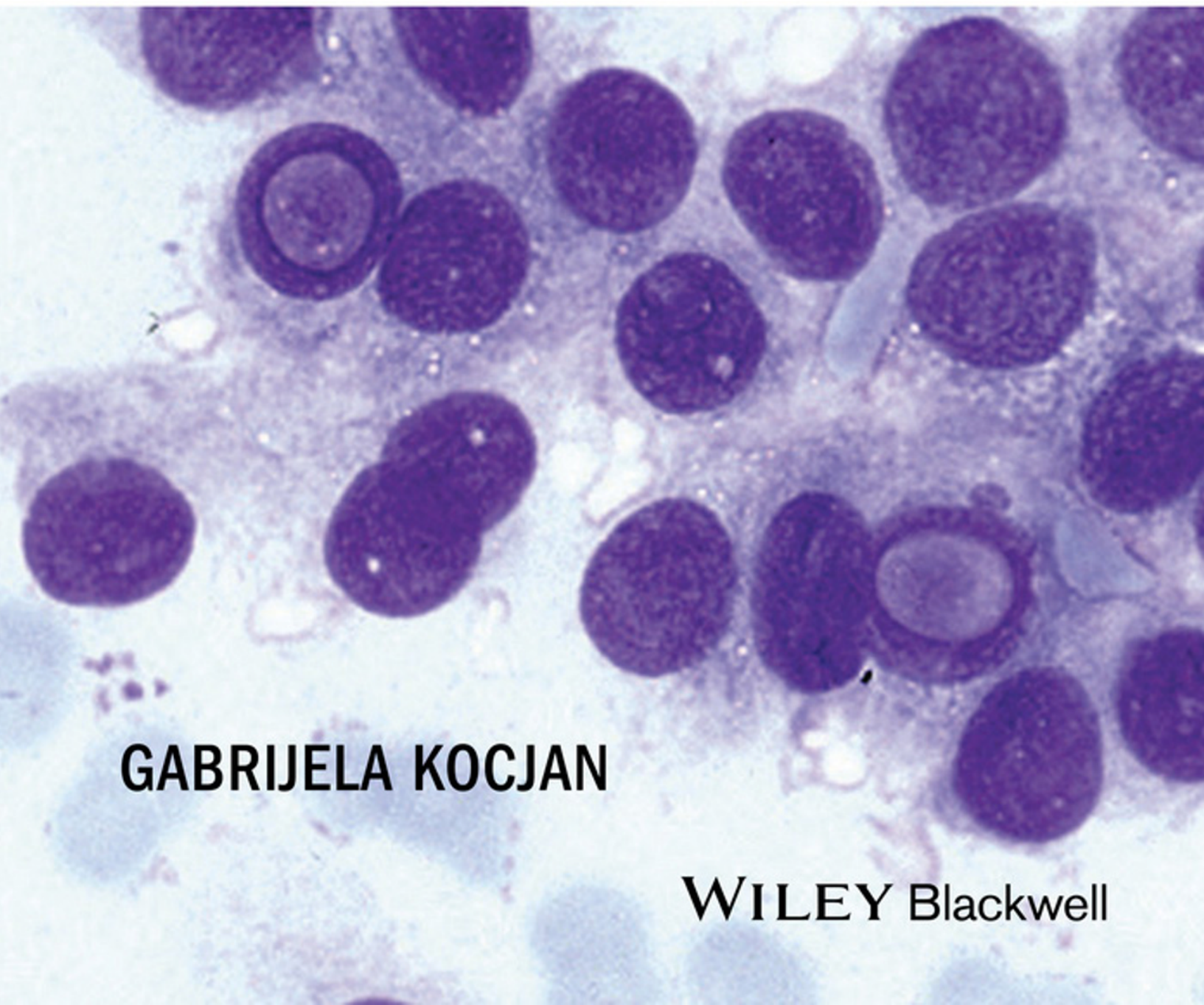


SECOND EDITION

CYTOPATHOLOGY OF THE HEAD AND NECK ULTRASOUND GUIDED FNAC



GABRIJELA KOCJAN

WILEY Blackwell

Cytopathology of the Head and Neck

Ultrasound Guided FNAC

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Ultrasound Guided FNAC

Second Edition

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MAXIMUM EFFECT WITH MINIMUM INTERVENTION

To all those striving to optimize diagnostic process and realize efficiency in the health service

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Preface to the first edition

The past century has seen Cytopathology as a discipline, and Fine Needle Aspiration Cytology as a method of obtaining material, become established. From the pioneering work of Martin and Ellis, through the enthusiasm of Zajicek and his colleagues, to the perseverance of numerous cytopathologists throughout the world, this simple technique has become part of the routine diagnostic investigations. To this end, this book summarises recent experiences with the role Cytopathology is playing in current clinical practice, particularly in relation to Head and Neck. This work is based on the experience drawn from a large referral practice from ear, nose and throat, maxillofacial and general surgeons, endocrinologists, oncologists and others. From sceptical beginnings the FNAC service has grown and developed, to the extent that it is now accepted as a routine investigation and cytopathologists are considered as the best people to deliver the service. Mutual understanding and trust between clinical colleagues and cytopathologists has led to the further development of skills and a desire for further improvement in respective disciplines. Clinically oriented

work is a valuable source for the education and training of laboratory and junior pathology staff. To a cytopathologist, who meets new patients daily, this work is enormously satisfying. In the new millennium, our efforts should not be spent any more at proving the validity of FNAC. Instead, with the advances in new technologies, our aim should be the refinement of morphological diagnoses in order to match the existing or improve future treatment options.

My sincere thanks for producing this book go, firstly, to the patients who posed for the, sometimes, unflattering photographs; to the medical and technical colleagues in the Cytopathology Laboratory, University College Hospital London, to eminent clinical colleagues for their advice and support and to Mr Paratian for processing the photographs. Lastly, I would like to thank Tony and Arabella for putting up with my absences during the long gestation of this book.

Gabrijela Kocjan
London, May 2000

Preface to the second edition

The idea for the second edition of this book arose through realization that the working practices of Head and Neck (HN) diagnostic and clinical teams have changed dramatically in the last 15 years, not only in terms of organization of health service with its aims for provision of HN cancer care but also in their diagnostic input. The publication of high profile professional guidance documents highlighted the importance of specialist Multidisciplinary Teams (MDT) with the intention that these should bring together all the services and organizations to provide high quality care. Written protocols, that specify investigations for each type of presentation of possible HN cancer as well as specific guidelines for investigation and diagnosis of each form of HN cancer, have emerged. The aim of reducing cancer waiting times meant that Rapid Access (One stop) diagnostic clinics have become a requirement not only in the base hospital of the specialist multidisciplinary teams (MDT) but also in many District General Hospitals and that these clinics are required to provide same day diagnosis by Ultrasound and Fine Needle Aspiration Cytology (FNAC), tissue/cell sampling thus becoming an essential function of these clinics. Pathologists within the HN networks now have to ensure that conditions under which FNAC and rapid diagnosis clinics services are provided follow the professional guidelines and are also part of the local network guidelines.

The initial experience with One stop Clinics found widespread diagnostic difficulties including a high non-diagnostic rate highlighting the need for a particularly high level of expertise required to achieve a precise and reliable diagnosis in HN through the involvement of specialist radiologists and cytopathologists. To achieve high levels of diagnostic accuracy, special training and commitment to cytopathology are required in addition to histopathology. There is a need for recognition of the new skills expected of practicing pathologists and a comprehensive approach to cytopathology training, to include performing FNAC, with or without ultrasound guidance and interpreting them on site, as is the case with frozen section specimen training in histopathology. Ancillary techniques that have become available in the past 10 years are now a mainstream requirement for diagnosis and sometimes prognosis of various conditions and can be applied to FNAC material. Trainee pathologists specializing in cytopathology require a secondment to centres where on site evaluation and rapid access clinics are in place and where molecular techniques are available. This may require pathologists to be absent from routine work at their institution in order to learn new skills and adopt different ways of working.

Ultrasound (US) guidance has emerged as an essential adjunct to either FNAC or needle core biopsy, and its use is expected to increase. US combined with US guided FNAC can be recommended as a method for evaluating regional metastases in HN patients, for both those with and those without palpable lumps. US and, if necessary, FNAC should continue to be the investigation method of

first choice for HN lesions. US-guided FNAC sessions benefit from attendance of cytopathology medical and non-medical staff to perform the procedure, assess adequacy of the samples and make decisions about collecting appropriate material for ancillary tests.

In our own practice, the emergence of MDT Meetings (MDM) where radiologists, oncologists, radiotherapists, surgeons, speech and language therapists, pathologists and other support staff meet regularly once a week and discuss individual cases in a formal meeting, meant a significant improvement in HN service. MDMs contributed to the understanding of the role each discipline plays in the clinical management and helped improve patient outcomes. This collaboration in a quest for successful outcomes has also helped drive the progress in using ancillary techniques in diagnosis thus enabling the so called personalised medicine. One stop HN clinics and MDMs are a model of service delivery that hopefully can be used as an example in successful health management.

My thanks for the publication of this book go primarily to all patients whose conditions served as an inspiration for education, training and research. I am extremely appreciative of all members of the MDT for their input, patience and support; our sessions were as much fun as they were informative. Thanks to my colleagues Simon Morley, a contributor to this book, and Timothy Beale, both radiologists, I managed to obtain a desk and a chair in the One Stop Clinic. Collaboration with surgeons, in particular Paul O'Flynn and Francis Vaz, went beyond the HN to tennis and golf tournaments. My gratitude goes to all my colleagues and staff in the Department of Cellular Pathology, who skeptically tolerated my indulgence in cytology, provided there was a Summer Party at the end. It is through cells that I met so many wonderful people, travelled around the world and made lasting friendships. It is a testament to Wiley editorial and production teams, headed by Claire Bonnet and Eswari Maruthu that this book is presented in such a clear and constructive manner which I am proud of and thankful for. Finally, my lasting devotion goes to my family who were a source of pride and encouragement throughout. I hope that this textbook justifies the sacrifice they made.

As I am approaching the end of my working life, this book represents forty years of experience working as a diagnostic cytopathologist in a prestigious institution, a tertiary referral and a Cancer Centre. As such, it is a summary of the most interesting clinical examples where FNAC made a real difference to the management. It is my life long ambition that this legacy continues and that, by using cells alone, maximum diagnostic effect is achieved with minimum of intervention. I believe that this is achievable in not too distant future.

Gabrijela Kocjan
Cavtat, January 2017

About the companion website

This book is accompanied by a companion website:

www.wiley.com/go/kocjan/clinical_cytopathology_head_neck2e

The website includes:

- Over 20 exclusive to website studies of head and neck ultrasound case histories, with description of essential diagnostic features and differential diagnosis, compiled by Dr Simon Morley.
- Powerpoints of all figures from the book

The password for the site is the last word in the caption for Figure 4.1.

Introduction

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1.1 Introduction

The Head and Neck (HN) area is one of the most complex regions of the body because of its anatomical and functional diversity. Diseases of the HN, both primary and systemic, rarely go unnoticed; patients either notice changes themselves, or are alerted to them by the diagnostic investigations, often done for unrelated conditions.

HN cancer is the ninth most common cancer in the USA, accounting for 3.3 % of all cancers. The incidence of HN cancer has plateaued recently; however, morbidity and mortality continue to remain high. Despite the decline in overall mortality rates since 2001 a racial disparity between the whites and the African Americans, both in incidence and mortality, still exists [1].

Tobacco and alcohol use are the most important risk factors for most HN cancers. In addition, infection with certain types of human papillomavirus (HPV) is thought to be the cause of an escalating incidence of HPV-related oropharyngeal squamous cell carcinoma predominantly among middle-aged adults [2].

1.2 Fine needle aspiration cytology of the head and neck

Fine needle aspiration cytology (FNAC) has been recognised as one of the core activities for the management of HN disease [3–23]. Sites in the HN that are amenable to FNAC include the thyroid, cervical masses and nodules, salivary glands, intraoral lesions and lesions in the paraspinal area and base of skull [24].

FNAC has a high overall diagnostic accuracy: 85–95% for all HN masses, 95% for benign lesions, and 87% for malignant ones [25, 26]. Diagnostic accuracy is dependent on the site of aspiration as well as the skill of the individual performing and interpreting the FNAC [24]. Each site undergoing FNAC within the HN is associated with its own set of differential diagnoses and diagnostic challenges. There are virtually no contraindications, and complications are minimal [27].

FNAC allows an immediate diagnosis to be available to the clinician so that appropriate treatment can be discussed with the patient. It is recommended as a first line of investigation in palpable HN masses. FNAC is the preferred first-line pathological

investigation of salivary gland and thyroid lumps because of the risk of recurrence and complications, respectively, associated with tissue biopsies [28].

The majority of aspirates from the HN will be to confirm an otherwise suspected diagnosis, for example a reactive lymphadenopathy or to confirm clinical staging for a metastatic carcinoma. However, there are a number of occasions where an unsuspected condition may be revealed, such as lymphoma or a salivary gland tumour. Whilst the diagnosis of lymphoma may need further tissue work up, the diagnosis of salivary gland lesions is often definitive in that it guides the surgical or non-surgical management. FNAC can diagnose majority of thyroid enlargements and help reduce the rate of surgery for benign thyroid disease. Ancillary techniques, namely immunocytochemistry, flow cytometry and 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 [29].

1.3 Ultrasound guided FNAC

Ultrasound imaging is a dynamic and readily available technique that is particularly useful in the examination of superficial structures. Modern machines combined with high frequency linear probes (7.5–12 MHz) produce high definition images in multiple planes. The spatial resolution that is achieved surpasses that of both multislice computed tomography (CT) and magnetic resonance imaging (MRI). Images are rapidly acquired, artefacts are few, and the technique is highly acceptable to most patients. As an adjunct to structural imaging, colour (directional) and power Doppler (non-directional but more sensitive) are often used to assess blood flow and the vascularity of tissue. These techniques add value in detecting abnormal peripheral or chaotic flow patterns in malignant lymph nodes, in assessing the patency of normal vessels, and in the investigation of vascular and lymphatic malformations.

Ultrasound (US) guidance is a useful adjunct to either FNAC or needle core biopsy (CB), and its use is expected to increase. US combined with US guided FNAC, rather than a tissue biopsy, can be recommended as a method for evaluating possible regional metastases in HN cancer patients, for both those with and those without a known primary tumour [30, 31].

US and, if necessary, FNAC, should continue to be the investigation method of first choice for HN lesions. The main indication for CB is after repeated failures of FNAC to provide a diagnosis. It can also be performed in patients who are not surgical candidates or in those who refuse surgery. Kraft et al. found CB was superior to FNAC in providing a specific diagnosis (90 vs 66%), and achieved a higher accuracy in identifying true neoplasms (100 vs 93%) and detecting malignancy (99 vs 90%). However, the sensitivity and specificity did not differ significantly between the two methods [32]. Khalid et al. found that the use of US-guided FNAC as the initial modality for tissue sampling of a thyroid nodule is more effective than traditional FNAC at an additional cost of \$289 per additional correct diagnosis [33].

In our own experience, the adequacy rate of US guided FNAC is critical for the success of the service. In our institution the adequacy of US guided FNAC in the HN clinic is 97% [34]. Computed tomography and magnetic resonance imaging do not appear to add any advantage to FNAC in terms of specificity, sensitivity or accuracy of a malignant diagnosis [34]. As with rapid-diagnosis clinics, US-guided FNAC sessions benefit from attendance of cytopathology medical and non-medical staff to assess adequacy of the samples and make decisions about collecting appropriate material for ancillary tests.

1.4 A Combined US/FNAC approach

Recently, some cytopathologists have learned to use ultrasound machines to assist them in performing FNAC procedures. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were 96, 50, 98, 33 and 94% in palpation guided (PGFNAC) versus 100, 86, 97, 100 and 97% in the US guided (USGFNAC) group, respectively. USGFNACs performed by a cytopathologist could significantly improve the specificity and NPV ($P=0.04$) while preserving virtually the same excellent sensitivity and PPV as those of PGFNACs. With US guidance, a cytopathologist is able to perform FNACs in smaller, non-palpable lesions and target complex lesions with confidence and accuracy, thus achieving a better outcome [36].

In our experience, even better results are obtained when an experienced radiologist takes the FNAC and has a cytopathologist close by to process the material and interpret the slides (Fig. 1.1).

According to the British Society for Clinical Cytology (BSCC) Code of Practice, the combination of physical examination/clinical history, radiological assessment, careful needle sampling, appropriate cell preparation, subsequent interpretation and multidisciplinary clinical discussion are essential for a successful outcome [37]. The lack of skill, clinical information and communication can be detrimental to the result.

1.5 Sampling technique

Sample collection is a major factor influencing both the adequacy and the accuracy of FNAC [38,39]. It is our experience and experience of others that a good sampling technique is essential for

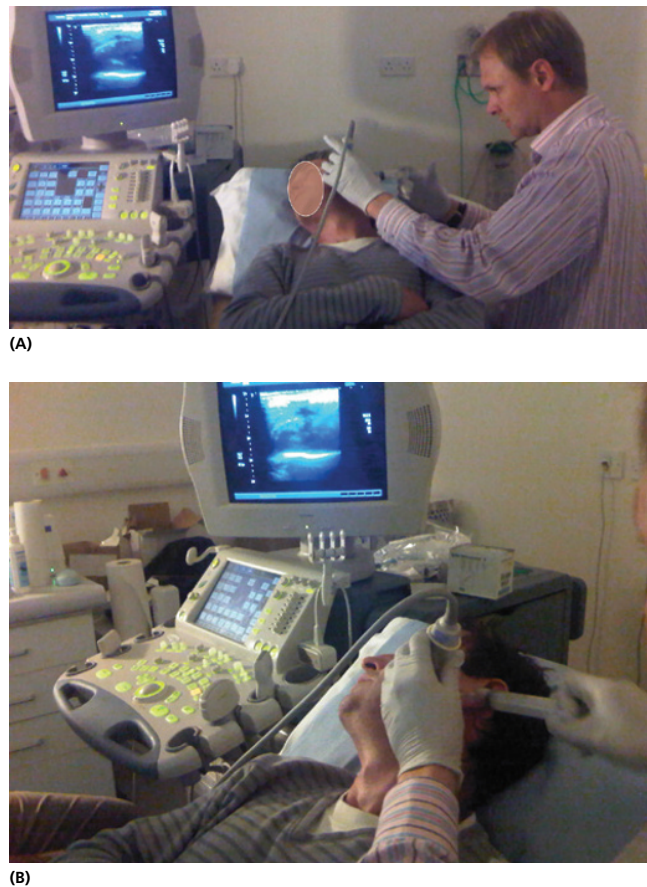


Figure 1.1 Ultrasound guided FNAC. (A) The radiologist, Dr Morley, uses the ultrasound probe in the left hand and injects the anesthetic into the lesion with the right hand. (B) Observing the monitor and using the ultrasound probe for guidance, the aspirator uses negative pressure to extract cystic fluid from the parotid gland lesion.

successful interpretation of FNAC. Comparing the material obtained by the cytopathologist with the material sent from various aspirators, Wu et al. found that the sensitivity of HN FNAC procedures is significantly better in the cytopathologist-performed group than in the non-cytopathologist-performed group (96 versus 67%) [40, 41]. Greater experience of the operator appears to improve the accuracy rate [42–44]. In experienced hands, palpation-guided FNAC is an excellent diagnostic tool. However, there is a movement towards using imaging guidance to target all masses [45].

The best results are obtained with a cytopathologist-led FNAC service, where the pathologist reviews the specimen immediately, in relation to the clinical context, thereby deciding on adequacy and the need for further sampling (Fig. 1.2) [46, 47].

With the FNAC procedure having been explained (Fig. 1.3), the patient is put in a supine position. The choice of whether to apply anaesthetic or not 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 needle-phobic or a child, or if the site is particularly tender, for example, 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



Figure 1.2 Examination of the glass slides in the clinic. This gives an orientation of adequacy and indicates whether further samples need to be taken for special techniques and/or for microbiology cultures. Results are usually not discussed with the patient at this preliminary stage of the investigations.



Figure 1.3 Clinical history and examination. The pathologist at the bedside examines the area referred to by the specialist and also asks the patient further relevant questions about the duration of the swelling, level of pain and any other associated systemic symptoms.

been using a needle free syringe where the pressurised air expels the anaesthetic, penetrating the skin, without the needle (Fig. 1.4A) [48]. Anaesthetic forms a small white ring through which the subsequent test needle is applied, once or more (Fig. 1.4B). Patients do not experience any pain on application of the anaesthetic and experience no or minimal pain at FNAC.



(A)



(B)

Figure 1.4 (A) Needle free anaesthetic system. Designed for patients who need daily injections, e.g. insulin, but also applicable to the local anaesthesia. This is particularly useful in children, in needle-phobic patients, in sensitive sites and where multiple needle passes are anticipated. (B) Needle-free anaesthetic system. A pale ring indicates the area of subdermal infiltration. A test needle should be passed through this area.

The palpable area in question is cleaned with an antiseptic agent and fixed between the two fingers of the non-dominant hand. 22 G, 23G or smaller needle is then passed into the lump using a non-aspiration technique (capillary sampling) with the aid of a needle only (without the syringe attachment) (Fig. 1.5). In a meta analysis comprising over 2000 thyroid FNAC samples, there was no difference between the aspiration and non aspiration technique in assessing thyroid nodules [29]. In cases where a fluid aspirate is expected, a syringe and a syringe holder are attached to the needle to help aspiration (Fig. 1.6) [29, 49–54]. The needle is passed round in a fan-shaped manner 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 representative material. When exiting the lump, if using syringe attachment, it is important



Figure 1.5 Free needle FNAC procedure. This, so called 'capillary technique', is particularly useful in very small, mobile lesions, e.g. lymph nodes. An 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.

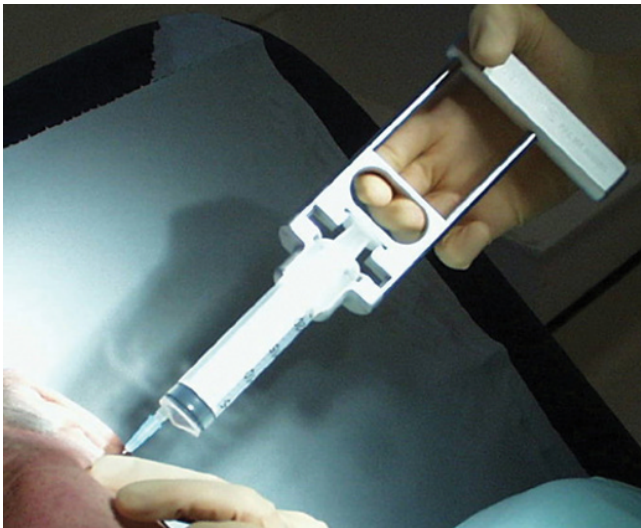


Figure 1.6 FNAC of fluids. A 20 ml syringe attached to a CAMECO (BELPRO MEDICAL, Canada), syringe holder A 23 G needle is used.

to release the negative pressure before exiting, otherwise the material is aspirated into the syringe and can only be retrieved by the aid of a needle wash.

Whilst adhering to the traditional technique of smearing the material ejected from the needle onto a slide and then either air drying or fixing it in alcohol, if necessary we also suspend the material from a separate needle pass in a liquid medium that can then be used for ancillary techniques including cell block



Figure 1.7 Rapid staining and examination. Slides are stained with one of the rapid stains and examined under the microscope for cellularity. This gives a good orientation if more material is needed or if different cell preparation technique should be applied.

(CBL). Air dried smears can be stained by a rapid staining technique to assess material adequacy in the One Stop clinic (Fig. 1.7). CBL provide a method for immunocytochemistry (ICC) that has revolutionised cytopathology by making it possible to apply panels of antibodies to multiple sequential sections of aspirated or exfoliated cellular material [55]. CBL can be prepared from virtually all varieties of cytological samples. CBL sections offer advantages over conventional cytological smears with respect to cellular architecture and archival storage. They also provide several sections, which can be utilised to perform special stains, immunophenotypic analysis, ultrastructural studies and molecular tests, including cytogenetic and polymerase chain reaction (PCR)-based techniques [56–59]. In today's era of personalised medicine, the ability to perform these tests augment the utility of cytological samples in analysing the molecular alterations as effectively as surgical biopsies or resection specimens. With the availability of molecular targeted therapy for many cancers, a large number of recent studies have used cytological material or CBL for molecular characterisation. Jain et al. described various methods of preparations of CBL and their application in cytology. The advantages and disadvantages of various methods of cell preparation are outlined in Table 1.1 [50].

One of the easiest way to prepare a CBL is the so-called 'Poor Man's cell block'. This method should be available to any laboratory and is able to produce very good results (Fig. 1.8) [61].

The final cytopathology report should be clear, written with the knowledge of the ultrasound and clinical findings, morphology and ancillary techniques (Fig. 1.9). Difficult cases should be discussed at the intradepartmental and multidisciplinary meetings and are also an important source of education and training [62] (Fig. 1.10).

Table 1.1 Comparison of different cytological preparation methods.

Direct smear ¹³			Cytospin ²⁵		Cell block ²⁻⁴		Liquid-based cytology ²⁶	
For	Against		For	Against	For	Against	For	Against
Diagnosis Fast			Optimal for cysts, urine and effusions	Cellular crowding	Additional to other slides	Needs time and histology skill	Easy to transport, collect and process	Low cellularity
Inexpensive	Needle handling		Multiple slides	Limited cellularity	Increased diagnostic yield		Reduced screening area	Cell shrinkage (methanol)
Routine process	Multiple slides		Air dried and/or alcohol fixed		More architecture		No air-drying artefacts	Less architecture
Permits rapid evaluation	Obscuring background				Archival storage		Clean background	Reduced or altered background material
Excellent cellular detail	Air-drying artefacts						Monolayer of cells	
ICC possible on fresh or destained slides	Adverse effect of stripped nuclei and cytoplasmic background		Routine laboratory method	Risk of FNs due to focal antigen expression	Easy to perform and compare with histology	Variable staining due to different fixatives	Equal or stronger than direct smears	Alcohol-based: may differ from formalin-fixed slides
Suitable for nuclear antibodies	Potential increased FNs				Routine histology controls		Equal distribution of staining	All antibodies not yet evaluated
					Dual ICC possible		Clean background	
Cytogenetic and molecular testing					Optimal results with current methods	Depends on cellular yield and fixation methods	Suitable for FISH and molecular analysis	Limited studies so far available
Potentially effective for preparation and storage	May be diluted by normal cells		Suitable for FISH	Cell crowding may hamper nuclear signals				
Routinely available and cost-effective	Cells of interest may be enriched by microdissection		DNA extraction possible	May be diluted by normal cells	Cells of interest may be enriched by microdissection	Partial cells in sections	Avoids cross-linkage of formalin fixation	May be affected by alcohol fixation (e.g. PR)
Whole cells			Whole cells			Sections	Whole cells	

FISH, fluorescence in situ hybridization; FN, false negative; ICC, immunocytochemistry; PR, progesterone receptors.

Source: Jain D, Mathur SR, Iyer VK. Cell blocks in cytopathology: a review of preparative methods, utility in diagnosis and role in ancillary studies. *Cytopathology*. 2014 Dec; 25(6): 356-71.

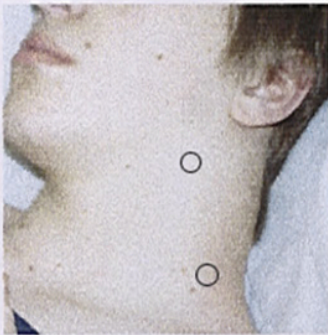
Cytology Report		CPA Accredited Laboratory
Department of Histopathology University College London Medical School Rockefeller Building, University St, London WC1E 6JJ		
Lab No.: NG00-0404	Hospital No.: 0000000	
Name : XXXXX,YYYYYY	Age/Sex : 18Y M	
Cons/GP : Doctor X	Ward : Maxillofacial Unit,	
.....		
SPECIMEN : Neck Fine needle aspirate		
EXAMINED BY: DR T HATTER		
SPECIMEN OBTAINED BY: DR G KOCJAN		
CLINICAL DATA: Swelling left neck and post auricular region. Fluctuant below angle mandible.		
MATERIAL RECEIVED: Nineteen slides from FNA 2 different sites in the left neck (see illustration).		
MICROSCOPIC DESCRIPTION: Smears show numerous single and small aggregates of cells. The cells have large oval nuclei with fine chromatin pattern, indistinct nucleoli, and scanty basophilic cytoplasm with prominent vacuolation.		
Immunocytochemistry: LCA and CD20 positive. Tdt, desmin, SMA, myoglobin, MYC2 negative.		
CYTOLOGICAL DIAGNOSIS: FNA left neck. High grade B cell non Hodgkin's lymphoma (? Burkitt's). Biopsy is advised.		
SNOMED : TY0600;M095903		
Signed :	Pathologist : DR G KOCJAN	
Date : 14/02/2000		
 PATHOLOGY DIRECTORATE		

Figure 1.8 Cytopathology report should contain patient's details, clinical history, number and position of the site(s) sampled (preferably illustrated by a photograph or a diagram), indicate the name of the aspirator, date of sample, number of slides made and/or other material obtained, microscopic description, special techniques used, cytological diagnosis, diagnostic code, pathologist's signature and date. This report is just an example for illustration. Today we would use flow cytometry and cell block immunocytochemistry to confirm and specify the diagnosis of lymphoma on FNAC.

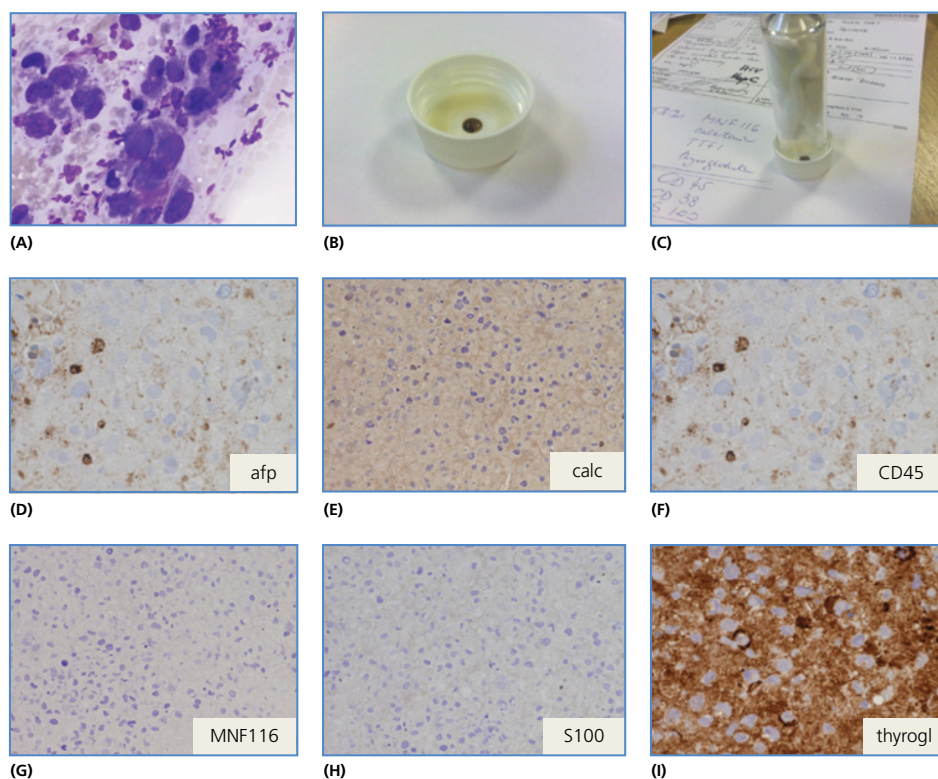


Figure 1.9 Images demonstrating the main steps in the preparation of a vapour fixed cell block. (A) The FNAC material from the thyroid is stained with May Grunwald Giemsa (MGG) and shows undifferentiated tumour cells. (B) The material is expelled from the needle into the well of the lid in a universal container. (C) It is left inverted for at least 6 h to allow the formalin vapours to fix it until solid after which it is handled as a histological sample. (D) afp negative, (E) calcitonin negative, (F) CD45 negative, (G) MNF 116 negative, (H) S100 negative and (I) Thyroglobulin positive. The conclusion was that this is anaplastic carcinoma arising from the thyroid.



Figure 1.10 Cytology education and training. Most interesting cases are discussed weekly at the multiheaded microscope; once a year, a traditional Christmas quiz, in addition to the seasonal jollity, provides a reminder of the most difficult cases.

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