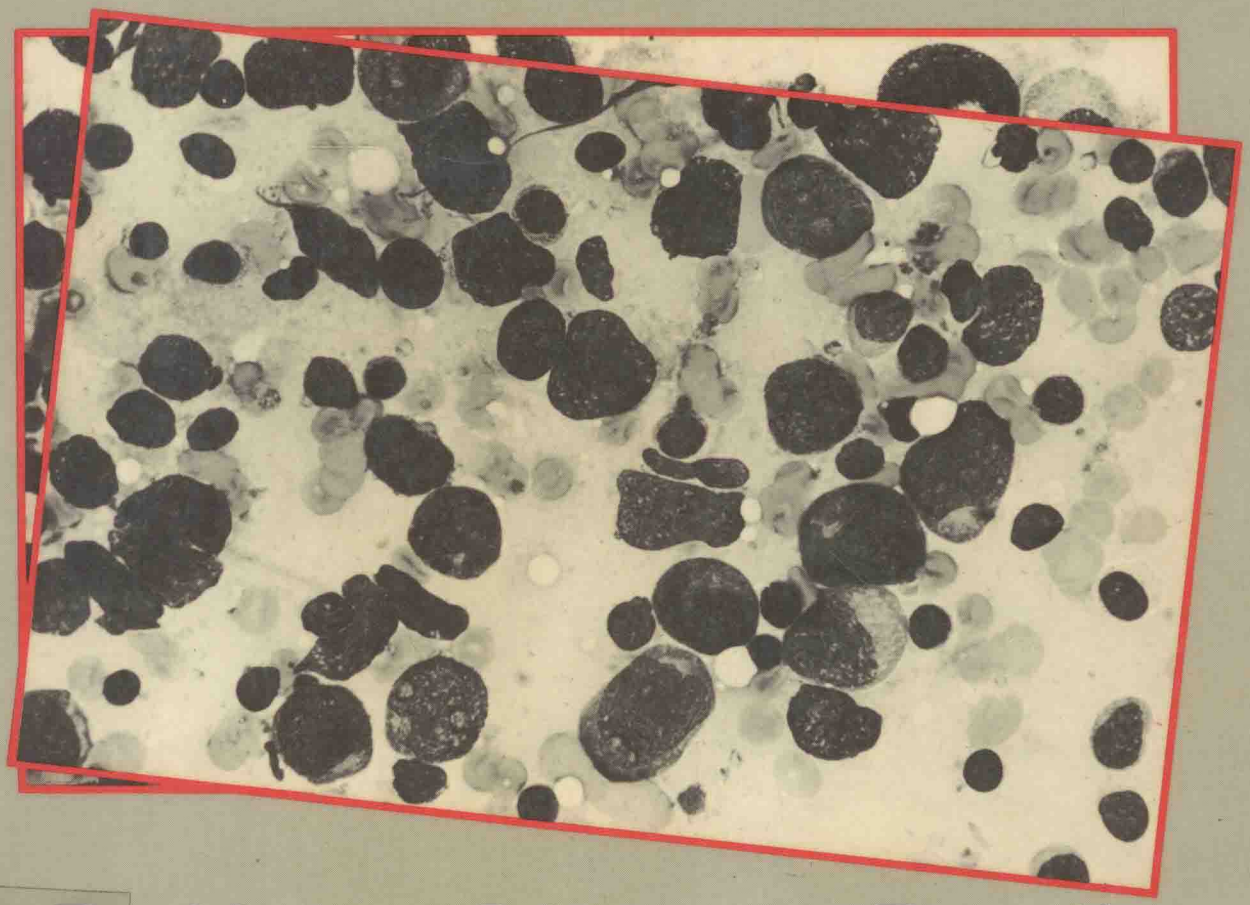
PRACTICAL ASPIRATION CYTOLOGY



David Melcher • John Linehan • Russell Smith

Churchill Livingstone

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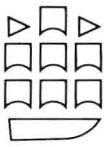

CHURCHILL LIVINGSTONE
EDINBURGH LONDON MELBOURNE AND NEW YORK 1984

CHURCHILL LIVINGSTONE
Medical Division of Longman Group Limited

Distributed in the United States of America by Churchill Livingstone Inc., 1560 Broadway, New York, N.Y. 10036, and by associated companies, branches and representatives throughout the world.

First published 1984

ISBN 0 443 02633 5

British Library Cataloguing in Publication Data
Melcher, D.H.
Practical aspiration cytology.
1. Diagnosis, Cytologic 2. Biopsy, Needle
I. Title
616.07'582 R43

Library of Congress Cataloging in Publication Data
Practical aspiration cytology.
Includes index.
1. Diagnosis, Cytologic. 2. Biopsy, Needle.
I. Melcher, David. II. Linehan, John. III. Smith, Russell.
[DNIM: 1. Biopsy, Needle. 2. Cytodiagnosis Methods. WB 379 P895]
RB43.P68 1984 616.07'582 83-7266

Printed in Great Britain by BAS Printers Limited, Over Wallop, Hampshire

Practical Aspiration Cytology

Preface

This book was planned as a laboratory bench book for the novice venturing into the fascinating, rewarding, practical world of fine-needle aspiration cytology. The essentials are enthusiasm and a supply of disposable syringes as used for venepuncture. If necessary a definitive diagnosis can be made while the patient is in outpatients or operating theatre.

We have attempted to be fully explanatory, with simple techniques an guidance charts, yet avoiding debatable issues (e.g. lymphoma classification). There are many photomicrographs to help the beginner, but to keep this book within a low price range we chose black and white rather than expensive colour reproductions.

We express our thanks to our colleague Rod Machling for his invaluable help in processing and printing many of the photomicrographs, and Mrs Jean Wiles for her excellent secretarial work.

To complete the comprehensive survey in this diagnostic art we invited collaborators, each expert in his or her own field, including that of immunology, with whom clinical cytology will become increasingly and inextricably linked as monoclonal antibodies and newer techniques become routine.

1984

D.H.M.
J.J.L.
R.S.S.

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Technique and interpretation

HISTORICAL DEVELOPMENT

Clinical cytology is now used world-wide; it does not supplant histology but augments it — they could be considered opposite faces of the same coin. Resistance to cytologic evidence under the microscope has a long history. There is a story that a keen French worker in the 18th century wanted to demonstrate the new instrument called a microscope, being newly used by botanists. He was assured by the French Faculty of Medicine that there was no place for the microscope in medicine. Again, almost a century and a half ago at a lecture in Oxford, a certain Dr Kidd was looking down a microscope whilst a younger colleague explained what he was looking at. Dr Kidd said, 'First, I do not believe in it, and secondly if it is true I do not think God means us to know it'.

The early microscopists had not only to satisfy their curiosity but teach themselves. Their descriptions and drawings have been well documented, including those of Robert Hooke, who in 1665 looked down the microscope he built and described the small elements seen as 'cells or little boxes'. He not only coined the word 'cell' but measured them: 'and in a cubick inch, above twelve hundred million, a thing almost incredible, did not our microscope assure us of it . . .'.

Of the many workers in many countries it was Johannes Muller who set the foundations of clinical cytology as we know it today.

In 1838 he published *On the Nature and Structural Characteristics of Cancer and those Morbid Growths that may be confounded with it*. Most microscopists of that day were still trying to recognise normal cells whilst Müller was already describing 'these minute globular cells which form the real seminium morbi in several forms of carcinoma' and distinguishing this from sarcoma!

One of Müller's disciples was the great Virchow, who twenty years later rejected the humoral theory of disease that had come down from the ancient Greeks. He published *Cellular Pathology* which claimed that all cells come from other cells, and that the origins of disease lie in the defects, the failure of the cell itself.

Then cellular pathology became 'formalised'. Instead of scrapes, pieces of tissue were immersed in formalin-based fixatives and the tissue was supported by clearing the intracellular juices and substituting them with molten paraffin wax, which was then allowed to cool and solidify. This technique was first used by Klebst in 1869; whereas Rutherford in 1871 employed a freezing microtome using ice as the supporting vehicle. Very thin slices of the tissue could now be cut and stained and mounted on a slide. The architecture of the tissue with its cellular relationships could be examined microscopically.

Thus cytology (from the Greek cytos = hollow vessel) became superceded by histology (from the Greek histos = web, tissue) and the study of stained, finely cut sections dominated

the next three score years and ten. But around the world isolated workers were using scrape or needle cytology. Martin & Ellis in New York began examining 1400 lesions in 1926. In Britain it was Dudgeon & Barrett in 1934, with Gibson & Smith in 1957 and Webb in 1970. The Herzen Institute in Moscow and the Curie Foundation in Paris used the technique but by far the greatest exponent was Zajicek in Sweden. At the Radiumhemmet in Stockholm about 12 000 fine needle aspirates are performed every year by experienced cytopathologists.

In most hospitals in the United Kingdom the aspirations are not performed within the cytology department but by the clinicians, who send the slides direct to the laboratory. As the equipment needed for a fine needle aspirate is minimal, the aspirate can be performed whenever and wherever necessary, in the clinic, ward, or even the patient's home. In most cases a definitive answer can be given, and if a cytological diagnosis of 'malignant cells' is made, histological confirmation prior to definite treatment is not required. On the other hand, a report of 'no malignant cells seen' does not exclude a cancer. If there is any doubt in the mind of the cytologist, it is indicated so in the report, and a formal biopsy or frozen section is performed.

Confirmation of metastatic tumour in a lymph node, or mastectomy scar, or skin nodule is amongst the easiest of cytological interpretations, and is of great value to the clinician. Seeding of tumour cells along the fine needle track is virtually unknown.

Trott & Randall (1979) summarised the relative merits of surgical biopsy and fine needle aspiration cytology (Table 1.1).

Table 1.1

	Surgical biopsy	*Fine needle aspiration cytology*
Diagnosis	Histopathological	Cytopathological
Diagnostic facility	Narrow	Broad
Anaesthetic	Yes	No
Length of procedure	More than 5 minutes	Less than 5 minutes
Report available	1–2 days	1–2 hours
False positives	None	Rare
False negatives	Few	Some
Cost	High	Low
Specimen obtained	In operating theatre	As out-patient anywhere
Trauma	Yes	Little, if any

In breast lumps, whereas neither cytology nor histology gives 100% accuracy, the initial use of cytology with frozen section back-up in doubtful cases gives a higher degree of accuracy than either frozen section or cytology alone.

As in other branches of laboratory investigation, a 'positive' result is significant, whereas a 'negative' may not be so, as the needle tip may have missed the lesion.

METHOD OF SPECIMEN COLLECTION

MATERIALS

1. Swabs with spirit or skin sterilising solution.
2. Disposable 10 ml or 20 ml syringe with fine needle, s.w.g. 21–23 (or 0.8 mm diameter) as used for venepuncture (Table 1.2). A pistol-grip syringe holder is preferred by some doctors (Fig. 1.1).

Table 1.2 Comparison of needle s.w.g. number and diameter in millimetres

Standard wire gauge number	*Diameter (mm)*
11	2.946
15	1.829
18	1.2192
19	1.0160
20	0.9144
21	0.8128
22	0.7112
23	0.6096
24	0.5588
25	0.5080
30	0.3150
40	0.1219

3. Several 76 × 26 mm microscope slides. It is essential that the slide be labelled clearly and correctly. Slides with ground and polished edges and the end inch 'frosted' (i.e. ground glass) are ideal, because if the patient's identification is written in pencil it is unaffected by the various solutions used in the subsequent staining process. Pencil carbon is insoluble in water, alcohol, acids and alkalies. Paper or ink labelling is unreliable and dangerous. If plain slides are used the patient's identification must be scratched on the surface with a suitable instrument.
4. Small transport box for slide preparations in which the specimen slides are held separately, so that the face of the slide is not damaged or contaminated in transportation or postage. Those supplied for use with cervical smears are adequate.
5. Completed laboratory request form with full clinical details.

TAKING THE SPECIMEN

Many workers on the European continent (Schöndorf, 1978; Zajicek, 1979) prefer to hold their syringe in a holder with a pistol grip handle such as the Cameco syringe holder* which holds disposable 10 ml or 20 ml plastic syringes that are fitted with a Luer off-centre needle (Fig. 1.1). With the syringe fitted in the pistol grip, by firmly closing the fist, the syringe-

* Obtainable from Cameco AB, Tabyvagan 71–180 Enebybang, Sweden

plunger is pulled up the barrel and suction is exerted through the lumen of the needle. Others (Gardecki, 1980) prefer not to use the syringe holder. They claim they get a better sensitivity and 'feel' by holding the disposable syringe.

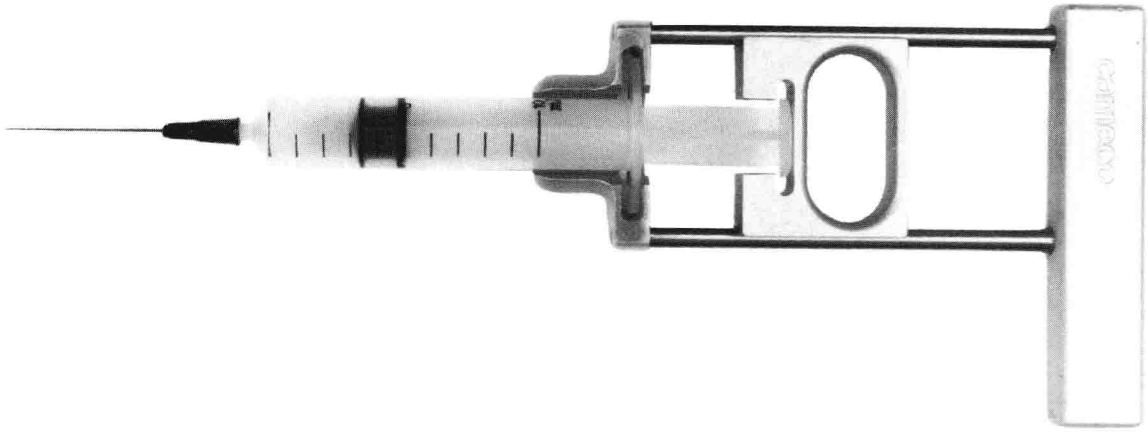

Fig. 1.1 'Pistol grip' syringe holder with syringe and needle in place

TECHNIQUE

The skin is cleaned and the 'lump' is located and firmly held between the thumb and forefinger of the free hand. The syringe is held by the outside of the barrel or the pistol grip, and the needle tip is pushed into the lesion. The plunger is partially retracted creating a negative pressure. Without losing the pressure or pulling the needle tip out of the skin, the whole syringe is rotated by a movement of the wrist and gently moved in and out. The cutting edge of the needle tip frees cells inside the lesion which are sucked into the fine bore of the needle. Using continuous negative pressure by pulling firmly on the plunger of the syringe, guide the cutting tip of the needle forwards and backwards obliquely through the firmly held lump (Fig. 1.2), and twisting the wrist to apply a rotating as well as a forward and backward action, cells are sucked into the lumen of the needle.

Nothing is usually visible in the body of the syringe between the bottom of the plunger and the needle head. A cyst being an obvious exception.

Now slowly release the pressure on the plunger so that there is no more suction effect. Shift the fingers to hold the outside barrel of the syringe well away from the plunger. Withdraw the syringe and needle gently from the skin. If this important relaxing of the pressure is not performed before removing the needle, air will rush up the needle and lose the specimen into the body of the syringe!

After the needle is withdrawn from the patient it is removed from the syringe, the syringe filled with air, and the needle replaced firmly. However, some workers using an air reservoir technique before commencing the aspiration, withdraw about 1 ml of air into the barrel of the syringe. This avoids removing the needle after the cells have been aspirated from the lesion.

The syringe is held vertically with the needle-tip just above the surface of the microscope slide. The plunger is pushed down and the contents of the needle are blown gently on to the slide, or slides. Some claim that wetting the inside of the needle with a saline-heparin solution

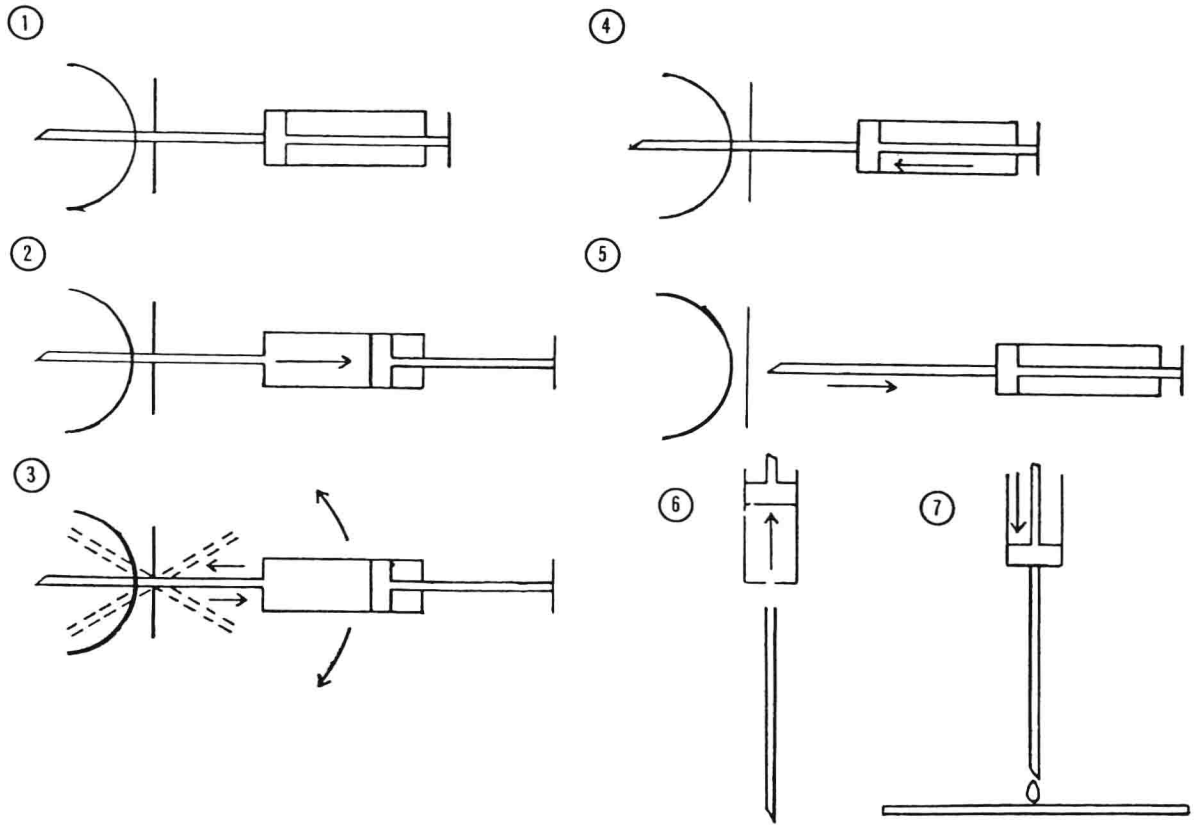

Fig. 1.2 Technique of fine needle aspiration cytology

inhibits cells from adhering, and a greater yield of cells is obtained (Duguid et al, 1979), but we have not found this an advantage.

The cellular preparation on the microscope slide should be thin and even. If there is a small blob of specimen a coverglass or another slide held at an oblique angle can be used to spread the specimen, using the same technique as making a blood film. If the sample varies in thickness the needle tip may be used to tease out those thicker parts of the specimen. A large specimen must be distributed thinly on several slides.

When aspirating a vascular organ such as the thyroid, a bloody aspirate may be obtained. A technique such as that used at the Karolinska Institute in Stockholm may be used: the syringe contents are emptied quickly on to one or two slides and the excess blood absorbed using gauze pads, and the residual tissue fragments transferred to another clean slide before the blood clots. Smears are prepared initially as for blood films. If there is semi-solid material on the specimen slide, the spreader slide is turned over and the flat surface is pressed gently on to these tiny fragments and the slides pulled apart, ending up with evenly spaced cells on both slide surfaces.

Making the cellular preparations should be rapid but with as little trauma to the cells as possible. Then the slides should be vigorously waved in the air to air-dry them as quickly as possible for subsequent staining by the May-Grünwald Giemsa method.

If Papanicolaou Stain is preferred then the specimen slide should be wet-fixed, by spray or immersion *before* the cells can dry.

The May-Grünwald/Giemsa method of staining is preferred by many cytologists looking at fine needle tissue aspirations. It is the same method as used in haematology laboratories for staining bone marrow smears. The colour range of the cytoplasm varies from the palest blue through the slate blue of keratinising squamous cells to the dark blue (basophilic) of plasma cells and the red to deep purple of the nuclei. There are morphological changes too. The air-dried cells appear larger than wet-fixed cells which are often shrunken, and in appearance like a boiled egg. An air-dried cell more resembles a fried egg, with its cytoplasm well spread out and cellular detail is enhanced. If the specimen has already been stained by May-Grünwald/Giemsa and the results indicate a keratinising squamous cell carcinoma, it is relatively simple to rapidly decolourise the slide in 3% hydrochloric acid in 70% alcohol, wash in water and then re-stain using Papanicolaou technique. The cell morphology is less satisfactory than a wet-fixed preparation, but is adequate and keratin is clearly demonstrated. We have used this restaining method many times to confirm a keratinising squamous cell lesion.

May-Grünwald/Giemsa staining method

Although we have tried staining machines that automatically stain blood films with this type of stain, we have not had much success with tissue aspirates due to the unevenness of the preparations, with cell clusters and adipose tissue leading to patchy staining. We therefore stain tissue aspirates by hand on staining rods across the laboratory sink.

All the stains in this Rowmanowsky group, e.g. Giemsa, Leishman's, Wright's, and Jenner, are composed of eosin and methylene blue mixtures, precipated, and resuspended in methanol, together with the oxidation products of methylene blue (i.e. azures). Principally used in haematology these commercial stains produce a range of colours varying from shades of blue, through mauve and purple to red. Constancy of colours is maintained by using water buffered to the appropriate pH.

Some methods, as in May-Grünwald/Giemsa, use two of the dyes in succession. May-Grünwald stain gives the subtle cytoplasm shades whereas Giemsa is a traditional stain for the cell nucleus.

The first stain applied is *very* important. The pure methyl alcohol solvent present in the May-Grünwald 'fixes' the air-dried cells. Subsequent dilution with the buffered water takes the stain from an alcoholic solution into a predominantly aqueous solution which stains the cells. If the stock bottle of May-Grünwald is large, the frequent opening of the bottle, especially near the staining sink, allows the methyl alcohol to absorb vapour *which reduces its effectiveness as a cell fixative*. Some workers initially dip the slide into pure methyl alcohol, but, *unless it is fresh*, the hygroscopic character of pure methyl alcohol absorbs water vapour and diminishes the cell fixation potential.

Any practice of staining slides by May-Grünwald/Giemsa in pots or jars along the laboratory bench should be avoided, unless the solutions in the pots are absolutely fresh for that batch of slides.

To maintain the cell fixation quality of May-Grünwald, we always buy it in small volumes of 100 ml quantity and the stain is used up before there is time for it to 'go off'. Thus, the next batch of May-Grünwald, freshly opened, is used on subsequent batches of specimen slides.

With this method we get crisply defined cells, well fixed, stained with a wide spectrum of colour and demonstrating where they occur, granules, or nuclear or cytoplasmic inclusions.

The final stain appearances can be altered to suit personal tastes by purchasing the stains

from different manufacturers; by varying staining dilutions or the pH of the buffered water; the length of time of staining; and the final rinsing.

The authors use British Drug Houses stains in solution and find the following technique gives very good results:

1. With a pasteur pipette flood slides with May-Grünwald stain and leave for two minutes to fix.
2. Add an equal volume of water buffered to pH 6.8, mix with pipette and leave to stain 9 minutes.
3. Rinse off the diluted May-Grünwald stain with buffered water (at pH 6.8), drain slide and immediately flood with freshly diluted and mixed Giemsa stain (1 part Giemsa and nine parts buffered water).
4. Briefly wash off stain with buffered water, shake slide free of excess, wipe back of slide and stand on edge to dry. Do *not overwash* otherwise some of the stain within the cells will come out. Only rinse briefly, then leave to air dry.
5. Mount in suitable medium.

CELL SIZE

The total magnification is determined by the lenses in the eyepiece, the objective, the reduction lens built into the camera, and the length of tube. When printed the magnification of the negative from the enlarger on to the printing paper must also be considered. Subsequently the photograph may further be changed in size for the convenience of fitting into the printed page.

Therefore, whenever measuring cells under the microscope, where possible use a red cell or neutrophil as a standard for size comparison. Remember that in an air-dried preparation stained by May-Grünwald/Giemsa the red blood cell will measure a little over 7 μ and the neutrophil leucocyte 12μ. Wet fixed cells may shrink which, though it will make the cells appear smaller, are still relative in size to the neutrophil leucocyte.

Photomicrographic illustrations of the various cells described are reproduced in this book, with the magnifications given in the caption.

PRINCIPLES OF REPORTING

For there to be full confidence in a cytology report, there must be a close liason between the laboratory and the clinicians who understand the implications of each comment. To issue a report saying, 'no malignant cells seen' when, in fact, the specimen material is scanty and unsatisfactory for cytological assessment, can be misleading.

Weigh the implications of everything said in the report and suggest a repeat of the investigation if not entirely convinced.

It is much better to have a false negative result than false positives.

Our reports fall broadly into four main categories:

1. *No epithelial cells seen.* This indicates an inadequate specimen, that where a carcinoma was suspected the lesion was missed, or failed to aspirate, and only blood and a little

* British Drug Houses, Poole, Dorset

fat or adipose tissue obtained. For example, in a breast aspirate where no ductal cells are present.

2. *No malignant cells seen.* This report is issued when benign cells expected from this site are present, and this presumes a representative aspirate. A description of the types of cells, and their condition and numbers, may be of help to establish a benign diagnosis, e.g. an abscess, apocrine metaplasia or fat necrosis in a breast, or numerous lymphoid cells suggest that the lump was a swollen lymph node.

A report of no malignant cells seen does not exclude the patient from having cancer. It merely indicates there were no malignant cells in this preparation.

3. *Malignant cells present.* This report must only be used when there is no doubt that the lesion is malignant and corroborated by a colleague within the department. Such a report should result in the patient receiving treatment for cancer.

4. *Suspicious but not diagnostic of malignancy.* This report should be avoided whenever possible, because it is of little help to the clinician. It may, however, be necessary and unavoidable if the specimen is very scanty, or the specimen is cellular and suggests well differentiated malignancy, but it is not sufficiently clear cut to submit the patient to definitive treatment for cancer.

The aspirate should be repeated, and preferably a biopsy recommended.

It is possible:

a. Not only to diagnose malignancy, but also to report whether the tumour is well, moderately or poorly differentiated

b. Sometimes to identify the type of malignancy, e.g. a keratinising squamous cell carcinoma; a clear cell carcinoma from a kidney, or a myeloma

c. To identify the site of the primary tumour from an aspirate of a metastatic tumour, e.g. recognition of an oat cell (small cell) metastatic carcinoma in a lymph node, or renal cells from a bone aspirate

a, b and c are questions asked by the clinicians and where we can give more information than just 'malignant cells present' we do so, but we only answer question c with extreme reluctance. Our experience shows that the accuracy of cytological diagnosis must not be undermined by overconfidence.

CONCLUSION

The reason for the rapidly increasing popularity of fine needle aspirate cytology is the simplicity of the technique, the rapidity of an answer, often whilst the patient is still attending the out-patient clinic, and the low cost. Thus, treatment can be planned and explained to patients before they leave the clinic, and therefore there is no delay.

Whether as a pre-operative investigation or as post-operative monitoring, this simple test is carried out with very little stress or risk to patients. In fact, they are often relieved to find that something is being done.

The scope of aspiration has widened and workers now use long needles to obtain cell samples deep within specific organs. The problem of accurately guiding the needle tip to the lesion within the organ is accomplished by close collaboration with the staff of X-ray departments or ultrasound specialists (Chs 6 & 7).

Cytology and histology together give a more accurate diagnosis than either method on their own.

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2
The breast

Fine needle aspiration cytology (FNAC) of the breast is an addition to the well established techniques of clinical examination, palpation, biopsy and frozen section, and more recently mammography, thermography and 'Tru-cut' needle biopsy.

Various techniques in cytology have been applied by many workers in the past, e.g. Ward (1912), Martin & Ellis (1926), Dudgeon & Patrick (1927) and Dudgeon & Barrett (1934), but FNAC has only recently become, in many centres, a routine diagnostic procedure. Zajicek (1979), Webb (1970) and Gardecki et al (1980) are keen advocates of the techniques to be used as part of the routine diagnostic methods.

The inclusion of FNAC as a routine in Brighton began in 1974, and is now employed almost without exception in patients with a palpable breast lesion enabling the reduction of frozen section by approximately 80% (Gardecki et al, 1980). Other advantages using the technique are that it is simple to perform and causes little discomfort to the patient. In some cases local bruising may occur but the authors have never encountered a subsequent infection or seen tumour spread due to needling.

Other workers (Berg & Robbins, 1962; Engzel et al, 1971) have exhaustively examined this aspect of the technique and have reported no evidence that FNAC produces increased metastases or local spread along the needle track.

ANATOMY

The breast in mature women consists of 15–20 lobes surrounded by fibrous and adipose tissue with each lobe containing multiple secretory acini and lobules embedded in a dense 'intralobular' fibrous stroma (Fig. 2.1). The lobes drain separately through lactiferous ducts to the nipple (Fig. 2.2). Lobules and ducts are lined by columnar epithelial cells which appear as a double or pseudostratified layer in the larger ducts down to a single layer in the acini. In ducts and acini a layer of cells can sometimes be seen between the columnar cells and the connective tissue (Fig. 2.1); these are the myoepithelial cells. Within an individual lobule the intralobular connective tissue is composed of spindle cells arranged in a loose, lightly stained delicate network (Fig. 2.1). This connective tissue differs from that between the lobules (interlobular connective tissue), which is compact, denser, collagenous fibrous tissue with few nuclei (Fig. 2.3). Stratified squamous epithelium covers the nipple and areola and extends only superficially into the main lactiferous ducts.