The Pathology and Surgery of the Salivary Glands

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Chapter 2: Investigation of salivary gland disease

Many techniques of investigation are research tools and do not, as yet, have an established role in diagnosis. The chief promise of some, such as immunohistochemistry or electron microscopy, is that they may contribute to more precise categorization of difficult tumour types. However, this book is not intended as a comprehensive treatise on salivary gland research. Investigational techniques will therefore be discussed only in relation to the practical problems of diagnosis and management.

Investigation has two main purposes. The first is to establish as precise a diagnosis as possible to guide the clinician towards the optimal mode of treatment. The second, which particularly applies to autoimmune disease, may only be applicable postoperatively but may be helpful in assessing the patient's ultimate prognosis. Benign lymphoepithelial lesion is the main example. The diagnosis is likely to be made only after operation but it is important then to discover whether there is any evidence of autoimmune disease (Chapter 4). The latter in turn may increase the likelihood of development of lymphoma or other complications. In addition, variants of lymphoepithelial lesion may be indicative of HIV infection.

Clinical Investigation

Much depends on the duration of the history and the clinical features of individual lesions. These may make it clear whether or not a tumour is present and if so, may suggest whether it is benign or malignant. The age and sex of the patient, in conjunction with knowledge of the demographic features of salivary gland lesions, should also be taken into account in the process of diagnosis. A history of systemic disease and of drugs being taken should also be carefully assessed. For example, a salivary gland swelling in a women of 60 years, with a history of rheumatoid arthritis or other connective-tissue disease has a high chance of being due to Sjögren's syndrome. In such a patient, the possibility of lymphoma needs also to be considered. In a male particularly if between the ages of 20 and 40 years, a cystic salivary gland swelling should suggest the possibility of HIV infection.

Rarely, drugs can give rise to salivary gland swellings but more often they are the cause of xerostomia (Chapter 5). The presence of endocrine or metaboic disease may be useful in recognizing sialosis (Chapter 6).

The following are the main investigatory measures available to the clinician or pathologist:

1. Imaging techniques

    Radiography and sialography

    Computerized tomography (CT)

Magnetic resonance imaging (MRI)

Ultrasound

Scintiscanning

2. Histopathology and related methods

Frozen sections

Aspiration cytology

Histochemistry

Immunohistochemistry

Electron microscopy

3. Salivary gland function tests

Flow rates

Sialochemistry

4. Tests for related or contributory systemic disease

Bacteriology

Haematology

Autoantibody studies and other immunological tests.

Imaging

Detailed imaging of diseased salivary glands is neither cost-effective nor necessary in all cases. In the majority, a thorough clinical examination yields adequate surgical information to permit safe removal once consent has been obtained. However, for some patients, imaging is necessary to stage their disease and plan surgical treatment. The first group of tests, namely radiography and related techniques, is useful to locate tumours and calculi, or abnormalities such as sialectasis in Sjögren's syndrome. The relative merits of each modality are discussed below. Ultrasound has not as yet been widely applied or become of established value in the investigation of salivary gland disease, but some have found it useful in the assessment of both inflammatory and neoplastic disease. Scintiscanning has also not proved to be of great value in diagnosis, requires bulky and expensive equipment, and is not without some risk to the patient. The methods currently in use are sialography, computerized tomography (CT), nuclear magnetic resonance imaging (MRI) and ultrasound.

Sialography

Sialography remains the most popular method of assessment of ductal inflammatory and degenerative disease despite the more sophisticated imaging techniques currently available (Figure 2.1). Focal or diffuse ductal strictures and ectasia are easily demonstrated and give useful information concerning the probable outcome of conservative management. However, its use is limited by the fact that space-occupying lesions are neither reliably detected nor localized. Sialography should be restricted therefore to cases with recurrent parotid or submandibular swelling in which no discrete mass, other than a calculus, can be palpated (Figs 2.2 and 2.3).

The technique is not difficult, but some expertise is required to cannulate the parotid and submandibular ducts in an atraumatic fashion. The injection of contrast medium occasionally causes some discomfort and it is for this reason alone that sialography is rarely possible, even if indicated, in an unsedated child. Over-filling of the duct system by excessive injection pressure may result in extravasation of contrast medium into the surrounding parenchyma. This produces a brisk local reaction, causing discomfort for several days.

CT Scanning and MRI

There are clear indications for CT or MRI which can be summarized as follows:

Major glanda

1. Masses confined to the deep lobe of the parotid gland (Figs 2.4 and 2.5).

2. Tumours with involvement of both the deep and superficial lobes of the parotid gland (dumb-bell tumours).

3. Parotid tumours presenting with facial weakness, other neural deficit or indication of malignancy (Figs 2.6 and 2.7).

4. Congenital parotid masses.

5. Submandibular gland tumours with neural deficit or fixation to the mandible.

6. Recurrent disease (Figs 2.8 and 2.9).

Minor glands

1. Tumours of the palate with suspected involvement of the nose or maxillary antra (Figs 2.10 and 2.11).

2. Any tumour with clinically ill-defined margins.

Several studies have assessed the reliability of both CT and MRI in the detection of malignant salivary disease. Neither is, nor can be expected to be, completely reliable, though reasonable specificity rates are claimed for both. However, it is not for this purpose that

imaging is generally required but rather for detecting suspected lesions within the salivary glands and providing surgically useful images of the extent of disease (Figs 2.12 and 2.13). Sensitivity rates approaching 100% are claimed for both modalities and image quality is influenced mainly by the generation of scanner or image protocol adopted.

The majority of tumours within the major glands examined by CT give higher attenuation values than surrounding normal glandular tissue (Figs. 2.14 and 2.15). The resolution of soft-tissue attentuation in thin slices achieved by modern generation scanners is exceptional, improved by intravenous contrast and is perfectly adequate for the examination of salivary glands. There is no longer the need for CT sialography, which was advocated at a time when scanning resolution was considerably inferior. Unfortunately, disruptive artefacts may be introduced by extensive dental restorations which can significantly detract from the image quality.

Occasionally, CT scanning will show calcifications in a salivary gland tumour and this strongly suggests that it is a pleomorphic adenoma. They are unlikely to be seen in conventional radiographs.

MRI has proven to be equally sensitive in the detection of salivary neoplasms (Fig. 2.9). Within the major glands they give low signal intensity with T_1-weighted protocols and relatively high signal intensity with T_2 and balanced protocols. Although both T_1 and T_2 images show the margins of the lesion with equal clarity, the tumour composition is better defined with T_2 sequences and the detection of subtle areas of disease by short inversion of tau inversion recovery (STIR) sequences (Figs 2.8 and 2.9). The introduction of paramagnetic contrast materials has improved this aspect of MRI further, but increased the expense.

Both CT and MRI have proved useful in demonstrating cystic AIDS-related lymphoepithelial lesions (Fig. 2.17).

Unlike CT, the quality of MRI is not influenced by the state of the patient's dentition. Occasionally also, MRI will indicate whether a parapharyngeal tumour has originated in the parotid gland or from minor pharyngeal glands, but this distinction can rarely be reliably demonstrated.

In clinical terms therefore, there is essentially little to choose between CT and MRI for the diagnosis of salivary gland disease: the ultimate decision will therefore be mainly influenced by the local availability of scanners, cost and personal preference.

Ultrasound

The most attractive feature of ultrasound is that most hospitals have access to suitable equipment and expertise in its use for the assessment of less accessible structures, for example heart valves and the foetus. It is relatively simple to obtain good, diagnostic quality scans of the major salivary glands as they are, to a large extent, subcutaneous structures and there is the additional advantage that the technique is non-invasive. Several studies have shown that the parenchyma of the glands can be depicted in detail and, in expert hands, an accurate picture of the duct system can be obtained. Small tumours can be detected and, it is suggested, a degree of suspicion about their malignant potential aroused (Figs 2.18 to 2.20).

The retromandibular vein is reliably seen and its relationship to a tumour mass gives some information about possible involvement of the facial nerve.

Despite these attractions, ultrasound has yet to become established as a useful clinical investigation for salivary disease. The reasons are, first, that the images do not provide sufficient operative information for the surgeon and, second, there are areas of the salivary glands that may be obscured by bone from ultrasonic assessment.

Histopathology and Related Techniques

Histopathology is generally understood to apply to microscopy of paraffin-blocked sections. The most commonly employed and most generally useful stain is haematoxylin and eosin. Though the picture is two-dimensional and largely artefactual, histopathology remains the most reliable diagnostic method because of the existence of a vast body of knowledge of the microscopic appearances of an enormous variety of diseases. In particular, of course, salivary gland tumours and other lesions have been categorized in terms of their histopathology. The latter allows extensive examination of many areas of a large specimen such as a parotid tumour and may therefore reveal localized malignant change which may be missed in frozen sections or fine-needle aspiration. Block specimens and mounted sections also provide a permanent record for retrospective evaluation when necessary.

The great disadvantage of conventional histopathology of course, is that it provides only a postoperative diagnosis. However, this is still valuable in confirming or contradicting the choice and extent of surgery and the later stages of postoperative managament. As discussed below, fine-needle aspiration biopsy or frozen sections can be used for pre- or intraoperative diagnosis, but their accuracy is less than, and must always be checked against, conventional histopathologic examination of the specimen afterwards.

The histopathology of salivary gland tumours is, nevertheless, a difficult area and indeed a major purpose of this atlas is to try to help pathologists who do not have extensive experience in this field. However, the fact remains that the categorization of a few salivary gland tumours is still a subject of some doubt.

Histochemistry

Histochemistry has limited applications in salivary gland tumour diagnosis. The demonstration of minute amounts of intracellular mucin may, however, be useful in distinguishing poorly differentiated mucoepidermoid from squamous-cell carcinomas, which are likely to have a worse prognosis. Histochemistry is also valuable in the preliminary investigation of neuroendocrine tumours by use of silver (Grimelius) and other stains. Silver staining may also be useful in demonstrating the reticulin pattern in some lymphomas. Other suggestes uses of histochemistry are confirmation of the nature of oncocytic cells by use of phosphotungstic acid haematoxylin (PTAH) and the identification of crystalline and other inclusions.

Immunohistochemistry

Immunohistochemistry, though sometimes invaluable, has many more limitations than were earlier anticipated. The chief difficulties include the sharing of markers by cells of different origin and the loss of markers as a result of neoplastic change. However, immunohistochemistry is valuable for differentiating difficult tumours such as dysplastic or anaplastic carcinomas from lymphomas or sarcomas, for distinguishing non-neoplastic lymphoproliferative lesions from lymphomas and for differentiating B- and T-cell lymphomas. Another use for immunohistochemistry is in the identification of the rare neuroendocrine tumours. Their small cells are positive for epithelial markers such as epithelial membrane antigen but negative for common leukocyte antigen. They are typically chromogranin and neurone-specific enolase-positive. Specific hormones or their precursors can be identified, if required and the facilities are available. Rarely also, a paraganglionoma may appear in the parotid region and be mistaken for a salivary gland tumour such as an acinic cell carcinoma with many clear cells. Paraganglionomas stain in a generally similar manner to neuroendocrine cells but are negative for epithelial markers.

Rarely, an adenocarcinoma of a salivary gland may mimic a thyroid tumour but can be readily recognized by its failure to stain for thyroglobulin.

Another use for immunohistochemistry is in the identification of amelanotic melanomas which are typically neurone-specific enolase, S-100 protein- and vimentin-positive, but negative for epithelial membrane antigen.

Spindle cell tumours (myoepitheliomas) of salivary glands may occasionally be difficult to differentiate from connective-tissue tumours. Myoepitheliomas are usually recognizable by the presence of obviously epithelial components of the tumour, but if these are lacking, the myoepithelial cells should be identifiable by their staining with both epithelial markers and vimentin, and actin or myosin. This is unlike most other spindle cell tumours which are positive for vimenting but not epithelial markers. An exception is the exceedingly rare synovial sarcoma which can appear in the parotid region, and may be positive for both of these markers. The apparent staining of myoepithelial cells for S-100 protein has been discussed earlier (see Chapter 1). Immunohistochemistry has the great advantage over electron microscopy that it can frequently give an answer more quickly and be carried out in a histopathology laboratory without bulky and expensive equipment. It can also be applied to frozen sections for intraoperative diagnosis. Indeed some markers can only be used on frozen sections as the relevant epitopes are destroyed by conventional specimen processing.

Though there are many immunohistochemical studies on the cell populations of salivary gland tumours and though a case has been made for differentiation of low-grade polymorphous adenocarcinomas from adenoid cystic carcinomas by immunohistochemistry (see Chapter 7), it cannot be said that this method is as yet of proven usefulness in the microscopic diagnosis of the more difficult salivary gland tumours, apart from those already mentioned.

Frozen Sections

Surprisingly, Gnepp (1988) states that the first use of frozen sections was in Holland in 1818. It was not until the introduction of the cryostat over 130 years later, and improvements in technique that frozen sections became a valuable and widely accepted ancillary diagnostic measure. By consultation between pathologist and surgeon during the operation, frozen sections should provide answers to three important questions which bear directly on management, namely:

1. Is the lesion benign or malignant?

2. If malignant, is the tumour high or low grade?

3. Are the margins of excision tumour-free?

Unfortunately, Gnepp (1988) in his extensive review of the literature, has confirmed that of all sites in the head and neck, frozen sections of salivary gland lesions have yielded the least reliable results. His analysis of 1629 salivary gland tumours from 17 reports since 1958, showed that the failure rate for differentiating benign from malignant tumours by frozen sections (false-negative diagnoses) was 3.7% and that false-positive diagnoses (benign tumours misdiagnosed as malignant) were made in 1% of cases. Mistakes in categorization were made in 2.6%. In the series of 229 salivary gland tumours reported by Gnepp *et al* (1987), mucoepidermoid carcinoma was the single most common source of false-negative results (34%), whilst pleomorphic adenomas were the most common cause of false-positive results (58%). Not unexpectedly, benign lymphoepithelial lesions also caused difficulties and this problem is likely to be greater in HIV-positive patients with cystic lesions.

The great variety of salivary gland tumours and their subclasses makes the chances of incorrect categorization by frozen sections greater than by conventional sections. However, it may be argued that this may be less important than first appears, since the optimal mode of treatment of individual tumour types is frequently uncertain.

Factors affecting accuracy of diagnosis by frozen sections

Assuming that the technique is adequate, sampling errors form the greatest obstacle to accurate diagnosis of salivary gland lesions by means of frozen sections. This is a result of the variety of configurations within a single specimen and, in particular, the presence in many of these tumours of ducts which do not appear to be neoplastic.

Another major difficulty is caused by a localized area of malignant change in a pleomorphic adenoma. This problem is still greater in a large tumour. Even if dysplastic cells are found, it is not possible to make the diagnosis of carcinoma in pleomorphic adenoma unless an area of invasion is found. If this fails, diagnosis may have to be deferred until more extensive sampling can be carried out with conventional sections.

Sampling errors of this sort can be reduced by close naked-eye examination of as many areas as possible of the whole tumour for ill-defined sectors of its borders. In any case, the specimen for frozen-section examination should include part of the capsule and

immediately contiguous gland tissue.

To distinguish non-neoplastic from neoplastic cysts, it is essential to examine any areas of mural thickening. This is particularly important in the case of mucoepidermoid carcinomas which, more frequently than any other type of tumour, can consist of mural thickenings in the wall of a single large cystic cavity.

Necrosis is a feature of malignant rather than benign tumours. Its presence should be regarded as a warning sign and area immediately adjacent to foci of necrosis should also be sampled. The main exceptions are the rare infarcted (infected) variant of Warthin's tumour (see Chapter 6) and occasional necrosis in plemorphic adenomas.

Fine-Needle Aspiration (FNA) Cytology

Fine-needle aspiration cytology offers at least the possibility of preoperative diagnosis of parotid tumours without the risk, associated with open biopsy, of seeding tumour cells in the incision. Its limitation, which is even greater than for frozen sections, is that of missing a critical area within, or at, the border of the tumour mass and thus failing to obtain a truly representative sample. Using FNA, a small area of malignant change in a pleomorphic adenoma could easily be missed and it would not, for example, be possible to differentiate follicular from diffuse lymphomas. Aspirates of non-neoplastic lymphocytes could come from a variety of lymphocyte-rich lesions such as Warthin's tumour, tuberculosis or benign or HIV-associated lymphoepithelial lesions (Figs 2.21 to 27).

Apart from special problems such as these, it should be possible to differentiate malignant from benign salivary gland tumours with over 90% accuracy (Chen *et al*, 1988). Accuracy of diagnosis may be improved by application of immunohistochemistry, but electron microscopy of the aspirated material is of no value for rapid diagnosis.

A more important consideration, however, is whether fine-needle biopsy is as safe as has been believed. Increasing experience with other tumours suggests that seeding of tumour cells along the needle track is real, rather than a theoretical possibility. This has not as yet been shown in the case of salivary gland tumours, but the slow growth of pleomorphic adenomas in particular may mean that in due course such recurrences may yet be reported.

Salivary Gland Function Tests

Flow-rate studies may be useful in the investigation of xerostomia, the complaint of which is not always substantiated by objective measurements. Conversely, salivary flow may be impaired but not mentioned or may go unnoticed by the patient. Salivary flow rates, unless the mouth is obviously dry, may therefore be valuable in the diagnosis of Sjögren's syndrome as may autoantibody studies and haematological investigation. Frequently, this has been done by cannulation of the parotid duct and measuring the amount of saliva over a standard period, before and after stimulation with 10% citric acid. A stimulated parotid flow rate for normal adults over 40 years of age is approximately 1.5 mL/min. Flow rates of 0.5 mL/min or less indicate significant xerostomia. Sialometric methods are discussed in Chapter 5, but measurement of unstimulated flow of whole saliva over a defined period is adequate for most purposes and may be at least as informatiove as more complicated methods.

Sialochemistry

Changes in the chemical constituents of saliva have been described in Sjögren's syndrome for example. However, sialochemistry has more value in the investigation of systemic diseases such as cystic fibrosis, than in the diagnosis of primary salivary gland diseases. It has also been suggested that compliance with instructions about the taking of some drugs such as lithium can be readily monitored by saliva concentrations. Some of the uses of sialochemistry are discussed by Seifert *et al* (1986) and diagnostic uses of saliva have been reviewed by Mandel (1990) and is also discussed in Chapter 5.

Bacteriology

Bacteriological investigation of saliva is obviously important in the management of acute bacterial sialadenitis. Many such cases are caused by penicillinase-resistant staphylococci. Nevertheless, to use a drug such as flucloxacillin on this assumption, may delay resolution if the causative bacteria is insensitive to this narrow-spectrum antibiotic.

A fresh specimen of saliva from the duct of the affected gland should therefore be obtained before antibiotic treatment is started, as discussed in Chapter 4.

The presence of a variety of viruses in the saliva, most notably the Epstein-Barr virus, has been reported, but such investigations are generally of research value only.

Haematology

Haemoglobin levels and routine blood pictures are a necessary preoperative investigation when salivary gland surgery is contemplated. Occasionally, the blood picture may help in the diagnosis of salivary gland disease. For example the erythrocyte sedimentation rate may be raised and there may be a normochromic normocytic anaemia in Sjögren's syndrome (Chapter 4). Alternatively, lymphopenia may suggest that a salivary gland lesion is the result of HIV infection.

Autoantibody and other immunological investigations

Autoantibody studies are of particular value in patients with benign lymphoepithelial lesion to determine whether or not a connective-tissue disease is associated or whether the patient has Sjögren's syndrome. The findings of Gleeson *et al* (1986) also suggest that the risk of development of lymphomatous change in benign lymphoepithelial lesion is greater in patients with rheumatoid arthritis. This finding is in keeping with the significantly higher incidence of lymphoma in patients with rheumatoid arthritis compared with the normal population.

Detection of immunodeficiency is of theoretical value in recognizing patients with HIV-related salivary gland disease. However, it is simpler and more informative to determine (if possible) whether the patient is HIV antigen- or antibody-positive. If not, a simple blood picture showing otherwise unexplainable lymphopenia is strongly suggestive.