Introduction to clinical cytopathology of the head and neck

Introduction
Fine needle aspiration
INTRODUCTION

Very few areas in the body are so exposed to our scrutiny as the head and neck. Lumps and bumps in this area rarely go unnoticed; patients either notice changes themselves, or are alerted to them by others. Fine needle aspiration cytology (FNAC) is a valuable technique in investigation of nodules and masses arising within the head and neck. FNAC is particularly helpful because biopsy of this area should be avoided unless all other diagnostic modalities have failed to establish a diagnosis. As such, FNAC represents an accurate, inexpensive and rapid investigative technique of head and neck masses.\textsuperscript{1-22}

The pattern of referral to FNAC is usually from the general practitioner, via the specialist to the pathologist. The referral pattern via the specialist reduces the need for FNAC in a majority of cases that are due to infection and have settled after the initial treatment. This particularly relates to the paediatric age group.

The majority of aspirates from cervical lymph nodes will disclose either reactive lymphadenopathy or metastatic squamous cell carcinoma. Occasional nodules will be due to lymphoma. While primary diagnosis of lymphoma by FNAC is generally not considered definitive, it is helpful in further management. Similarly, establishing the presence of carotid body tumours, branchial cleft cysts or epidermoid cysts excludes metastatic carcinoma and avoids the open biopsy as well as reassuring both clinician and patient. FNAC of lesions within the mouth, oropharynx, nasopharynx and nasal sinuses eliminates squamous cell carcinoma as its primary objective. FNAC can establish diagnosis of salivary gland tumours, which should not be biopsied prior to definitive treatment. It can diagnose the majority of thyroid enlargements and helps reduce the rate of surgery for thyroid lumps. In addition, FNAC can diagnose many specific conditions, both of local origin or a reflection of a more generalised disease. Each of these represents an important clinical entity with a specific therapy. Ancillary techniques, namely immunocytochemistry, flow cytometry and sometimes molecular techniques, can greatly broaden the diagnostic range and specificity of FNAC. They are particularly useful in the diagnosis of lymphoproliferative processes and in determining the precise nature of lesions as variable as rhabdomyosarcoma, olfactory neuroblastoma and granular cell tumour. The prudent use of these techniques can be cost-effective and avoid the need for more invasive diagnostic procedures. FNAC has a high degree of accuracy for the diagnosis of both primary and metastatic disease.\textsuperscript{23}

FINE NEEDLE ASPIRATION

Clinical history

Clinical history forms an integral part of FNAC investigation. In our institution, the specialist refers patients to the FNAC clinic (Fig. 1.1). A pathologist (who examines patients, takes the samples and reports them) runs the FNAC clinic. Patients bring a request form or a letter from the referring physician stating a short clinical history and indication for FNAC. The pathologist confirms the patient’s identity, takes the past and present clinical history, examines the patient and confirms the site of investigation (Fig. 1.2). The procedure is explained to the patient. In cases where a need for multiple passes is anticipated, a local anaesthetic is applied. As a result, most patients do not experience any significant degree of pain. Clinical history and physical properties of the lesion (anatomy, consistency, mobility, pain) are very useful information helping to reach a correct diagnosis.

Physical examination

The pathologist examines the patient, focusing on the area indicated by the referring specialist but also any other areas indicated by the patient or discovered by thorough scrutiny. This is a very important part of the consultation since

Figure 1.1 FNAC clinic room. Consulting room with an examination couch, work surface, waste disposal, sink, microscope and facility for rapid staining.
not to apply anaesthetic largely depends on the patient, the site involved and the extent of FNAC sampling planned. Since the average FNAC does not involve more than one pass with a 22+G needle, most patients do not require local anaesthetic. However, if the patient is a child or a needle-phobic, or if the site is particularly tender (e.g. lip, nose, areola), or if it is expected that several passes will be necessary, a local anaesthetic is applied in the form of subcutaneous injection of 0.5 ml of 2% lignocaine. More recently we have been using a needle-free syringe where pressurised air expels the anaesthetic, penetrating the skin, without a needle (Fig. 1.3). The anaesthetic forms a small white ring through which the subsequent test needle is applied, once or more (Fig. 1.4). Patients do not experience any pain on application of the anaesthetic and experience no or minimal pain at FNAC.

The palpable area in question is cleaned with an antiseptic agent and fixed between the two fingers of the non-dominant hand. A 22G, 23G or smaller needle is then passed into the lump. Usually, a syringe and a syringe holder are attached to the needle to help aspiration (Fig. 1.5). More recently we have been using a non-aspiration technique (capillary sampling) with the aid of a needle only (without the syringe attachment) (Fig. 1.6). The needle is passed round several times in the cases of non-thyroid lumps. In the case of thyroid, several vertical movements in the same direction are usually sufficient to gain representa-

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Figure 1.2 Clinical history and examination. Pathologist at the bedside examines the area referred to by the specialist. Patient is asked further relevant questions about the duration of the swelling, level of pain and any other associated systemic symptoms.

valuable data can be obtained about the anatomy and nature of the lesion as well as getting relevant clinical history, e.g. a patient with a submandibular swelling will readily describe that the swelling changes in size. Palpable lumps can be hard or soft, mobile in different directions or adherent to surrounding tissues and skin, painful, oval, round or irregular, or pulsating. On needling, they can feel soft, hard, gritty, fatty, can yield clear fluid, purulent fluid, keratinous viscous fluid, mucoid material and blood. All these signs are significant and are noted on the patient’s request form.

FNAC procedure

With the FNAC procedure having been explained, the patient is put in a supine position. The choice of whether or

Figure 1.3 Needle-free anaesthetic system. Initially designed for patients who need daily injections (e.g. insulin), but applicable to local anaesthesia. This is particularly useful in children, needle-phobic patients, in sensitive skins and where multiple needle passes are anticipated.
tive material. Whilst using a syringe attachment, it is important to release the negative pressure before exiting the lump, otherwise the material is aspirated into the syringe and can only be retrieved with the aid of a needle wash.

**FNAC MATERIAL PREPARATION**

FNAC material obtained with the needle is expelled onto glass slides or into a solution (Fig. 1.7). Material on the slides is smeared by sliding another glass slide over it gently (Fig. 1.8), resulting in an evenly spread thin smear with the

**Figure 1.6** Free needle FNAC procedure. In recent years, a free needle technique of aspiration has been used. This is particularly useful in very small, mobile lesions (e.g. lymph nodes). The aspirator has a much better feel of the tip of the needle and better control of the area sampled. It is not the method of choice for cystic or very sclerosed lesions.

**Figure 1.7** FNAC procedure. After the needle is withdrawn from the patient, a 20 ml syringe is attached to the needle with the air having been already drawn prior to attachment. The material is expelled from the needle onto the centre of a glass slide. The process is repeated, each time making sure that the needle is removed from the syringe when the air is drawn. Excess material expelled may be imprinted onto another slide thus multiplying the slides obtained.
Figure 1.8 FNAC smearing procedure. Although there are various ways of spreading material onto the slide, we find that the most uniformly spread material is obtained when two glass slides are pulled over each other parallel. The top slide should not be at an angle but flat on top of the bottom slide. Otherwise it creates uneven thickness of the smear and ridges with accumulation of cells and various fixation artefacts.

majority of material in the centre of the slide (Fig. 1.9). This enables easier stain penetration and clearer microscopic image. Excess of blood is undesirable since this dilutes cellular material. Fibrin clots tend to aggregate cellular material and make them uninterpretable (Fig. 10). The solution is spun in the laboratory with the aid of a centrifuge. Material on the slides, either smeared directly or spun, is either air dried or immediately fixed in 95% alcohol. Air dried smears are stained with May Grunwald Giemsa (MGG) (Romanowski). Alcohol fixed preparations are stained with Papanicolaou (PAP) stain. In the outpatient clinics, a rapid staining method is used to obtain an immediate microscopic image (Fig. 1.10). In our practice, we use mostly air dried smears since they are amenable to rapid staining techniques and immunocytochemistry. When deciding which method of cell preparation and fixation to use, the aspirator is guided by the clinical and pathological findings at the time of FNAC.

Some of the material at the time of aspiration may be sent for bacterial culture or washed in saline for molecular techniques.

WHO SHOULD BE PERFORMING FNAC?

It is our experience and that of others, that a good sampling technique is essential for the successful interpretation of

Figure 1.9 FNAC spreading. Material spread on the slides should be placed centrally and spread evenly so that the staining is even and screening easier (see bottom row of slides). Bloodstained smears (see top row) are unevenly spread, contain many blood clots and empty areas. When stained, much of the material may be washed off (see Fig. 1.10).

Figure 1.10 FNAC artefacts. Poorly spread bloodstained smears, as seen in Fig. 1.9 (top row), result in thick material, in which cells are trapped in the strands of fibrin or detached in the staining process as in this case. Material obtained in this way is unusable.
FNAC. Comparing the material obtained in the FNAC clinic by the cytopathologist with the material sent from various aspirators, we found that the rate of material unsuitable for diagnosis was 9% and 46% respectively. It is this that has prompted us to centralise the FNAC service where a cytopathologist performs aspiration. Similar experience was described by Cajulis et al, where the cytopathologist performed 59% of FNAC and clinicians performed 49%, achieving 100% and 77% diagnostic accuracy respectively. The greater the experience of the operator the more improved the accuracy rate.

The FNAC clinic, where the pathologist takes and reports optimally prepared and stained specimens, provides a high-quality and accurate service on which clinicians can confidently base clinical management decisions (Figs 1.11–1.13). Unnecessary investigations and operations are avoided, allowing resources to be released for other procedures.

The triad of physical examination/clinical history, appropriate cell preparation and subsequent interpretation is essential for a successful FNAC service and is in our view best performed by fewer persons. Like others, we have experienced difficulties when different people have undertaken one or more components of this triad. Lack of skill, clinical information and communication has on occasions been detrimental to the result. The success rate of FNAC

**Figure 1.11** Rapid staining and examination. Slides are stained with one of the rapid stains and examined under the microscope for cellularity. This gives a good indication of whether further material is needed or if a different cell preparation technique should be applied.

**Figure 1.12** Examination of the slides in the clinic. This gives orientation of cellularity and indicates whether further samples need to be taken for special techniques and/or microbiology cultures.

**Figure 1.13** Cytology report. The report should contain the patient’s details and clinical history and the number and position of the site(s) sampled (preferably illustrated by photograph or diagram), as well as indicating the name of the aspirator, the date of the sample, the number of slides made and/or other material obtained, the microscopic description, the cytological diagnosis, the diagnostic code and the pathologist’s signature and date.
diagnosis in material obtained by an experienced cytopathologist is far in excess of that where specimens are taken and sent to a laboratory for interpretation.

References


Salivary glands

Introduction
Non-neoplastic and inflammatory conditions
Benign tumours of the salivary gland
Malignant tumours of the salivary glands
Miscellaneous tumours
INTRODUCTION

Cytological diagnosis of salivary gland lesions is becoming one of the most sought after requests in the preoperative clinical management of patients. Therefore, cytopathologists have to be familiar with the main principles necessary for safe practice. First, the anatomical site of the material is important. If it is within the area of major and minor salivary glands, it is important to establish if the lesion involves salivary gland or has arisen in adjacent tissues such as lymph nodes or skin (Fig. 2.1). Second, it is important to decide whether or not the lesion is neoplastic. In many series, non-neoplastic lesions make for over 50% of fine needle aspiration cytology (FNAC) requests and fewer than 10% of these had subsequent surgery. Third, if the lesion is neoplastic, the cytopathologist has to decide whether it is benign or malignant (low or high grade). The distinction is desirable since benign tumours may not require surgery and highly malignant tumours may require the planning of a more radical surgical procedure. Cytological diagnosis of malignancy is based on well-established cytological criteria: nuclear enlargement, pleomorphism, chromatin pattern, nucleoli, cytoplasmic differentiation, cell cohesiveness and arrangement and presence of background stromal material, as well as on the clinical history and presentation. From a practical point of view, cytological subtyping of malignant salivary gland tumours is unnecessary since it rarely influences management. Although diagnosis of some common tumours is not difficult in most cases, sampling error may cause difficulties in interpretation.

Diagnostic accuracy

Cytopathologists interpreting FNAC of the salivary gland should be aware of the diagnostic features of common conditions. However, as large series have shown, even in the best hands, cytological interpretation of salivary gland cytology does not have a 100% sensitivity and specificity (Table 2.1). False-negative and false-positive diagnoses are described in most series. However, if type-specific diag-

Table 2.1 Sensitivity and specificity of salivary gland FNAC in some of the series carried out between 1990 and 2000

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>No. of cases</th>
<th>Sensitivity</th>
<th>Specificity</th>
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<tr>
<td>Orell (1995)</td>
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<td>Candel et al (1993)</td>
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<td>95.7</td>
<td>100</td>
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<td>Frable &amp; Frable (1991)</td>
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<td>93.3</td>
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<td>79</td>
<td>84</td>
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noses are made only when all diagnostic criteria are present and any uncertainty clearly conveyed to the clinician, FNAC is a safe and accurate tool in the investigation of salivary gland lesions. The pitfalls in cytological interpretation can be avoided with increased practice. Special training is required to become proficient in both obtaining and interpreting aspirated material. It is not sufficient for pathologists to apply personal experience from tissue pathology to the diagnosis of cytological specimens.

Role of FNAC in management of salivary gland lesions

Although it is accepted that clinical and radiological assessment (including two-phase helical CT) of parotid masses cannot distinguish reliably between benign and malignant lesions, the value of FNAC in the evaluation and management of salivary gland pathology has been controversial for a long time. The major reasons for this controversy are the difficulty in cytological evaluation and the fact that the extent of surgery can be easily defined based on clinical judgement, particularly at a cancer referral centre (where clinicians may be experienced at diagnosing parotid gland malignancies). However, a preoperative diagnosis is helpful in discussions with patients regarding the extent and type of surgery necessary. Most centres using salivary gland FNAC do so selectively, in an attempt to obtain a preoperative diagnosis in order to distinguish between neoplasms and non-neoplastic conditions and thus avoid surgery in conditions that clinically mimic neoplasm. A combination of MRI findings and cytology results is optimal for diagnosing malignancies of the parotid lesions.

Apart from the fact that FNAC can distinguish benign from malignant conditions, it is also very useful in distinguishing between salivary and other non-salivary pathology. Preoperative diagnosis of Warthin’s tumour, lymphoma or benign lymphoepithelial disease is essential to the correct management of these patients. With FNAC, surgical excision is often unnecessary.

When assessing the impact of FNAC of salivary gland masses in clinical decision making, Heller et al. compared the clinician’s initial clinical impression with the FNAC diagnosis and the final diagnosis in each case. Overall, FNAC resulted in a change in the clinical approach to 35% of the patients. As a result, they recommend the performance of FNAC in almost all patients with salivary masses.

FNAC provides accurate diagnosis of most salivary gland lesions and contributes to conservative management in many patients with non-neoplastic conditions. By using FNAC, an operation was avoided in 70% and 79% of patients with a non-neoplastic lesion and a metastasis respectively. FNAC plays an important role in the preoperative and postoperative assessment of parotid masses by aiding in the evaluation of tumours in poor surgical candidates and unresectable tumours, and by identifying metastases from other sites. Although definitive subclassification of some lesion types remains poor, FNAC is invaluable in patient triage.

Diagnostic difficulties

The wide spectrum of benign and malignant tumours as well as the heterogeneity of many tumours poses a formidable task for any cytopathologist with an ambition for a good histological correlation of his or her findings. The original series of salivary gland tumours described by Zajicek and co-workers at the Karolinska hospital in the 1960s and 1970s described the tumour types known at the time. Since that time, many new entities have been described. The newest World Health Organization classification of salivary gland tumours now includes 9 benign tumours (adenomas) and 18 malignant tumours (carcinomas), with the addition of non-epithelial tumours, malignant lymphoma, tumour-like lesions and metastatic tumours (Box 2.1). There is sometimes overlap between different conditions showing similar appearances.

Problem areas in FNAC of salivary gland lesions are: cystic lesions (neoplastic and non-neoplastic); atypical cells in pleomorphic adenoma; cellular smears with epithelial cells and no stroma; squamous or ‘squamous differentiation’, tumours with a ‘clear cell’ pattern, hyaline stromal globules and prominent lymphoid component in some lesions. These present potential pitfalls, of which the cytopathologist needs to be aware and, if necessary, include differential diagnosis as part of the final report. The clinician will then plan further management and decide about the need for surgery on clinical grounds, knowing that only the final histology will give the full answer.

Salivary gland tumours are composed of epithelial cells, myoepithelial cells and stroma in various proportions. Cytological smears lack the architecture that is particularly important in differentiating some of the tumours.