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Macroscopy of specimens from the head and neck

Brendan Conn¹, Miranda Pring², Adam V Jones³

¹Pathology Department, Royal Infirmary of Edinburgh, Edinburgh, UK

² Department of Oral and Dental Sciences, Bristol Dental School, University of Bristol, Bristol, UK.

³Department of Cellular Pathology, University Hospital of Wales, Cardiff, United Kingdom

Abstract

Macroscopic examination of surgical resections from the from the head and neck may be difficult due to the complex anatomy of this area. Recognition of normal anatomical structures is essential for accurate assessment of the extent of a disease process. Communication with the surgical team, correct specimen orientation and sampling are critical for assessment and the importance of radiological and clinical correlation is emphasised. Tumour involvement at each subsite is highlighted with reference to where there are implications on pathological staging and the potential need for adjuvant therapy.

Introduction

Macroscopic assessment is the initial step in pathological analysis and is just as important as microscopic examination when constructing a histopathology report; if not more so. In contrast to microscopy, there is limited published evidence to support inclusion of specific macroscopic data items and a clear lack of consensus regarding methodological assessment. The literature including cancer datasets has limited information on approach to macroscopic technique (1-8). A frequent cause for concern in head and neck is a failure to recognise normal anatomical structures. This is required for accurate assessment of the extent of the disease process and accurate pathological staging. It is for this reason that the pathologist trimming the specimen should have a sound awareness of the anatomy and pathology at this anatomical sub-site. Delegating this procedure to an inexperienced trainee in the absence of appropriate specialist supervision is not only unfair to the individual but may compromise the clinical decision-making process and ultimately the patient. In the face of a particularly complex specimen, or one where specimen integrity has been compromised intraoperatively it is appropriate to request that the surgeon attends the cut-up bench to discuss and jointly orientate the specimen. Megablocks may facilitate assessment of laryngeal and segmental resections of the maxilla and mandible, but the pathologist should be sensitive to the potential impact on the laboratory in terms of additional workload and the technical skill required to process and section the material. They will not be available in all laboratories and under such circumstances the pathologist should sample critical areas with a 'jigsaw puzzle' technique, subdividing the tissue slices into multiple standard size cassettes. Appropriate use of specimen photography and a block key is important particularly as the material may be subject to decalcification and extended processing; histology sections may not be available for several weeks.

Mucosal biopsies

Superficial floor of mouth, gingival and laryngeal mucosal biopsies tend to fold on fixation leading to suboptimal orientation, tangential sectioning and difficulty in assessment.

Surgeons should place samples on blotting paper or suture card prior to receipt by the lab to ensure orientation is maintained, tissue embedding is simplified and sectioning is optimised.

Odontogenic Cysts

Odontogenic specimens may arrive in the form of a completely intact cyst attached to a tooth but are often composed of loose fragments of curetted fibrotic tissue. Inflammation can compromise microscopic interpretation by masking characteristic histological features with loss of discerning features. The point of attachment of a cyst to the tooth must be determined and is required for diagnosis. (9) Attachment of a cyst to the root apex of a non-vital tooth suggests a likely radicular cyst and envelopment of the crown with attachment at the amelocemental junction (ACJ) often a dentigerous cyst. Access to and interpretation of radiographs alongside an assessment of the anatomical relationship to the dentition is fundamental to histopathological interpretation and a correct diagnosis. Decalcification and sampling of teeth in these situations generally offers no additional benefit. The soft tissue components should be trimmed and orientated in the cassette in a way that allows optimum assessment of the epithelial lining and cyst wall. Odontogenic tumours such as an adenomatoid odontogenic tumour and ameloblastoma may mimic and present in a dentigerous relationship; however, would not be adherent to the ACJ and present with a capsule that is thicker +/- a solid component. The latter should alert the practitioner to a potential tumour and need for thorough sampling.

Oral cavity & oropharyngeal tumours

Resection specimens should be orientated by the surgeon and critical margins illustrated using sutures or tags. Margins can be inked to facilitate the proximity of the tumour and the specimen cut into 3–5 mm parallel slices to demonstrate its relationship to mucosal margins and measurement of the maximum depth of invasion. Specimens from the central and lateral parts of the oral cavity should be cut in the coronal plane and those from the anterior sliced in the sagittal plane. For tumours close to or involving bone, soft tissue blocks should be obtained prior to decalcification to expedite communication of margin status and preserve tissue integrity for further investigations including genetic studies; however, it is prudent to retain some soft tissue in situ for orientation. A bandsaw is preferable to a bone saw as the former can provide a better appreciation between soft and hard tissue. Extensive sampling of bone is not necessary, only blocks to confirm or refute the presence of bone invasion as well as cruciate margins. Resections from the oropharynx and tonsil follow the same general principle as oral cavity. Occasionally these lesions may require resection of retromolar mandible and maxillary tuberosity to enable surgical clearance. Traditionally oral and oropharyngeal resections were managed in a specimen driven approach; however, a defect driven approach is increasingly used where surgeons sample the tumour bed following en-bloc resection. It is essential that surgeons orientate marginal biopsies and the pathologist interprets these in the context of radial margins. (10, 11)

Macroscopic measurements essential for staging purposes include maximum diameter, depth of invasion and bone involvement. (12) Awareness of pre-operative radiological findings can assist macroscopic evaluation of oral cavity resections; however, imaging may overestimate stage due to inflammation and scarring secondary to biopsy, overestimate depth through inclusion of the exophytic component and underestimate early bone invasion. Maximum tumour diameter directly influences tumour stage and should be

measured in millimetres during dissection. Small tumours may require correlation with microscopic diameter. Histological depth also has a direct influence on stage and must only include tumour invading beneath the normal level of mucosa (fig 1). Depth of invasion is reliant on microscopic assessment and measured from the basement membrane at the normal anatomical level of mucosa to the deepest extent of the tumour. (13, 14) The exophytic part of the tumour should be excluded. In large ulcerative and exophytic tumours, the normal level of mucosa is best appreciated macroscopically and representative tissue blocks taken without over or underestimating tumour depth. A methodical text-based block key and/or photographic record should be included.

Identification of genuine bone involvement is essential in staging oral cavity tumours, with those invading bone marrow staged as pT4a. (12) Blocks should be selected with the aim of differentiating between erosion and marrow invasion (fig 2). Incorrect staging could result in over treatment with subsequent risk of therapy related complications that may further impact on quality of life. These include osteoradionecrosis, xerostomia, dental caries and periodontal disease secondary to radiotherapy. Floor of mouth and small gingival tumours with mandibular rim resections (alveolar process) require close attention. Tumours frequently run along the lingual plate (where they may involve the periosteum) and the nearest margin in these resections tends to be the base of the rim resection. Assessment of soft tissue without attached bone may lead to an overestimate of surgical margins. Particular attention also needs to be paid to differentiating sino-nasal from oral cavity tumours involving the maxilla as staging criteria differ significantly. Involvement of the pterygoid fossa (pT3) and pterygoid plate (pT4) are best demonstrated by a transverse section through the posterior maxilla. (12)

Salivary

Parotid resections are rarely received oriented and a sound knowledge of normal anatomical structures is therefore important. The facial nerve bisects the gland into superficial (where salivary tumours are more common) and deep lobes. The deep margin (where masseter and mandible are located) is usually smooth, whereas the superficial surface rough. Superficial and deep surfaces should be inked and the specimen serial sliced. The deep aspect of the specimen is usually the closest margin due to attempts to preserve the facial nerve at the time of surgery. Routine sampling of background salivary gland tissue is not necessary as it should be represented in tumour blocks. Intra-parotid lymph nodes should be sampled. Inflammatory conditions such as chronic sialadenitis associated with sialoliths require a single block.

On slicing, benign and low-grade malignancies frequently present as circumscribed nodules. Pleomorphic adenomas (PA) are by far the commonest salivary neoplasm and are given special attention here. These are typically well-circumscribed, rubbery to firm with translucent cut surface and may feature a bosselated appearance with satellite nodules. (15) Capsular retraction is common and recognition is essential to reduce risk of communicating false margins. Bosselated PAs can appear multifocal under histology and an appreciation of this on macroscopy is essential to exclude over-diagnosis of invasion. Unlike follicular thyroid neoplasms, the tumour-capsular interface does not need sampling in entirety as the criteria for invasion differs significantly. The presence of a central scar or

hyalinised nodule should raise the index of suspicion for malignant transformation alongside haemorrhage, necrosis, and ill-defined border. These features should prompt the pathologist to undertake additional sampling. Two blocks will usually suffice to confirm a diagnosis of a Warthin's tumour.

Tumours in which the preoperative FNA/core biopsy has failed to render a firm diagnosis should be extensively sampled as this may pertain to a low-grade malignancy which is not immediately obvious until histology sections can be examined. Highly infiltrative tumours such as adenoid cystic carcinoma often extend at some distance beyond that appreciable under macroscopic assessment. This is one of the reasons these tumours maybe understaged on radiological assessment, where gnathic bones may appear normal, but histology demonstrates wide infiltration of tumour islands in marrow spaces with little to no effect on bone trabeculae. For these reasons thorough sampling of apparently normal tissue is required if the diagnosis has been established preoperatively. Sampling of proximal and distal ends of nerves is necessary in malignant tumours and surgeons should mark these. Identification of the presence of macroscopic, rather than microscopic, extraparenchymal extension, including involvement of cervical fascia and masseter, directly influences tumour stage (T3) and is a core item in cancer datasets. (6, 12)

Distinguishing benign from low grade malignant in minor glands and in particular the palate can be difficult as both typically present <2cm in size. PAs often lack a capsule at this site and some malignant tumours such as polymorphous adenocarcinoma can, macroscopically, appear circumscribed. 3mm slices should be sectioned and submitted in their entirety. Staging of malignant minor salivary gland tumours is contentious with UICC utilising the staging criteria for oral cavity squamous cell carcinomas, with a depth of invasion >5mm upstaging a small T1 tumour to T2. (12) This approach is curious as minor glands occur deep to mucosa and the epithelial surface. The authors query the significance of depth of invasion to inform staging. Maximum tumour dimension may be more appropriate to inform staging of minor salivary gland tumours.

Laryngectomy

In the larynx, the anatomical extent of the primary tumour is key to determining the pathological stage and potential need for further oncological intervention. (1, 12) Some laryngectomy specimens will have an accompanying neck dissection which is typically bilateral (see neck dissections below). In comparison to oral cavity resections, trimming procedures of the laryngectomy specimens do not vary significantly from case to case but the approach should always be determined by the location of the primary. (16) Supraglottic and pyriform fossa tumours may be visible on receipt however it may not be possible to directly visualise a tumour of the glottis or subglottis. The left and right aspects should be differentially inked - especially when megablocks are employed as it is easy to lose orientation of symmetrical anterior commissure tumours under the microscope. The laryngeal cartilages generally show degrees of ossification and therefore decalcification is required. It is prudent to obtain a fixed tumour block prior to this to ensure suitable material is available for immunohistochemistry and molecular studies should these be required. (1) Inferior margins of the trachea (and oesophagus in the case of pharyngolaryngectomy) and margins from the superior and peripheral pharyngeal aspect of a laryngopharyngectomy can also be taken along with sampling other soft tissue structures

including thyroid and tracheostomy fistula if present. Units with a band saw may prefer to section the fixed specimen transversely to ensure optimum fixation, consistent thickness of slices and sampling of fixed tumour. This does however carry a risk of damage to the circumferential soft tissues. To aid fixation and gross tumour description, some pathologists prefer a single sagittal slice through the posterior aspect of the specimen. (17, 18) This approach carries a risk of distortion of the posterior specimen limit.

Once decalcification is complete, the specimen should be sliced in the transverse plane from superior to inferior with PM40 or feather blade knife. The superior and inferior horns of thyroid cartilage can be removed, as they are anatomically unimportant and can complicate access with the knife. Some pathologists prefer to dissect the hyoid bone and strap muscles from the specimen to enable assessment of the preepiglottic space and to sample the hyoid only if tumour is present. (16-18) The authors prefer to keep the hyoid in situ for specimen integrity. The laryngeal prominence may require prolonged decalcification. Dissection should not be compromised because of time pressure as premature slicing can result in tearing of sections with introduction of artefact.

The authors prefer that the superior-most slice should be taken beneath the border of the hyoid bone to enable sagittal sections of the epiglottis, pre-epiglottic space and superior tongue base margin. A single midline section is often all that is necessary. In the case of an exophytic supraglottic tumour, several sagittal sections may be taken for superior and tongue base margin assessment and megablocks may be employed. For most tumours, the subsequent transverse slices should be systematically numbered from superior to inferior and named according to the anatomical compartment (i.e. supraglottis, glottis, subglottis). Specimen photography of the slices is critical. Pathologists must carefully inspect the slices, with magnification if required, for the presence or absence of tumour. The maximum diameter of the tumour should be measured remembering that the largest diameter may be superior-inferior with the slices added together. Uniquely to this site, size itself has no influence over tumour stage but is important in conveying the volume of disease removed from the patient. Older texts advocate taking a single longitudinal/vertical section to include false cord, ventricle, true cord and the paraglottic space. (18) This may result in underestimation of total disease burden however.

Block selection must focus on the sampling of tumour involving relevant anatomical structures that have direct effect on staging as specified in the current UICC staging criteria. (12) Typically, the presence or absence and extent of cartilage invasion is key to determining the overall stage; the thyroid cartilages and cricoid cartilages should be closely inspected for involvement (fig 3). Cartilage invasion may not be visible macroscopically and therefore it is important to sample tumour directly abutting cartilage to assess for microscopic spread. The arytenoid cartilage is not considered important for staging purposes but may be described. It is not possible to reliably comment on vocal cord mobility/fixation and this requires correlation with clinical findings.

Blocks may be taken to sample the nearest circumferential margin. It should be noted that the closest margin is often 'posterior' in the region of the glottis; however, this is usually disputed in multidisciplinary team meetings by the surgeon protesting that it's an 'air'

margin and further resection posteriorly is not anatomically feasible. In personal experience it is better to refer to this as the 'specimen limit' rather than 'margin'. (1)

Thyroid

Thyroid gland specimens should be received in formalin and ideally orientated by the surgeon. Dimensions should be measured and preferentially weighed and inked. In the case of a total thyroidectomy right and left lobes should be identified along with the pyramidal lobe if present. It is important to ensure adequate fixation prior to dissection and ideally without slicing through a nodule as this may complicate interpretation of invasion and margin status secondary to artefact. When fixed it is recommended that transverse parallel slices are taken from superior to inferior. Location, appearance and size of any abnormality should be recorded, and representative sections submitted for processing. A combination of regular size and megablocks may be used with adequate sampling of the peripheries of the nodules to assess for invasion. In the case of malignancy only gross extrathyroidal extension informs staging. For non-neoplastic pathology including Grave's disease, the cut surface of slices may appear unremarkable. Random sections should be submitted without over sampling (19, 20) - guidance is set at one block per centimetre or one per 10g. (8) Level VI lymph nodes may be submitted with the gland and may require microscopic assessment to distinguish from parathyroid gland material.

Neck Dissection

Neck dissections are performed for both therapeutic and staging reasons and may be submitted attached to a primary resection or as a separate specimen. They should be submitted by the surgical team with individual anatomical levels that are easily identified. Separation between level II/III is the most difficult in non-orientated specimens as this requires knowledge on the level of hyoid bone and carotid bifurcation. Other levels are easily delineated by using the submandibular gland to separate the posterior border of level I from II and the omohyoid muscle to delineate the inferior border of level III from IV. In recent years, there is a tendency for the surgical team to separate each level into separate containers and this may be preferable to reduce ambiguity in interpretation of anatomical boundaries. (21) The pathology request form should include clinical N staging with details of location, size and number of clinically positive nodes to allow pathological correlation. The importance of getting the anatomical level correct cannot be overstated as it may form the basis for deciding future radiotherapy fields.

Once fixed, neck dissections may be separated from the main specimen and sampled. Submandibular gland is a useful structure to identify for separation in oral cavity resections as it will reduce the possibility of sectioning through an involved node or critical deep margin. The point of separation may be inked a different colour from the primary site to prevent confusion with margin interpretation later. Comprehensive neck dissections feature a significant volume of muscular tissue as well as large vessels, including jugular vein and nerves in comparison to a selective neck dissection. This can be bewildering for the inexperienced pathologist; however, a consistent, meticulous and systematic approach can simplify the process. Each level should be palpated first for larger lymph nodes which are more likely to be involved by metastasis. Larger nodes can be bluntly dissected from fat and bisected or serially sliced depending upon size. (9) Macroscopically abnormal nodes should be described and size measured. While tempting to save on number of blocks, each bisected

node should be allocated its own cassette to simplify the counting process. Lymph node yield is considered a quality indicator of the surgery (22); recent literature suggests a potential value of lymph node ratios on prognosis. (23)

A multinodular appearance may indicate fusion of lymph nodes secondary to extranodal extension and it is appropriate to give an estimate of the number of involved lymph nodes. Larger metastases can be selectively sampled with particular attention to identifying extranodal extension as it has direct influence on staging, prognosis and requirement for adjuvant therapies. (12) The remaining fibroadipose tissue can be bread sliced to identify smaller nodes. Lymph nodes 5 mm or less may be embedded whole with multiple nodes submitted in the same cassette. A comment on pathological margins is not usually required unless therapeutic removal of a clinically apparent tumour mass has been performed. In this scenario, the deep margin should be sampled.

To simplify the process, some pathologists prefer to embed all tissue received as a time saving measure and to maximise nodal yield. The authors do not advocate this approach as it can result in excessive consumption of resources, prolonged technical and microscopy times and a risk of double counting nodes. Close attention is required with epithelial lined cysts presenting at level II in the neck and especially those where an FNA test was equivocal. These should be serial sliced and sampled in their entirety to ensure that a cystic metastasis has been excluded. Cystic metastases are frequently associated with carcinoma of unknown primary; significant proportion of these are later found to arise in the oropharynx. The use of transoral robotic surgery (TORs) in head and neck has led to a significant increase in the number of mucosectomies of the tongue base and tonsillectomies to identify a primary site. The reader is referred to the recent publication by Robinson et al (24) for a comprehensive review.

Specimens of limited diagnostic value

Finding significant pathology in nasal polyps that are not clinically or on gross inspection concerning is rare, (25, 26) therefore one could question the value of extensive sampling of these. Tonsils other than those submitted for clinical concern such as unilateral enlargement or suspicion for malignancy are also of questionable value. (26, 27) Serial slices and a single representative section is usually sufficient in a grossly normal tonsil. Sampling of a submandibular gland in a neck dissection submitted for cancer management is recommended in most reporting guidelines; however, there seems little diagnostic value in the absence of any macroscopic abnormality. Extensive sampling in cases of osteoradionecrosis is not required unless there is clinical suspicion for malignancy. Representative sections through necrotic area(s) as well as anterior and posterior margins will suffice, with specific comment on bone vitality at the resection margin. Cases of osteonecrosis may be complicated by ingression of oral epithelium into bony defects. This may appear alarming with potential for an erroneous diagnosis of recurrent squamous cell carcinoma. Teeth submitted for diagnosis and assessment for developmental conditions such as amelogenesis and dentinogenesis imperfecta are best referred to specialist centres.

Summary

We outline areas of potential oversampling and highlight the importance of transfer of relevant clinical and imaging information prior to specimen trimming. The complexity of

H&N anatomy and relevance for staging and implications on patient management are highlighted.

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Contributors

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AVJ: conceptualisation, original draft writing, review and editing.

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Figures

Figure 1: Bone invasion in an exophytic well differentiated squamous cell carcinoma – bone invasion is highlighted in the yellow circle. The decalcified H&E section (right) shows invasion of the bone marrow spaces via the periodontal ligament. The extent of invasion is greater than is evident on the corresponding macroscopic image of the same slice (left). Line 1 demonstrates the tumour thickness from the exophytic tumour surface to the deepest invasive island and is 12.9mm according to the digital tool. Line 2 demonstrates the depth of invasion from the anatomical gingival surface and is 9.8mm. While this is not critical in this case which is pT4a for bone invasion, this would mean the difference between a pT3 (line 1) and a pT2 (line 2) in a lesion of oral soft tissue.

Figure 2: Bone erosion and invasion. The left image demonstrates a squamous cell carcinoma detached from the mandibular alveolar ridge. Microscopy revealed superficial erosion of cortical bone only. On the right side the carcinoma has breached the cortical surface with involvement of bone marrow. Microscopy confirmed the presence of invasion with involvement of trabecular bone and a pT4a tumour, which raises the potential need for post-operative radiotherapy.

Figure 3: Transverse section of glottis from a laryngectomy specimen for a right transglottic squamous cell carcinoma. Tumour (T) is seen to arise on the right vocal cord and extensively infiltrate the right thyroid cartilage (Tc – black star). Microscopy confirmed extension beyond the external aspect into peri-laryngeal tissues and confirms staging of at least pT4a (TNM8). Thickness and depth of invasion may be given for information but are not considered 'core' data items in the current RCPATH laryngeal dataset (2023). The strap muscles (Sm), left thyroid cartilage (Tc), left arytenoid cartilage (Ac) and cricoid cartilage (Cc) are shown but are not directly involved by tumour. The left thyroid cartilage shows extensive ossification with expansion in the mid-posterior region. The left paraglottic space is highlighted by the red broken line and includes the thyroarytenoid muscle. The right arytenoid cartilage and paraglottic space have been completely obliterated by tumour. While the tumour extends close to the right and posterior circumferential aspects it should be noted that further clearance is typically not anatomically feasible and should therefore not be considered a 'true' margin without discussion with the surgical team.