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Guidelines for the laboratory handling of laryngectomy specimens

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These guidelines are intended to facilitate the dissection and reporting of those tumours of the larynx, hypopharynx, and piriform fossa that may be treated by laryngectomy. The guidelines incorporate the core data for histopathology reports on head and neck carcinomas previously published by the Royal College of Pathologists[1] which indicate the information required, in addition to clinical data, for the consistent management of these diseases and to give patients as accurate a prognosis as possible.

The optimal reporting of specimens from the head and neck area requires a partnership between the pathologist and surgeon/oncologist. The surgeon can help the pathologist by the appropriate handling and labelling of the specimen in the operating theatre. The discussion of cases at clinicopathological meetings, and correlation with preoperative imaging studies, are important in maintaining and developing this partnership.

The larynx is a difficult specimen to dissect owing to its complicated anatomy and to the mixture of soft and calcified tissues that are present. A careful, methodical approach is required to provide an accurate record of the extent of spread of laryngeal neoplasms. The pathologist needs to understand the normal anatomy of the larynx, the types of operation performed by surgeons for laryngeal cancer, and the nature of the information required by surgeons for patient management and audit.

Normal anatomy of the larynx and hypopharynx

The skeleton of the larynx is formed by a group of cartilages that are connected by ligaments and fibrous membranes and moved by intrinsic and extrinsic muscles. The cricoid cartilage forms a complete ring around the airway with a narrow anterior arch and a broader, flatter posterior lamina that forms most of the posterior wall of the larynx. The cricoid cartilage articulates with the inferior horns of the thyroid cartilage which is formed by two quadrilateral laminae that are fused in the anterior, median plane at an angle of about 90° in males and 120° in females to form the laryngeal prominence. The laminae form the lateral wall of the larynx and are connected by the thyrohyoid and cricothyroid ligaments to the hyoid bone and cricoid cartilages, respectively. The arytenoid cartilages are roughly pyramidal in shape, articulate with the cricoid cartilage, and support the vocal cords. The corniculate and cuneiform cartilages lie in the aryepiglottic folds and, like the epiglottic cartilage, are fibrocartilage. The thyroid, cricoid, and most of the arytenoid cartilages are hyaline cartilage that undergoes patchy calcification and ossification after the age of 25 years.

The hypopharynx comprises:

- the *posterolateral pharyngeal wall*, which extends from the level of the floor of the vallecula to the level of the inferior border of the cricoid cartilage;
- the *postcricoid oesophagus*, which only has an anterior wall and extends from the level of the arytenoid cartilages superiorly to the inferior border of the cricoid cartilage;
- the *piriform sinuses*, which lie lateral to and below the opening of the larynx. Each is bounded laterally by the medial aspect of the thyroid lamina and medially by the aryepiglottic fold.

The mucosal surface of the anterior epiglottis is reflected onto the base of the tongue. A prominent median glosso-epiglottic fold divides this region into the right and left valleculae.

Surgical anatomy of the larynx and hypopharynx

Laryngeal carcinomas are classified according to their site of origin:

- *supraglottic carcinomas* arise above the true vocal cords from the epiglottis, the false cords, or from the ventricles which lie between the false and true cords;
- *glottic carcinomas* arise from the vocal cords, the anterior and posterior commissures, or from the vocal processes of the arytenoid cartilages;
- *subglottic carcinomas* arise from the region extending inferiorly from the vocal cords to the lower border of the cricoid cartilage;
- *transglottic carcinomas* extend across two or three of these regions.

A careful description of the site, size, and extent of spread of a tumour (particularly

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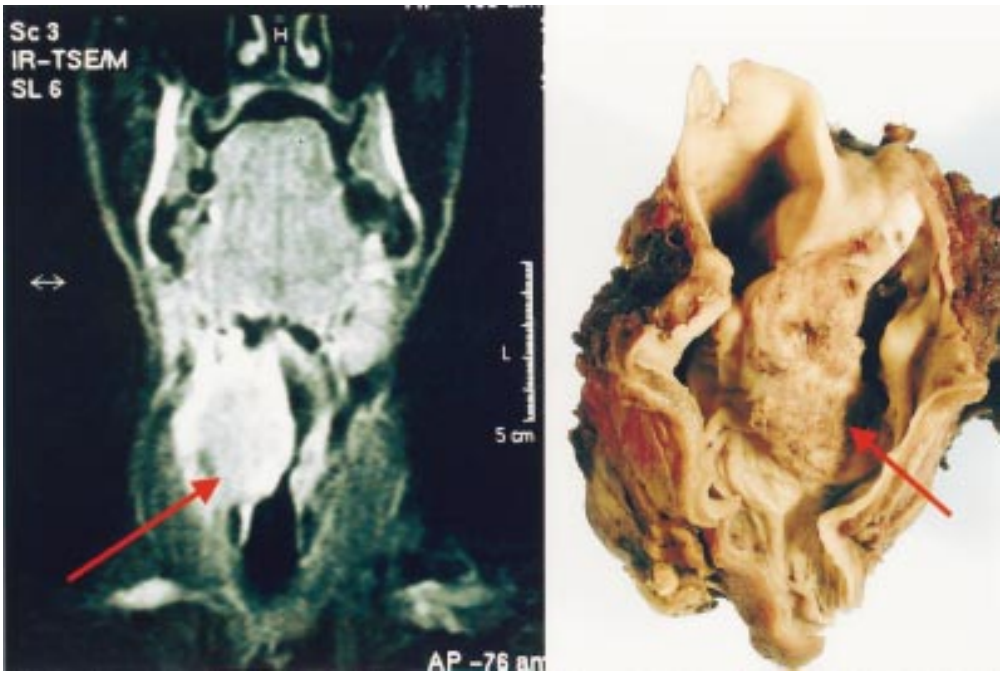

Figure 1 Coronal magnetic resonance image of a carcinoma of the right piriform fossa, seen macroscopically on the right. The specimen has been opened posteriorly to demonstrate the tumour.

between regions) is important in determining the prognosis for a patient and the need for adjuvant postoperative treatment. The spread of laryngeal carcinomas is largely determined by the anatomy of the cartilages and ligaments.[2 3] Carcinomas spread easily within the supraglottic and subglottic compartments, but are restricted by the elastic ligaments and cartilages. Carcinomas of the true vocal cords metastasise relatively infrequently owing to the poor lymphatic drainage of this area. In contrast, carcinomas of the supraglottis frequently metastasise.[4] Carcinomas of the true cords may spread from one side to the other at the anterior commissure, remaining confined to the cords, or may extend upwards towards the base of the epiglottis, usually invading the thyroid cartilage in the process. Carcinomas of the epiglottis may be confined to the epiglottis or may involve its base, where fenestrations in the cartilage allow invasion of the pre-epiglottic space.

The spread of carcinomas arising in the hypopharynx is less well determined.[5] Small carcinomas of the medial wall of the piriform fossa tend to spread into the supraglottic larynx, and spread through the cricothyroid membrane to structures outside the larynx. Carcinomas of the lateral piriform fossa often extend through the thyroid cartilage into the thyroid gland, and also extend through the submucosa into the posterior pharyngeal wall.

Surgical procedures for laryngeal cancer

The treatment offered to a patient with laryngeal or hypopharyngeal cancer is determined by the extent of local spread of the tumour, and by local practices that determine whether small laryngeal carcinomas are treated by radiotherapy, laser ablation, or partial laryngectomy.

Total laryngectomy is usually performed for advanced carcinomas that have crossed the ventricle to involve both the true and false cords, and is the usual surgical option for carcinoma of the piriform sinus. This is also the salvage procedure for carcinomas that have failed to respond to radiotherapy.

Partial laryngectomy may be horizontal or vertical. *Horizontal supraglottic laryngectomy* allows the removal of tumours in the anterior supraglottis without disturbing the true vocal cords. This operation removes all supraglottic structures in continuity with the pre-epiglottic space. A 3–4 mm clearance inferiorly is usually adequate. *Vertical hemilaryngectomy* may be performed for carcinoma of the true vocal cord, and may or may not include part of the body of the arytenoid cartilage. If carcinomas of the vocal cord recur after this procedure, this is usually because of involvement of the inferior margin.[4]

Dissection of the larynx

Historically, whole organ sections were prepared after decalcification of the intact organ and embedding in celloidin or paraffin wax.[2 6] Although effective, this method is too time consuming for most laboratories and the prolonged exposure to acid decalcifying agents impairs immunohistochemical and molecular techniques. The use of computed tomography and magnetic resonance imaging to obtain horizontal slices through the larynx preoperatively makes it preferable for the pathologist to provide histological preparations of the tissue in a similar plane (figs 1 and 2). The method introduced by Michaels and Gregor provides the paradigm for this approach.[7]

EQUIPMENT REQUIRED

Photographic equipment is required to record the macroscopic appearances and to provide a

print onto which the location of histological blocks of tissue can be marked. A digital camera linked to a computer allows this to be done electronically. A rotary meat saw ("bacon slicer") is helpful, although a long bladed knife and hacksaw will often suffice.

DISSECTION PROCEDURE

1. The larynx is opened vertically in the midline posteriorly, so that the mucosal surface can be inspected, described, and photographed if necessary (fig 1). It is useful to mark surgically critical margins (mucosal and lateral soft tissues, and soft tissues at the base of the tongue) with Indian ink or a suitable artist's pigment.

If required, fresh tissue can be taken and frozen for DNA or RNA analysis, and small samples of tissue may be fixed immediately in glutaraldehyde for electron microscopy.

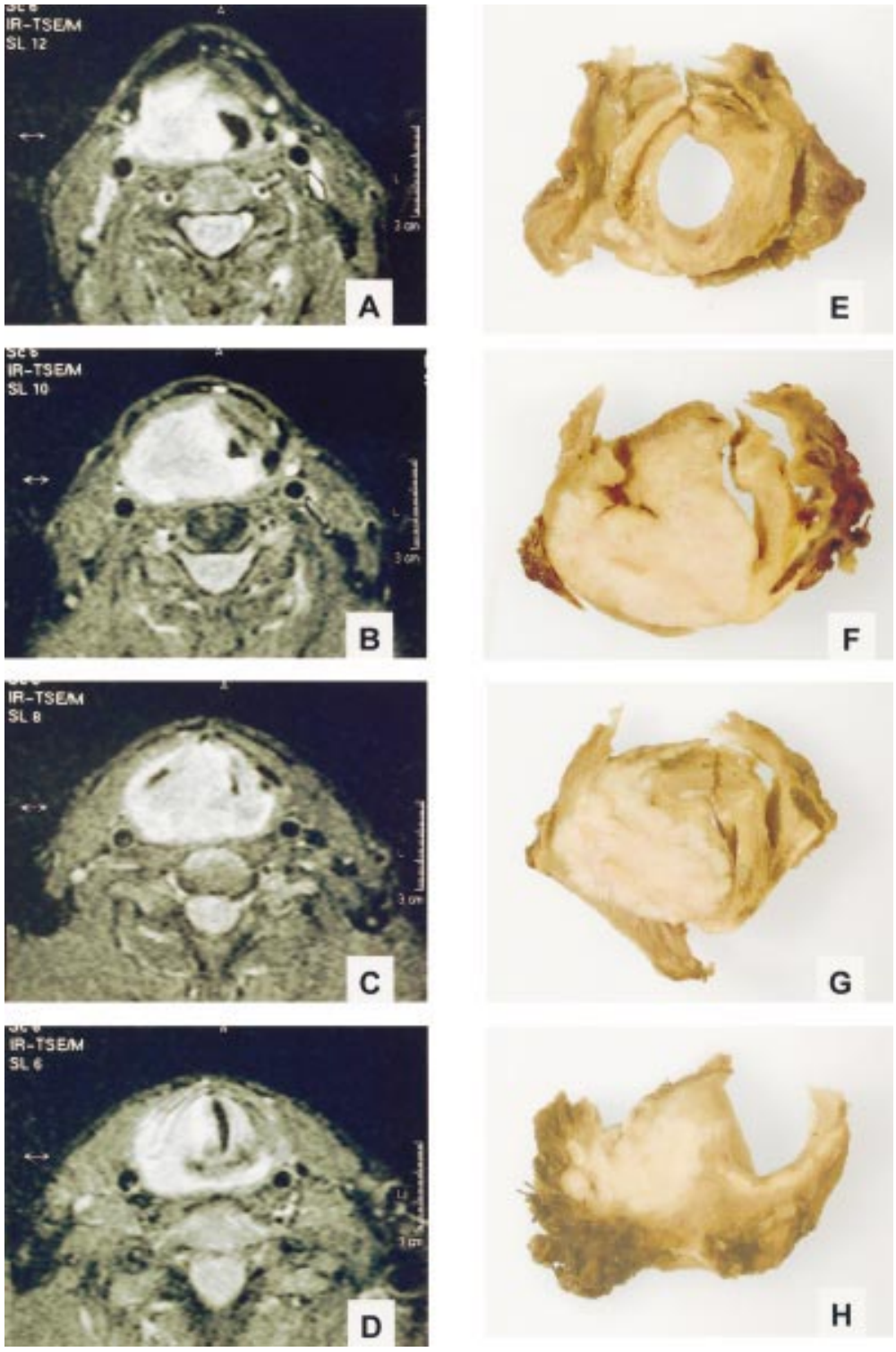

Figure 2 (A)–(D) Horizontal magnetic resonance images of the carcinoma seen in fig 1, showing invasion of the tumour along the right side of the larynx. The corresponding macroscopic slices of the larynx are shown in (E)–(H).

It is often easier to take blocks showing the relation of a hypopharyngeal carcinoma to the nearest mucosal margins before distortion of the soft tissues during fixation and slicing.

2. The larynx is fixed for 24–48 hours in 10% buffered formol saline, or other routine fixative.
3. The hyoid bone is removed and the pre-epiglottic tissue is inspected. If it is suspected that the tumour is in the soft tissue, the hyoid should be cut transversely by sawing, and histological samples taken.
4. The larynx is sliced transversely. This is most easily performed with a rotary meat cutting machine,[7] producing 4 mm thick slices. Alternatively, slightly thicker slices can be obtained using patience, a sharp long bladed knife (which will deal with lightly calcified and soft tissues), and a hacksaw. The transverse slices should be photographed so that the location of the histological blocks can be recorded (fig 2).

   This method ensures that the extent of invasion of tumour around or through the laryngeal cartilages and into perilaryngeal tissues is identified. The vertical extent of tumour spread is assessed by following the tumour through sequential slices.
5. For supraglottic and hypopharyngeal carcinomas, blocks should include the relation between the carcinoma and the anterior (submucosal) resection margin at the base of the tongue. The extent of spread of carcinoma into the base of the tongue can be assessed by continuing the axial slicing of the specimen superiorly with a long bladed knife.
6. Partial laryngectomy specimens should be received pinned onto cork board and oriented. As the inferior margin is usually the most surgically critical margin, the specimens should be cut parallel to the supero-inferior axis of the tissue, marking the margin with ink.
7. Histological blocks are trimmed to size with a scalpel, decalcified (in formic acid or EDTA), processed to paraffin wax, sectioned, and stained with haematoxylin and eosin. Special stains are rarely required for typical squamous carcinomas.

MACROSCOPIC DESCRIPTION

This should include:

1. The overall dimensions of the specimen and a note of other tissues, for example the hypopharynx and thyroid, included with the larynx.
2. The anatomical site of the tumour (glottis, supraglottis, subglottis, or transglottis), its relation to the midline (left, right, or both) and mucosal margins.
3. After slicing, record the largest tumour diameter, the depth of tumour invasion, the structures invaded, and the longitudinal extent of the tumour, particularly noting spread between regions in the larynx.
4. Abnormalities in the specimen that are apparently unrelated to the main tumour, for example thyroid nodules.

SELECTION OF BLOCKS FOR HISTOLOGY

- Tumour: At least one block per 10 mm diameter of tumour, including one selected to demonstrate the maximum depth of invasion; whole tumour if less than 10 mm;
- Blocks of defined mucosal and soft tissue margins, including pharyngeal, oesophageal, and tracheal margins;
- Non-neoplastic mucosa;
- Bone or cartilage, if grossly-involved by tumour;
- Thyroid if present;
- Lymph nodes, if present in the paratracheal region adjacent to the thyroid;
- Tracheostomy site.

Minimum dataset for histopathology reports

The core dataset produced by the Royal College of Pathologists[1] applies to the reporting of primary squamous carcinomas of the upper aerodigestive tract including the hypopharynx and larynx. Similar principles may be applied to the reporting of other mucosal malignancies arising in this area. The College guidelines are presented as a proforma that lists the core data items that may be applied across the head and neck region; most features have evidence to support their inclusion in the reporting of laryngeal and hypopharyngeal carcinomas.

CLINICAL AND MACROSCOPIC DATA

1. Site(s) and side(s) of the carcinoma(s). For carcinomas that involve more than one site, the principal site of involvement should be recorded; this may not be the site of origin. The involvement of associated sites can be noted if required. Sites and subsites should be recorded according to the UICC nomenclature (see appendix).
2. Type of resection specimen, for example total or partial laryngectomy.
3. Clinical TNM stage. The final pathological T coding in the larynx will be determined by clinical features such as vocal cord mobility.[8]
4. Previous radiotherapy or chemotherapy; this may influence the interpretation of the histological changes in the current specimen and should prompt a free text comment on the response to treatment.

PATHOLOGICAL DATA

1. *Maximum diameter of tumour* (mm). The macroscopic diameter should be used unless the histological extent is greater than macroscopically apparent. As for other tissues, measurements are made pragmatically, acknowledging distortion of tissues by fixation and processing.
2. *Maximum depth of invasion* (mm) below the luminal aspect of the surface; if the tumour has ulcerated then the reconstructed surface should be used. A more detailed comment on the nature of the tissues invaded (mucosa, cartilage, muscle, and so on) should occur in the comments sections.
3. *Histological type of carcinoma*. The guidelines specifically apply to typical squamous carcinomas. Subtypes of squamous carcinoma,

such as papillary, verrucous, basaloid, adenosquamous, and spindle cell carcinoma, should be recognised[9] and potential prognostic implications noted in the comments section.

4. *Degree of differentiation*. Grading is based on the degree of resemblance of the carcinoma to the normal epithelium and follows the descriptions in the WHO classification.[9] The most aggressive area (medium magnification field) is graded as well differentiated, moderately differentiated, or poorly differentiated. This system is widely used and prognostically useful, even though it suffers from interobserver variability and sampling problems.[10 11] While most squamous carcinomas will be moderately differentiated, it is important to separate well differentiated and poorly differentiated tumours for prognostic purposes.
5. *Invasive front of the carcinoma*. The pattern of invasion by the carcinoma at its deep margin is of proven prognostic value for oral carcinomas.[12 13] Studies of laryngeal tumours suggest that a similar approach may be of value.[14]

   Scoring systems for histopathological features of squamous carcinomas include features related to differentiation and to the tumour/stromal interaction.[14 15] While these may improve the consistency of reporting, they are not in widespread use and it is suggested at present that the recording of differentiation and invasive pattern is made separately.

   It should be recognised that the pattern of tissue invasion by carcinoma is a continuous spectrum of changes. For prognostic purposes, the cut off appears to be between broad cohesive sheets or groups of cells and narrow strands of cells (> 15 cells across) or non-cohesive small groups and single cells.[13]
6. *Distance from invasive carcinoma to surgical margins* (mm). Measure the distance histologically for both mucosal and deep margins. From a surgical point of view, > 5 mm is clear, 1–5 mm is close, and < 1 mm is involved.
7. *Vascular invasion*. The presence or absence of vascular invasion should be mentioned if it is an obvious feature at medium magnification. The presence of carcinoma cells within an endothelium lined space is the essential criterion. It is not necessary to distinguish between small lymphatics and venous channels.
8. *Severe dysplasia/in situ carcinoma*. Epithelial dysplasia forms a continuous spectrum of appearances from mild to severe dysplasia/carcinoma in situ.[9] Severe dysplasia/carcinoma in situ is associated with a high risk of progression to carcinoma and its presence both adjacent to the primary carcinoma and at the resection margins (where it may predict local recurrence) should be recorded.[16]

OTHER FEATURES THAT MAY BE REPORTED

These features should be included as part of a comprehensive description of a carcinoma and the surrounding tissues. Some are preferences of individual centres, but are considered to be of uncertain prognostic significance and therefore are not part of the minimum dataset at present.

1. Type and intensity of inflammatory infiltrate and desmoplastic stromal response.
2. Involvement of a tracheostomy (if present).
3. Response to previous treatment (if applicable).
4. Results of other investigations, for example flow cytometry, and molecular and immunohistochemical studies. While molecular markers predictive of tumour behaviour or response to treatment may be required in the future, current surgical practice does not demand their inclusion in the minimal dataset.

The role of immunohistochemical techniques

A wide range of antibodies is available to help resolve diagnostic problems. Most lack a precise tissue or neoplasm specificity, so that a combination of appropriate results is required to make a diagnosis. These results should always be consistent with the haematoxylin and eosin appearances.

DIAGNOSTIC CODING OF PRIMARY CARCINOMAS

pT status should be recorded according to the UICC guidelines,[8] and SNOMED T codes should be recorded (see appendix).

Appendix

TNM classification of malignant tumours[8]

HYPOPHARYNX

T1 Tumour limited to one subsite and 20 mm or less in greatest dimension;
T2 Tumour involves more than one subsite or measures 21–40 mm in size;
T3 Tumour > 40 mm in size or with fixation of hemilarynx;
T4 Tumour invades adjacent structures.

LARYNX, SUPRAGLOTTIS

T1 Tumour limited to one subsite with normal vocal cord mobility;
T2 Tumour invades more than one adjacent subsite without fixation of larynx;
T3 Tumour limited to larynx with vocal cord fixation, and/or invades postcricoid area, pre-epiglottic tissues, or deep base of tongue;
T4 Tumour invades soft tissues of the neck, thyroid, or oesophagus.

LARYNX, GLOTTIS

T1 Tumour limited to vocal cords with normal mobility;
T2 Tumour extends to supraglottis and/or subglottis and/or with impaired vocal cord mobility;
T3 Tumour limited to larynx with vocal cord fixation;
T4 Tumour invades extends into soft tissues of neck, thyroid or into oesophagus.

LARYNX, SUBGLOTTIS

T1 Tumour limited to subglottis;
T2 Tumour extends to vocal cords with normal or impaired mobility;
T3 Tumour limited to larynx with vocal cord fixation;
T4 Tumour invades extends into soft tissues of neck, thyroid or into oesophagus.

Sites and subsites with SNOMED 'T' codes

T-60300 HYPOPHARYNX

T-60320 Piriform sinus
T-60350 Posterior pharyngeal wall
T-24080 Pharyngo-oesophageal junction (post-cricoid area)
T-24100 LARYNX
T-24010 Epiglottis
T-24310 Aryepiglottic fold, laryngeal aspect
T-24320 Ventricular bands (false cords)
T-24440 Glottis
T-24400 Vocal cords
T-24470 Commissures
T-24450 Subglottis

1 Helliwell TR, Woolgar JA. Minimum datset for the reporting of head and neck carcinomas. London: The Royal College of Pathologists, 1998.
2 Tucker GFJ. A histological method for the study of the spread of carcinoma within the larynx. *Ann Otol Rhinol Laryngol* 1961;**70**:910–21.
3 Gregor RT. Tumours of the larynx. In: Jones AS, Phillips DE, Hilgers FJM, eds. *Diseases of the head and neck, nose and throat*. London: Arnold, 1998:207–29.
4 Kirchner JA. The relationship of the pathologist to the laryngologist. In: Ferlito A, ed. *Surgical pathology of laryngeal neoplasms*. London: Chapman and Hall Medical, 1996:1–8.
5 Jones AS. Tumours of the hypopharynx. In: Jones AS, Phillips DE, Hilgers FJM, eds. *Diseases of the head and neck, nose and throat*. London: Arnold, 1998:230–49.
6 Tucker GFJ. *Human larynx coronal section atlas*. Washington, DC: Armed Forces Institute of Pathology, 1971.
7 Michaels L, Gregor RT. Examination of the larynx in the histopathology laboratory. *J Clin Pathol* 1980;**33**:705–10.
8 Sobin LH, Wittekind C. *TNM classification of malignant tumours*, 5th ed. New York: John Wiley and Sons, 1997.
9 Shanmugaratnam K. *Histological typing of tumours of the upper respiratory tract and ear*, 2nd ed. Berlin: Springer-Verlag, 1991.
10 Roland NJ, Caslin AW, Nash J, *et al.* Value of grading squamous cell carcinoma of the head and neck. *Head Neck* 1992;**14**:224–9.
11 Henson DE. The histological grading of neoplasms. *Arch Pathol* 1988;**112**:1091–6.
12 Bryne M, Nielsen K, Koppang HS, *et al.* Reproducibility of two malignancy grading systems with reportedly prognostic value for oral cancer patients. *J Oral Pathol Med* 1991;**20**:369–72.
13 Odell EW, Jani P, Sherriff M, *et al.* The prognostic value of individual grading parameters in small lingual squamous cell carcinomas. *Cancer* 1994;**74**:789–94.
14 Jakobsson PA, Eneroth CM, Killander D, *et al.* Histologic classification and grading of malignancy in carcinoma of the larynx. *Acta Radiol* 1973;**12**:1–8.
15 Bryne M, Koppang HS, Lilleng R, *et al.* New malignancy grading is a better prognostic indicator than Broder's grading in oral squamous cell carcinomas. *J Oral Pathol Med* 1989;**18**:432–7.
16 Lumerman H, Freedman P, Kerpel S. Oral epithelial dysplasia and the development of invasive squamous cell carcinoma. *Oral Surg Oral Med Oral Pathol* 1995;**79**:321–9.