ECS, and discriminating between enlarged reactive and metastatic nodes, is inadequate [29, 89], whereas detection of micrometastases and ITCs is at present impossible. Ultrasound-guided fine-needle aspiration cytology can detect tumour deposits greater than 5 mm with 90 % sensitivity and 100 % specificity in experienced hands, but with less than 50 % sensitivity in the N0 neck [89–91]. Moreover, it cannot establish the presence and extent of ECS. Even after work-up by an experienced head and neck oncology team, 20–30 % of "negative" necks harbour nodal metastases [92] and 17 % show ECS [30].

^{18}F-FDG PET is superior to CT or MRI in assessing morphologically-normal nodes, but anatomical resolution is poor and technical resolution limits mean that tumour deposits less than 5 mm are likely to be missed [93]. Hence, in the pre-operative "N0" neck, the sensitivity of FDG PET is only 50 % [94].

A meta-analysis of clinically N0 necks [95] showed similar diagnostic accuracy for CT, MRI, PET and US (pooled estimates for sensitivity on a per-neck basis were 52, 65, 66 and 66 %, respectively, while the corresponding values for specificity were 93, 81, 87 and 78 %). Even fused PET/CT which provides accurate co-registration of functional and anatomical images is constrained by technical resolution limits and cannot reliably detect deposits less than 5 mm in profile diameter [89]. Similarly, enhanced MRI techniques such as diffusion-weighted, dynamic contrast-enhanced and nanoparticle-enhanced cannot detect micrometastases [89].

Although of obvious value, the various types of preoperative assessment of the neck, currently in use, do not compare favourably with detailed and standardised pathological examination. Even in the present era of rapid advances in molecular biology, routine histology is still considered the gold standard [34, 89, 96] and offers the greatest likelihood of accurately "mapping" cervical metastatic disease.

Epilogue—the future is not here

Novel molecular techniques are exciting as they allow assessment of metabolic or replicative cell pathways significant in the pathogenesis of cancer. It is, however, disappointing how little they have so far affected therapeutic strategies in head and neck malignancy. Further technological advances may change the situation, but as regards prognostic information the scales are still tipped towards routine pathological assessment. The latter remains the gold standard, against which all other methodologies should be tested. It is, however, only applicable in tissues obtained after surgery and many patients with head and neck cancer are non-surgically treated. Contemporary imaging techniques, likely the second best, add value and

molecular biological information is promising. In order to achieve the optimal prognostic information, development of an index combining metastatic volume, distribution of metastases and the presence and pattern of ECS is desirable. All sources of information on prognosis (clinical, radiological, histopathological and molecular biological) are not competitive but complementary.

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