Surgical pathology of the mouth and jaws

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Preface

Surgeons have a natural interest in pathology, or if they haven't, they should. Only by understanding pathological processes involved in the diseases with which they have to deal can they manage them rationally and effectively.

Surgeons also have to understand the vocabulary of pathology and the full implications of a pathology report. Moreover, they need to appreciate some of the limitations of histopathological diagnosis. To provide helpful diagnoses, pathologists frequently need to know what the clinical findings are. Only by mutual understanding of their different tasks can surgeon and pathologist acquire that happy degree of cooperation that is ultimately in the best interest of the patient.

Vast numbers of publications on all aspects of oral and maxillofacial pathology continue to appear. To help the busy surgeon cope with this flood of material, this book aims to provide as much information as possible in concise, accessible and readily digestible form. Also, to serve the needs of the oral surgeon as well as possible, this book has been written jointly by a surgeon and two pathologists. Unusually great emphasis has therefore been placed on the principles of treatment of the conditions that have been discussed. Non-surgical conditions, particularly mucosal diseases, have been included as these so frequently come under the wing of the oral surgeon. We earnestly hope therefore that this book will help to ease the many difficult problems that the oral surgeon often has to face.

We are anxious to express our gratitude to our publishers for their patience in waiting for this text to be put together and their generous allowance of colour illustrations. Inevitably, however, to provide an adequate coverage of the histopathology, the constraints of cost have forced on us some restriction on the number of illustrations. We therefore made it a matter of policy to omit clinical pictures. While one kind of lump may look much the same as another, we accept that the lack of clinical illustrations of mucosal diseases may be regarded as regrettable.

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1. Investigational methods

Malignant disease is inevitably one of the most important conditions that must be confirmed or excluded. Many biopsies of the mouth are taken primarily for this purpose and, in most cases, early recognition leads to better treatment results. The approach to the diagnosis of oral tumours is straightforward and can be used to illustrate the path to the diagnosis of all types of oral disease. The oral cavity is accessible and requires no special facilities for its examination. Moreover, unlike those in many other sites, early, even minute,

oral lesions can cause symptoms. Biopsy is usually an essential adjunct to clinical and other investigations and is readily carried out under local analgesia. Nevertheless, between 25% and 50% of patients with oral cancer have late disease when referred for treatment and this may be, in part, due to failure to biopsy the lesion when first seen.

Provided that the biopsy is adequate, the microscopic diagnosis of most malignant tumours rarely presents major problems. Difficulties mainly arise in such conditions as dysplastic lesions, undifferentiated tumours and rare tumours, particularly if mesenchymal or lymphoreticular. In some cases, immunocytochemistry, as discussed later, can be decisive, but it must be appreciated that, on rare occasions, it may be difficult to reach a diagnosis with absolute certainty.

Clinical investigation

The provisional diagnosis of all disease must initially be clinical and a high index of suspicion is essential for all those attending patients with oral symptoms. It should be needless to add that a detailed history, with special care to record the dates of onset of particular signs and symptoms should precede clinical examination.

All areas of the oral mucosa should be closely inspected in sequence and any suspicious area palpated for texture, tethering to adjacent structures and deep induration. The World Health Organisation's *Mucosa Manual* suggests a technique for sequential examination of each site within the mouth. If a systematic approach such as this is used, there is little change of overlooking even a minute abnormality.

Accurate clinical records are essential for the care of any patient, and are valuable for any prospective or retrospective analysis of treatment methods and results. These notes must provide a detailed description of the lesion including site, precise measurements, appearance, extent, texture, fixation and induration. A photographic record is particularly valuable.

It is important to emphasize that this initial record is the basis upon which treatment is planned and progress assessed. Even a biopsy irrevocably changes the presenting lesion in some degree. Precise records are also essential should there be any medico-legal complications.

Surgical biopsy

Biopsy of any suspicious area in the mouth should be undertaken, not as a substitute for sound clinical diagnosis, but for objective confirmation of its nature and, in some cases, efficacy of excision (see later). If the clinician suspects a particular disease, but the pathology report is at variance, there should be no hesitation in repeating the biopsy to obtain a more adequate or representative specimen.

It is sometimes claimed that malignant tumours should not be biopsied before definitive treatment, on the grounds that surgical manipulation increases the risk of bloodstream or lymphatic spread or of seeding tumour cells into previously healthy tissue planes. However, there is little evidence to support such arguments for most tumours and, in any case, there is rarely any practical alternative to biopsy for establishing a definitive

diagnosis. Nevertheless, the biopsy should always be planned in such a way that, should surgical excision be necessary, then the resection can include the track of the previous biopsy.

Salivary gland tumours are an important exception to this rule, as pleomorphic adenomas are among the few that have a strong tendency to seed and recur if cells are released at biopsy or attempts at enucleation. This is a serious hazard in the parotid glands, and incisional biopsy should not therefore be carried out in this site.

It is often tempting to remove small lesions *in toto* by an excisional biopsy. However, this may be dangerous. Without histological confirmation of the malignant nature of the disease it is difficult to be certain of adequate margins. Moreover if the lesion is excised locally, subsequent management is compromised. It may also be difficult to persuade the patient to undergo further treatment for a lesion which appears to have been eliminated. Further, once the primary lesion has been excised, the surgeon no longer has a guide to the necessary extent of further excision and the radiotherapist cannot be certain of the field that needs to be treated.

For these reasons an incisional biopsy is recommended whenever cancer is suspected. For obviously benign lesions, such as most fibrous nodules, excisional biopsy is often curative as well as diagnostic.

When malignant disease is suspected, it is useful to tattoo the margins with Indian ink at the time of the initial biopsy. Any surgical resection must be planned according to the original dimensions of the tumour and the latter should be accurately delineated.

Biopsy technique

Biopsies in the mouth can usually be carried out under local analgesia. Infiltration of local anaesthetic solution should be made well away from the biopsy site. Distending the tissues with anaesthetic solution distorts them, with subsequent difficulties with diagnosis. It is also important not to crush the specimen with dissecting forceps or artery clips. This can be avoided by passing a suture through the specimen to immobilize it. Whenever possible, a representative piece of the lesion should be taken in continuity with adjacent normal tissue, as the changes at or near the junction may be important diagnostically. Specimens of lymphoid tissue need to be particularly gently handled. Lymphocytes are fragile and readily damaged to produce streaking artefacts.

Soft tissue biopsies should usually be taken using a double semilunar incision. This is not merely simple but also leaves a wound that is readily sutured. The triangular flaps that has been advocated in the past not only leaves a good deal of waste tissue after the main slice has been taken for processing, but also leaves an awkwardly shaped wound.

The incision should include an adequate depth of underlying connective tissue. It is not possible to lay down hard-and-fast rules as to how deeply the incision should extend, as the requirements for diagnosis vary from one condition to another. In many mucosal diseases no great depth of connective tissue is necessary, but in Crohn's disease, for example, granulomas may only be found deeply. Biopsies should also be extended more deeply when there is superficial necrosis, in order to reach living lesional tissue.

Thin biopsy specimens tend to curl up in the fixative and it may then be impossible to orientate the specimen for microscopy. Curling can be prevented by placing the specimen, deep surface downwards, on a square of blotting paper.

The specimen should be put in fixative as soon as possible. Delay can lead to autolysis and any drying distorts the tissues. Formal saline (10%) is generally acceptable unless electron microscopy is to be undertaken.

As important as any other aspect of biopsy procedure is completion of the pathology request form. Essential information includes:

- Name, age and sex of the patient. The sex may not be apparent from the given (first) name, especially in the case of immigrants.

- The provisional diagnosis and as much clinical information as possible (including, where relevant, details of nay previous treatment, medical and drug history), but particularly the precise biopsy site and some indication of the rate of growth of the lesion and symptoms.

- The name of the clinician, the date of the biopsy and the hospital number.

Though all this seems obvious enough, essential information of this sort is depressingly frequently left out.

A laboratory with automatic processing should usually be able to provide diagnoses withni about 48 hours after submission of the specimen. With purely manual processing, the answer may not be available for up to a week. With difficult material, immunostaining may also delay diagnosis. If decalcification is necessary, there may be a delay of anything up to a month according to the type and size of the specimen and the kind of information required. However, this sort of delay can often be avoided by removing some of the tumour tissue from the bone. Genuine urgency in getting a diagnosis should also be mentioned if a frozen section is not possible or feasible. In the case of fibro-osseous and intra-bony cystic lesions, the pathologist frequently needs to know radiographic or other findings and joint discussion in the light of such information may sometimes be decisive.

Toluidine blue marking. Vital staining for delineating malignant and dysplastic lesions depends on the binding of toluidine blue to DNA in the superficial cells. Binding should therefore be proportional to the amount of DNA present and the number and size of superficial nuclei in the tissues. Though this test has been widely advocated in the past, false negative are so frequent as to make it unreliable.

Frozen sections

Frozen sections are indicated under such circumstances as the following:

- to establish, at operation, whether or not the tumour is malignant and to determine the extent of the excision

- to confirm that excision margins are free of tumour at the time of operation

- on rare occasions such as in an infant when it has not been feasible to take a biopsy under local anaesthesia preoperatively

- for immunofluorescence microscopy or for immunocytochemistry when reagents are ineffective in paraffin sections

- if the patient is from abroad and needs an immediate diagnosis to decide whether to return or stay.

Frozen sections are only justifiable if the definitive operation will follow immediately. Fixed, paraffin blocked sections are always to be preferred for light microscopy if the elective operation is going to be carried out some days later.

With reasonable precautions and facilities, frozen sections for tumour diagnosis usually provide a rapid and highly reliable answer and the only problem may be that of conveying the specimen, from theatre to laboratory, simply, rapidly and without deterioration. The usual methods include the following:

- With a cryostat in a side room of the theatre, the specimen can be prepared for sectioning within minutes of having been taken.

- If theatre and laboratory are close, then the wet specimen can be taken direct to and frozen in the laboratory. It is not possible to generaalize about the maximum permissible delay when this is done, since lymphoma cells (for example) deteriorate faster than carcinoma cells. For most oral tumours, up to 20 minutes between excision and freezing is unlikely to cause serious deterioration, though it is clearly better to lose as little time as possible. The specimen can first be put in frozen section embedding material such as OCT, if the pathologist wishes, and then taken immediately to the laboratory.

- The specimen can be dropped into liquid nitrogen, kept in the theatre in a vacuum flask, or into isopentane chilled with liquid nitrogen. If the specimen is very small it should be put in a capsule.

If liquid nitrogen is not available, the specimen can be put on dry ice in an insulated container or snap-frozen inthe theatre using a CO_2 jet spray freezer and then transported on dry ice.

- Instant freezing aerosol sprays (using, for example, dichlorodifluormethane) will produce temperatures of -50°C and are suitable and convenient for small specimens apart from muscle.

Rapid freezing is important to prevent autolysis and to reduce freezing artefacts. It is far better to take a wet unfrozen specimen immediately to the laboratory where it can be snap-frozen at -50°C, rather than to put it in an ordinary deep freeze (about -40°C) where serious freezing artefacts will develop, and take it later.

Under normal circumstances, using a cryostat, a stained section is obtainable in about 5 minutes from receipt of the specimen.

Limitations and contraindications. Frozen section diagnosis is susceptible to error as a result of

- poor or inadequate sampling of the mass

- technical defects (freezing artefacts)

- difficulties of interpretation.

Sampling errors can result from sheer inadequacy of the specimen or from the fact that it may not be representative of the whole of the tumour. An obvious example is the case of a small area of malignant change in a salivary gland tumour. In other cases, the difficulties of diagnosis of some rare tumours may be such that immediate diagnosis cannot be made on a frozen section alone. Obvious examples are lymphoreticular diseases, including some of the midfacial granulomas (Chapter 14).

Finally, the initial verbal report on a frozen section must always be confirmed in writing and further confirmed by examination of paraffin sections which provide a permanent record. Misunderstandings can arise, especially if the message is relayed by a third party, and have unfortunate effects on the patient. This in turn can lead to medico-legal complications.

Limitations of histopathological diagnosis

In many cases, such as cysts, the diagnosis is made largely on the clinical and radiographic features together with the findings at operation and is usually confirmed by the histological findings. Nevertheless, the pathological findings are occasionally unexpected and if, for example, a mural ameloblastoma or other cystic neoplasm is found, the management and prognosis are affected accordingly.

Histological examination of as much excised material as possible is therefore essential and there can be important medico-legal implications if this is neglected.

On the other hand, the surgeon should be aware of the limitations of histopathology and should not expect the pathologist always to be able to give a definitive answer as to the prognosis or what may be the optimal line of management for all possible diseases.

The apparent failures of histopathology stem from several factors, namely:

- Limitations imposed by the specimen. In this connection the following problems can arise if

(a) the specimen fails to include a border with normal tissue and hence may fail to show (for example) peripheral invasion; in other cases, submission of no more than part of a cyst wall may prevent the identification of ameloblastoma in another area

(b) the specimen is too large to examine completely; in the case of parotid gland tumours in particular, even multiple blocks might fail to find a focus of

carcinomatous change

(c) the specimen has been damaged by diathermy, crushing with forceps, injection of local anaesthetic solution or other means

(d) the specimen is too thin and has been allowed to curl so that correct orientation is impossible

(e) poor fixation or autolysis; the specimen may have been pit in *normal* rather than *formal* saline or put into fixative after too long a delay.

- Microscopy provides only a two-dimensional view and strictly speaking may never give an answer as to whether excision of a tumour is complete. Though the tumour may have a wide border of normal tissue in the plane of section, it could extend beyond the excision margins in another plane. Pathologists may therefore report that 'the margins of the specimen are clear of tumour *in the planes of section'*, and try to take sections in as many planes as possible. This problem is perhaps greater in the mouth than in many other sites because of the lack of space for wide excisions.

- In a few cases, there are no absolute criteria for malignancy, particularly when the material shows no invasion. Some salivary gland tumours in particular can be cytologically benign, yet prove by their behaviour to be malignant.

- In some uncommon tumours, especially chondromas and neurofibromas, the borderline between being benign and malignant is indefinable.

- Some tumours are so uncommon that knowledge of behaviour is deficient. Prognostication is therefore impossible and there may be no consensus as to the optimal management.

- In the case of dysplastic leucoplakias, grading is no more than a subjective visual assessment of the degree of epithelial atypia. Though the diagnosis of severe dysplasia is frequently equated with inevitable development of frankly invasive carcinoma, this is no more than a probability as a significant number regress spontaneously.

- Occasionally, previously unrecognized tumours or other diseases still appear. These may have such unusual microscopic features as to fail to fall into any of the recognizable categories, and a group of even the most experienced pathologists will be unable to reach a consensus. Despite the fact that the current WHO typing of salivary gland tumours (Seifert, 1991) includes no fewer than 32 categories of epithelial tumours alone, it can be difficult to fit a particular tumour into one of these categories as van der Wal et al (1992) have pointed out.

Table 1.1 lists a variety of problems which may affect in histological diagnosis and prognosis.

Table 1.1 Some problems in histological diagnosis and prognosis

Keratocysts	Inflammation can destroy the characteristic appearances to simulate a simple cyst.
Ameloblastoma	Cystic expansion can flatten the epithelium to mimic a simple cyst over a large area.
Salivary gland tumours	
Pleomorphic adenomas	Rarely, an area of carcinomatous change can be missed in a large specimen. Even more rarely, a cytologically benign tumour can behave as a carcinoma.
Mucoepidermoid carcinoma	Behaviour not reliably related to histological features.
Acinic cell carcinoma	Histologically benign specimens can be invasive.
Papillary cystic adenomas	Cytologically benign tumors can metastasize widely many years after apparently adequate excision.
Clear cell tumours	Several different entities. Most are undoubtedly malignant.
Neurofibromas	Hazy borderline between benign and malignant.
Chondromas	Can prove to be sarcomas despite benign microscopic appearances.
Fibro-osseous lesions	A grey area where differentiation between dysplasias and tumours may only ultimately be confirmed by behaviour.
Fibrous histiocytomas, fasciites and fibromatoses	Differentiation from sarcomas or prediction of behaviour from the microscopic features may be difficult.
Dysplastic leucoplakias and erythroplasias	Degree of dysplasia cannot be objectively assessed and behaviour not entirely related to microscopic features.
Deep mycoses	Often no entirely specific features, and fungi or spores may be impossible to find.

Immunocytochemistry

Antigenic substances can be identified in cells or tissues by the use of specific antibodies. Binding of the antibody to the antigen is made visible by conjugating the antibody with a stain or a stainable substance and using fluorescence microscopy.

Fluorescence immunocytochemistry has long been helpful in the diagnosis of immunologically-mediated diseases, by identifying deposits of immunoglobulins or complement in the tissue and thus to provide presumptive evidence of immunologically-mediated damage. A well-established application has been in the diagnosis of pemphigus vulgaris (Chapter 8) where, using frozen sections of fresh unfixed material, localization of pemphigus autoantibodies along the intercellular junctions can be demonstrated by fluorescence of conjugated anti-human immunoglobulin. This technique is relatively non-specific in that it does not identify pemphigus autoantibodies specifically, but merely takes advantages of the fact that these autoantibodies are immunoglobulins, but are antigenic.

Immunocytochemical techniques have been extended by the introduction of monoclonal antibodies. In essence, the identification of cytochemical markers depends on the antigen being insoluble and on a suitably sensitive detection system; the most widely used at present is peroxidase-antiperoxidase, but there are increasing numbers of alternatives such as avidin-biotin, each with its attendant advantages and limitations. Horseradish peroxidase labelling, conjugated with a suitable antibody, enables many antigenic substances to be identified in fixed paraffin-blocked material, though some antibodies can only be used on frozen sections. The hope underlying the use of monoclonal antibodies is that each type of cell or structural component has sufficiently well-defined antigenic characteristics to differentiate it from every other. In tumours particularly, the cells of origin, however poorly differentiated, may therefore be identifiable by such means.

An early application was the identification of monocolonal immunoglobulin production by lymphoreticular tumours. Nevertheless, despite the availability of commercial monoclonal antibodies to an enormous number of tissue components, it can still happen that they fail to provide a definitive diagnosis. An important limitation is that, in addition to any difficulties of technique, tissue components from different types of cell may share antigenic determinants (epitopes). Thus some sarcomas, for example, stain positively for epithelial markers (keratins).

Frequently, a panel of monoclonal antibodies has to be applied. The panel chosen depends on general and personal experience. It is not feasible simply to apply all the monoclonal antibodies available, partly because of time and cost, but also because of the fact that the specificity of some of them is questionable and positive staining by one of these may only cause confusion.

The limitations of these techniques have become widely appreciated, but the continued stream of publications on unexpected findings testifies to the problems. Moreover, overconfidence in their value may lead to lack of care in assessment by light microscopy. Important difficulties are as follows:

- effects of fixation and other aspects of processing

- false-positive or false-negative results

- problems of specificity

- lack of adequate controls

- distinguishing between tissue (tumour) or infiltrating cells which mark positively

- loss of specific epitopes by tumour cells

- inability to distinguish malignant from benign cells by immunostaining alone.

Immunocytochemistry has become a speciality in itself, sufficient to fill substantial textbooks, and it is not feasible to discuss it in detail here but only to point out that the techniques demand considerable experience in their performance and in the interpretation of results.

Nevertheless, among tumours of the mouth or jaws, several points can be clarified. At the least, what used sometimes to be called small dark cells tumours, which might be anaplastic carcinomas or lymphomas, can now be distinguished with certainty by positive staining of carcinomas for epithelial antigens and lymphomas for leucocyte common antigen (CD45). B cell lymphomas can also be confidently differentiated from T cell lymphomas. Some of the latter are so pleomorphic as not to have been recognized as lymphomas in past (Chapter 14).

Surgeons, naturally enough, usually want answers in a hurry. Unfortunately immunostaining is slow and labour-intensive unless it can be reliably automated.

Relatively recently, the possibility of differentiating benign from malignant lesion has been studied by identifying markers of cell proliferation, oncogenes and mutations of the p53 oncogene-suppressor gene (Birch, 1992). Changes in the p53 gene lead to nuclear accumulation of p53 protein and this has been found in many different types of malignant neoplasms. Nevertheless, Dei Tos et al (1993) have shown that p53 protein can also be expressed in many benign neoplasms or non-neoplastic lesions.

Antibodies used to recognize cell proliferation of potential value in the diagnosis or prognosis of cancer include KiSi (Sampson et al, 1992), Ki67 (Sawhney and Hall, 1992) and JC1 (Garrido et al, 1992). However, immunocytochemistry will not reliably distinguish between all tumours of similar origin, such as the many varieties of salivary gland tumour.

Flow cytometry measures the DNA content of 10.000 or more nuclei in suspension. The presence of diploidy, haploidy or aneuploidy has been correlated with behaviour for some salivary gland neoplasms (Chapter 11). However, the results are mainly of statistical value and as yet of little use in predicting the behaviour of a particular tumour. The method itself also has limitations, as discussed by Robinson (1992).

Fine-needle aspiration cytology

This technique is applicable mainly to lumps in the neck, especially suspect lymph nodes in a patient with a known primary carcinoma, and to parotid gland tumours. It is valuable when open biopsy is inadvisable or contraindicated. It consists of percutaneous puncture of the mass with a fine needle and aspiration of material for microscopic examination. This technique needs no elaborate equipment and is quick, almost painless and without complications.

Nevertheless, the technique is often incorrectly performed and too little tissue obtained. Two aspects are critical, namely successful puncture of the mass and the transfer of material from the needle to a microscope slide. The mass is immobilized between finger and thumb and then punctured with a 21- or 23-gauge needle (depending on the size of the mass) on a 10 mL syringe. Important points to note are that

- the needle is tighlty fitted on to the syringe to prevent air leaking in when the plunger is withdrawn, and

- about 2 mL of air is already in the syringe before puncturing the node in order to expel all the aspirate from the needle on to the slide.

Aspiration should be forceful and, at the same time, the needle should be moved around different parts of the mass. Having aspirated for 10-30 s, the plunger is released and the needle withdrawn through the skin. The needle contents are then expelled on to a glass slide using the 2 mL of air drawn into the syringe beforehand. To do this, the tip of the needle must touch the slide to prevent splashing the material, which can be gelatinous or sticky, over a wide area. Blobs of material can be deposited on several slides and then smeared with a second slide to provide a thin film for staining and microscopy. Some pathologists prefer wet-fixed material which must be sprayed with an alcoholic spray fixative immediately. Others prefer thinner films which can be left to dry.

After aspirating the tissue, instead of making a smear directly on to the microscope slide, 2 mL of 95% ethanol can be aspirated as fixative into the syringe to avoid losing material. The syringe containing fixative and cells is then sent to the laboratory where the contents are centrifuged and a smear prepared from the deposit.

Fine-needle aspiration has become widely used to obviate the need for open biopsy and the risk of spreading malignant cells into the surrounding tissues where they cannot be detected at the time of radical surgical excision. Further, any displaced cells having lost their blood supply and rendered anoxic may be more resistant to radiotherapy. Schelkun and Grundy (1991) have reported the results of 213 fine-needle aspiration biopsies of head and neck lesions and found a false-positive rate of 0.5% and a false-negative rate of 2.3%. Sensitivity was 81.1% and specificity was 99% for malignant tumours.

Tumour implantation into the needle track has been reported when large (Vim-Silverman) biopsy needles have been used. It is widely believed that there is no risk of needle track dissemination of cancer after aspiration using 21- or 23-gauge needles. However, this hazard cannot be dismissed, as discussed by Hix and Aaron (1990) and Nankhonya and Zakhour (1991). Increasing experience with other tumours, particularly of the lung, shows that the risk of seeding of tumour cells along the needle track is real (though small) rather than theoretical. This complication has not as yet been shown in the case of salivary gland tumours, and Frable (1983) did not consider that there was any significant risk. However, the slow growth of pleomorphic adenomas in particular may mean that such recurrences may eventually appear.

Fine-needle aspiration cytology offers great potential advantages for the diagnosis of parotid gland tumours, since benign and malignant tumours are frequently not clinically distinguishable and open biopsy is contraindicated. However, the diagnosis of salivary gland tumours is by no means always straightforward even when the gross specimen is available. For example, malignant change in a salivary pleomorphic adenoma may occupy only a minute part of the mass and could be missed during needling. In other cases, dysplasia within a pleomorphic adenoma, in the absence of invasion of surrounding tissues, might be mistaken for carcinoma in a fine-needle aspiration biopsy. In any case, from the practical viewpoint, parotidectomy is the treatment required for pleomorphic adenomas, as for malignant tumours. In practice, the chief factor limiting the usefulness of cytologica diagnosis of salivary gland tumours is that it may be difficult for the cytologist to build up sufficient experience in the

appearances of the aspirates of the many different types of neoplasms, unless working in a specialist centre.

Punch/drillTrucut needle biopsy

Several techniques are available for obtaining samples of deep-seated solid lesions. These may be obtained using a small punch, a rotating trephine or a special wide-bore needle. Each of these techniques results in a solid core of material and may be useful for solid intra-osseous lesions. However, there is a high risk of seeding malignant cells along the tract of the instrument.

Simple aspiration

Simple aspiration of radiolucent areas to confirm whether or not they are cysts and if possible to make a precise diagnosis has been extensively used in the past.

This technique can be performed at the chairside using a wide-bore hypodermic needle and syringe. Infiltration with a local anaesthetic makes the procedure pain free.

If it is impossible to aspirate anything (assuming that the needle has entered the lesion), the lesion is probably either solid or the uncommon variant of keratocyst, filled with semi-solid keration. If the lesion is in the maxilla and air is aspirated, the needle is in the antrum. This may be confirmed by injecting a little sterile saline, when the patient will complain of a salty taste at the back of the throat as the saline passes into the nose via the antral ostium. Gas obtained from a mandibular lesion indicates a solitary bone cyst. These cysts sometimes contain serosanguineous fluid resulting from breakdown of red cells, and biochemical analysis may show a high bilirubin content.

Uninfected dentigerous or radicular cysts typically contain clear straw-coloured aspirates containing cholesterol crystals. The characteristic shimmering of these crystals may be seen by running some of the aspirate on to a dry swab and examining it in a strong oblique light. Alternatively, a smear on a slide enables cholesterol crystals to be identified by their characteristic notched rhomboid shape by microscopy. However cholesterol crystals are not pathognomonic of cysts.

If blood is aspirated, this suggests either a vascular tumour (haemangioma) or an aneurysmal bone cyst. This finding is probably the most valuable application of simple aspiration and may prevent torrential bleeding when an intraosseous haemangioma is unknowingly opened surgically.

Any infected lesion may yield pus on aspiration and occasionally yeast forms of a deep mycosis may be identifiable in the smear. An odontogenic keratocyst may contain cheesy material unlike the clear fluid from other odontogenic cysts. If this material can be aspirated, a smear can be made and stained (haematoxylin and eosin) to show keratin squames. Alternatively, the aspirate can be sent to the laboratory for protein analysis. Less than 4 g per 100 mL of total protein in the aspirate indicates a keratocyst, whereas a protein content of more than 5 g per 100 mL excludes the diagnosis.

In practice, however, competent clinicians are rarely mistaken in their diagnosis of jaw cysts on the basis of the clinical and radiographic findings. On rare occasions, a monolocular cystic ameloblastoma can be mistaken for a simple cystm, but even then aspiration is unlikely to provide useful information. Aspiration of cystic lesion is, therefore, increasingly rarely performed.

Exfoliative cytology

Cervical smears are a valuable mass screening method for detecting early cervical cancer, where Papanicolau staining helps to differentiate the varied appearances of epithelium during the oestrous cycle from malignant cells. Exfoliative cytology has also been used for suspected oral carcinomas, but for this purpose haematoxylin and eosin staining is simpler and satisfactory. The specimen is obtained by scraping the moist oral mucosa with a blunt-edged instrument such as a spatula. The scrapings are transferred to a clean microscope slide. A smear is made, allowed to dry briefly, then fixed and stained. Skill is required in interpreting the results, and a specialist cytologist should be consulted. A positive result is an indication for surgical biopsy.

However, the technique is unreliable and there can be a false-negative rate of over 30%. Since the mouth is so readily accessible for biopsy, it should always be used in preference of exfoliative cytology and a biopsy should also be taken for confirmation. As cytology can produce false-negative findings, and positive findings need to be confirmed by biopsy, there is virtually no justification for the use of this technique in the mouth for the diagnosis of tumours.

One possible exception is the appearance of ulceration following radiotherapy, when there is doubt as to whether this is due to recurrence of the tumour. In such cases, a biopsy wound can be very slow to heal or can precipitate serious infection. If cancer cells are found by cytological examination, an immediate answer is thus obtained and biopsy may be avoided. However, only positive findings are informative.

Another use for exfoliative cytology in the mouth is for the diagnosis of infections, particularly herpetic or candidal and for the preliminary diagnosis of pemphigus vulgaris. Haematoxylin and eosin stains are also satisfactory for smears of herpetic or candidal lesions. Virally damaged cells with ballooning degeneration and syncytial multinucleate cells are seen in both herpes simplex and zoster infections. A refinement of this technique is to use antibodies to these viruses to confirm the nature of the infection. The diagnosis of thrush is readily confirmed by the finding of many strongly Gram-positive hyphae in and among epithelial and inflammatory cells.

Aspiration and other ancillary methods - conclusions

Fine-needle aspiration cytology is a valuable technique for examining equivocal masses in the submental and submandibular areas, and the neck in patients whose primary tumour has been treated but who develop palpable nodes later. Metastases should always be suspected, but the lymph nodes sometimes show only reactive changes. Alternatively, it is sometimes difficult to distinguish such a mass from a chronically inflamed submandibular salivary gland. If aspiration biopsy confirms either the presence of tumour or salivary tissue,

the nature of the mass is certain. Failure to find tumour or salivary tissue leaves the same doubts as a negative biopsy and in particular whether the aspiration needle missed the mass. Fine-needle aspiration under computer tomography (CT) guidance may be helpful under such circumstances.

Exfoliative cytology and toluidine blue tests cannot be recommended. They are only applicable to surface lesions accessible to surgical biopsy which avoids the risk of false-negative results.

DNA hybridization and the polymerase chain reaction

The diagnostic applications of recombinant DNA technology are as yet limited to (a) the diagnosis of genetic disorders, (b) identification of some microorganisms in tissues or body fluids, and (c) phenotyping of some lymphomas and leukaemias (Arends and Bird, 1992). Of these, identification of microogranisms is most likely to be useful in the present context, because of the great sensitivity and rapidity. *In situ* DNA hybridization has the advantage that it is more readily applied to formalin-fixed tissues and can be used, for example, for identification of human papilloma virus (HPV) in papillomas, carcinomas and other oral lesions.

Radiography and other imaging techniques

Conventional radiography is essential for investigating any lesion of the mouth and jaws other than superficial soft tissue lesions. The facial bone structure is so complex that confusion with overlying structures sometimes makes X-ray diagnosis difficult. However, rotational pantomography of the jaws can be helpful in assessing alveolar and antral involvement, provided that the limitations are understood (Whaites, 1992).

Conventional tomography can be useful for assessing bony involvement by antral lesions where posterior extension beyond the pterygoids may render a lesion inoperable. However, CT scanning has largely replaced conventional radiographs for the pterygoid and orbital regions.

Chest radiographs are essential if metastases, sarcoidosis, tuberculosis or Wegener's granulomatosis are suspected. Skull radiographs or skeletal syrveys will help to confirm the diagnosis of multiple myeloma and are occasionally required in cases of hyperparathyroidism.

Sialography

Sialography depends on instillation of contrast medium into the salivary tree to demonstrate such changes as obstruction or tumours obliterating or displacing the duct system. Sialography is discussed in more detail in Chapter 11.

Computed tomography (CT) and magnetic resonance imaging (MRI)

The increasing availability of CT scanning and MRI has been of enormous benefit in the investigation of head and neck lesions and particularly for salivary gland tumours where they have displaced and are considerably more informative than sialography.

However, for intraoral tumours the value of CT scanning is more limited. For the evaluation of antral tumours, particularly assessment of the pterygoid regions, CT has largely displaced plain radiography and conventional tomography. CT is also of value in the investigation of metastatic disease in the lungs, liver and skeleton.

The principles of magnetic resonance imaging have been described by Komoroski et al (1992). The patient is placed in the structured magnetic field of the MRI apparatus and the field subjected to a radiofrequency pulse, to generate an MRI signal. The signal reflects the status and environment of hydrogen molecules within the body. Diseased tissue produces different signals from healthy tissue. These signals are computer processed to produce an image on a visual display unit. However, hard tissues produce no image.

The sensitivity or MRI to differentiate diseased from healthy tissues sometimes enables it to detect metastatic oral cancer in clinically negative necks and the absence of disease in suspicious necks. However, Kabala et al (1992) remain sceptical of the ability of MRI to detect micrometastases. Until this question has been satisfactorily answered by the use of gadolinium enhancement, use of STIR sequences or other means, the management of patients with oral cancer but clinically normal neck nodes (Chapter 10) remains controversial.

Ultrasonography

Ultrasonography provides a quick, safe and cheap method of imaging which provides the expert with remarkably detailed and informative pictures. For example, the valvular vegetations of infective endocarditis can be visualized in this way.

However, CT and/or MRI scanning are even more informative and provide readily interpreted images. Their value is limited mainly by their availability and cost.

Radionuclide scanning

Technetium pertechnetate bone scans of the facial skeleton are of little value in the diagnosis of primary oral cancers. Obvious clinical disease long precedes bone changes visible on a technetium scan and the procedure is not without risk to the patient.

Radionuclide scans for liver metastases have been used but have been displaced by CT scanning or ultrasound scans which are both non-invasive and more sensitive techniques.

Technetium scanning of the salivary glands has been advocated in the past, but is rarely diagnostic. Many tumours and functional disorders show up unpredictably as either hot or cold spots and the only lesion showing a consistent appearance in technetium scans is Warthin's tumour (adenolymphoma) which appears as a hot spot. Technetium scanning may be useful in the investigation of functional activity of salivary glands, although empyting films on sialography are more informative and do not require special apparatus.

Gallium-67 is taken up by leucocytes and may be useful in staging lymphomas. However, it is also taken up by leucocytes in inflammatory lesions and those passing through vascular tumours. It has therefore been used as an alternative to angiography.

Salivary flow measurement

Impairment of salivary flow, unless of extreme degree, is not usually detectable clinically and patients frequently make no direct complaint of it even when it is present. By contrast, other patients with normal salivary flow may complain of a dry mouth. Objective confirmation of complaints of dry mouth is frequently therefore necessary, particularly in the diagnosis of Sjögren's syndrome. Techniques are discussed in Chapter 11.

Laboratory investigations

Innumerable investigations can be asked for, but unless the clinician is clear about their relevance to the disease in question and the likely yield in terms of diagnostically useful information, the findings are unlikely to be helpful. Further, the habit of asking for unnecessarily many investigations is likely to eventually to antagonize laboratory staff and may result in lessened cooperation.

Important examples of investigations relevant to and often crucial in the diagnosis of oral lesions are shown in Table 1.2.

It should be emphasized that there are no tests which will provide a direct, positive diagnosis of immunologically-mediated disease, and autoantibody studies must be interpreted in relation to the clinical and other findings. In a few cases such as Sjögren's syndrome the autoantibody findings (associated with objective confirmation of a reduced salivary flow rate) may obviate the need for biopsy. However, even in this case a labial salivary gland biopsy may be just as informative.

With regard to bone diseases, newer methods for assessment of bone formation such as osteocalcin, procollagen, bone specific alkaline phosphatase have been developed but are not yet widely available. Similarly, markers of bone resorption, such as pyridinoline and deoxypyridinoline, are likely to improve accuracy of diagnosis. Lytic metastatic bone lesions may be associated with production of parathyroid hormone-related peptide, while secondaries from prostatic cancer may be identifiable by raised (up to 100 u/L) levels of prostate specific antigen (PSA).

Medical assessment of the patient

However certain the surgeon is of the diagnosis and his ability to eliminate the disease, these advantages will be of little value to a patient who fails to survive the operation. In general, the anaesthetist is responsible for the preoperative assessment, but it is advisable also for the surgeon to take a careful medical and drug history. Thus may be initiated by use of a proforma questionnaire, but needs to have any areas of doubt confirmed by clinical examination and further examination.

The latter may include haemoglobin estimation, blood picture, sickling test in Afro-Caribbeans and blood pressure estimation. The taking of an electrocardiogram (ECG), particularly for patients over 60, and a chest radiograph may seem to be desirable precautions, but rarely yield more information than can be obtained clinically or from the history. Similarly, the history is the most important guide to defective haemostasis. Wagner and

Moore (1991) reviewed an extensive literature on the value of many screening procedures and found that 60% of oral surgery patients were overinvestigated without benefit, and that some tests could even be hazardous to the patient.

Table 1.2 Examples of clinically useful laboratory investigations for oral diseases

Haematology

Lymphomas, leukaemias, myelomas, sore tongues, HIV infection (lymphopenia), recurrent aphthae (particularly in older patients).

Microbiology

Osteomyelitis, cellulitis, acute parotitis, deep mycoses (frequently mistaken for tumours).

Serology

Confirmation of some infections, particularly syphilis and theoretically for others such as herpes or mumps, HIV infection (rarely), infectious mononucleosis (Paul-Bunnel test).

Erythrocyte sedimentation rate

Raised in all inflammatory diseases, but especially important in cranial (giant cell) arteritis.

Blood chemistry (calcium or phosphatases)

Giant cell lesions of bone to exclude hyperparathyroidism, Paget's disease of bone, prostatic metastases.

Autoantibodies

Sjögren's syndrome, rheumatoid arthritis, lupus erythematosus, pemphigus vulgarisand mucous membrane pemphigoid (immunofluorescence of tissue specimen).

Urine

Bence-Jones protein in amyloid disease (myeloma or other paraproteinaemia).