

Cytology

Phillip Woodford

Cytopathologist

Rebecca Said

Cytologist

Hunter Area Pathology
Service

John Hunter Hospital
Newcastle, New South
Wales

Key words

cervical screening, fine
needle aspiration, Pap
smear

Aust Prescr 2012;35:94-7

SUMMARY

Cytology allows the diagnosis of malignancy from a small number of cells. Cervical Pap smears are the most recognised example of this kind of testing and have dramatically reduced the incidence of squamous cervical carcinoma.

Cytology provides a minimally or non-invasive means of obtaining samples and allows analysis of specimens such as exfoliated cells in sputum and urine, and pleural, pericardial, cyst and ascitic fluids.

Fine needle aspiration yields cell samples from palpable lesions, and under image guidance from inaccessible sites such as the mediastinum, lung, head of pancreas and para-aortic lymph nodes.

Specimens are often small and to obtain maximum value it is vital that the request clearly specifies the question that is being asked and gives all the relevant clinical information.

Introduction

Cytology is literally 'the study of cells' and is related to anatomical pathology. While histology uses tissue sectioning techniques, cytology uses various cell preparation technologies. Specimens are obtained mechanically, by scrapings or needle aspirates, or by collection of exfoliated material from various body fluids. Gynaecological cytology, in the form of Pap (Papanicolaou) smears, is the most recognised type of test. However a wide variety of specimens are analysed by cytologists and cytopathologists, including urine, sputum and other body fluids. Fine needle aspiration specimens are also taken from sites such as lymph nodes, breast, thyroid and liver.

As with many tests, appropriate clinical information and patient history are essential and enable directed ancillary testing of limited material. Unfortunately, when this information is not provided it can lead to delay or failure of diagnosis due to an exhausted specimen. Communication is important and patients will benefit if their doctor has a good relationship with the cytology department.

Gynaecological cytology (Pap smears)

The cervical Pap smear is a long established screening test for cervical carcinoma. It identifies premalignant changes and allows selection of women with at-risk findings for more intense clinical examination, treatment and follow-up. It is very successful for detecting squamous lesions and can prevent most squamous cell carcinomas. In 1991, squamous cervical cancer occurred at a rate of 12.4/100 000. This had fallen to 5.4/100 000 by 2006.¹ Some glandular abnormalities including adenocarcinoma *in situ* can also be identified on Pap smears, however this is not the main aim of cervical screening programs.

Australian guidelines^{2,3} recommend screening every two years for all women over 18 years of age or two years after onset of sexual activity. Women over the age of 70 with two negative screens in the preceding five years can reasonably stop. The biggest risk factor for cervical cancer in Australia is not being screened, with 65% of all carcinomas identified in women who have been underscreened. The current Medicare Schedule provides a rebate for a conventional Pap smear.

Human papillomavirus is the main cause of cervical cancer. The transformation zone of the cervix is the area most susceptible to this infection and is sampled when collecting a Pap smear. This involves scraping the cervix transitional zone with a spatula or brush, then smearing the sample onto a glass slide and fixing with alcohol while still wet. Sampling should be taken by rotating the device around the endocervical canal. Abnormality develops at the squamo-columnar junction. This is the edge of any visible eversion/ectropion and care should be taken to sample this area.

Alternative monolayer technologies are equally effective but are not eligible for a Medicare rebate. After sampling, the plastic, not wooden, collection implement is vigorously agitated into a fixative solution to produce a cell suspension. This is subsequently processed in the laboratory to generate a more homogeneous specimen and a slide with cells spread one cell thick (thin layer or monolayer), with reduced obscuring factors such as blood or inflammation. After preparation of a conventional Pap smear, the plastic sampling brush can be rinsed into solution which can then be used for monolayer technology. Microbiological testing for chlamydia, by

polymerase chain reaction, and DNA subtyping of human papillomavirus (HPV) can also be performed on this solution.

Currently, DNA human papillomavirus testing and subtyping for high-risk strains can be used as an indicator of risk of recurrence of high-grade abnormality and as a 'test of cure'. A concurrent negative Pap smear with no high-risk human papillomavirus subtypes detected for two consecutive years enables a patient with a previous high-grade lesion to return from yearly screening to routine two-yearly screening. This use is covered by Medicare.

Sputum cytology

Sputum cytology is a diagnostic test not a screening test. A series of three deep cough morning sputum specimens has a 66% sensitivity for confirming lung carcinoma.⁴ A single sputum specimen has a sensitivity of 0.1–0.7, averaging 0.22 in a review of published studies.⁵ There is a small gain in additional specimens (up to five). Post-bronchoscopy sputum samples sometimes give diagnoses not achieved by other means.

Critical to sputum specimens is 'deep cough'. Many specimens are oral and contain saliva, oral squamous cells and micro-organisms. Cytologists judge sample adequacy by the presence of pigmented pulmonary macrophages. Cytology can only examine a small quantity of material so technologists subsample by picking blood or streaked areas for slide production. Sputum is mostly mucus which interferes with the creation of a homogeneous representative subsample and limits the diagnostic yield. However, with some manipulation monolayer and similar technologies can be used.

Sputum is more likely to be diagnostic with large and central lung lesions and less likely with small and peripheral lesions. Cytology can usually make the therapeutically vital distinction between small cell and non-small cell carcinomas. There is greater difficulty in reliably distinguishing adenocarcinoma, squamous cell carcinoma and large cell carcinoma, particularly when poorly differentiated. The distinction has become important with oncologists wishing to screen adenocarcinomas for activating mutations of epidermal growth factor receptor to determine eligibility for specific inhibitors such as gefitinib. Currently this requires a tissue sample or a good cell block preparation (more than a hundred malignant cells on the slide). This would normally require fine needle aspiration or core biopsy.

Urine cytology

Urine cytology is very sensitive for detecting high-grade urothelial malignancy (papillary neoplasm,

invasive and *in situ*) as these cells are recognisably malignant and shed readily. Cytology may be correctly positive when cystoscopy is negative for these malignancies. However, cytology is insensitive for diagnosing low-grade urothelial neoplasms and papillomas as these cells appear normal or near normal. The only abnormalities found may be increased cellularity and increased cell groups which are sometimes papillary. When made, the diagnoses of low-grade urothelial neoplasms and papillomas are sometimes incorrect, reflecting the difficulties. Sensitivity for high-grade lesions makes urine cytology useful in screening high-risk groups such as workers with exposure to carcinogenic chemicals. It is also useful for investigating symptomatic patients and in follow-up of patients with previous urinary tract malignancy. There is no role in detecting solid renal tumours or prostatic carcinomas as these shed only small numbers of cells late in their natural history.

Confounding factors in diagnosis

Inflammation caused, for example, by therapeutic *Bacillus Calmette-Guérin* (BCG – the tuberculosis vaccination organism used to treat bladder carcinoma) can generate prominent reactive cytological changes. Instrumentation and catheters can result in increased cellularity and cell grouping. These can confound cytological diagnosis and it is helpful if this information is provided on the request form. Giving details of how the sample was collected (instruments used) is also vital.

Urine is a harsh environment for cells and degenerative change is common. In combination with inflammation and instrumentation, it leads to a moderate number of specimens being called 'atypical'. A short series of three morning 50 mL specimens is preferred from ambulatory patients, but not first void of the day, as cells in these samples may have been sitting all night in urine.

Other specimens for cytology

Cytologists also examine other body fluids including pleural, ascitic and cyst fluid, as well as fine needle aspiration specimens from sites such as lymph nodes, breast, thyroid and liver.

Pleural, pericardial and ascitic fluid

Aspirated fluids are commonly sampled when looking for a malignancy. The preferred sample volume is 50–100 mL. While 1–2 L may have been removed from the patient, such specimens are simply too hard to manipulate and store. In contrast, smaller samples do not allow for production of a cell block and immunohistochemistry.

Synovial fluid

Ordering cytology on synovial fluid specimens is often unnecessary or wasteful. Where possible, diagnosis should be made by other means.

Cerebrospinal fluid

Cerebrospinal fluid specimens are usually examined cytologically. The composition of any cell content can be identified, such as neutrophils in bacterial meningitis or lymphoid cells in viral meningitis. Less commonly, malignant cells from haematopoietic malignancies (leukaemia, lymphoma), epithelial malignancies (breast cancer) and brain (ependymoma, medulloblastoma) can be found. Cerebrospinal fluid is a poor medium for cells and is ideally processed for slides within an hour of collection.

Fine needle aspiration

This involves collecting a specimen from a mass using a fine needle, paradoxically, usually without aspiration. The quality of the sample depends on the operator and their experience. Many pathology services provide clinics or appointments for patients with palpable lesions so specimens can be collected by an experienced pathologist.

Fine needle aspiration can be a very successful diagnostic strategy. It is as accurate and successful as core biopsies in the diagnosis of breast malignancy, but not for distinguishing invasive from *in situ* neoplasms (ductal carcinoma *in situ*).^{5,6} This level of success can only be achieved with a sufficient flow of specimens through the laboratory to develop true expertise. Pathological assessment of a fine needle aspirate has been part of the triple test for breast malignancy with clinical and radiological assessment. When any of the assessments disagree, biopsy is performed – a strategy that has ensured quality. The tendency is for

more core biopsies with prognostic and therapeutic markers to be done on the samples enabling presurgical hormone treatment or chemotherapy.

Image guidance

Image guidance, including ultrasound, computed tomography and conventional X-ray (for example angiography) is increasingly used by radiologists to sample impalpable, deep, intra-abdominal or intrathoracic lesions, such as para-aortic lymph nodes and lung masses. Attendance by cytology staff at the procedure can be an advantage as they can provide immediate feedback on the adequacy of a specimen. This reduces the need for repeat biopsies and additional patient anxiety.

Image guidance for palpable lesions is sometimes appropriate. For example, ultrasound guidance for thyroid nodule fine needle aspiration helps ensure that the tissue comes from the targeted nodule and not adjacent thyroid tissue.

Most recently, ultrasound-guided fine needle aspiration via endoscope has become possible with the endoscope serving as the ultrasound probe and the needle channel. In the upper gastrointestinal tract, this is being used to access the pancreas, bile duct and upper abdominal lymph nodes and allows pathological confirmation of carcinoma of the head of the pancreas. Ultrasound-guided fine needle aspiration via bronchoscope is being used to access mediastinal lymph nodes.

Cell blocks

The amount of material obtained in cytological preparations is small – a fine needle aspiration pass might yield 10 microlitres. This sample is used not only to make slides, from what will hopefully yield a diagnosis, but also for supplementary testing.

Table 1 Examples of supplementary technologies used with cytology

Technique	Indication	Purpose
Flow cytometry	Suspicious lymphoid population	Lymphocyte clonality to prove lymphoma, lymphocyte subtyping
Microbiology	Granulomatous	Culture
Immunostains*	Malignancy in pleural fluid	Distinguish between carcinoma and mesothelial cells
	Unidentified malignancy	Distinguish carcinoma and melanoma
	Carcinoma, unknown primary	Identify primary site of carcinoma
	Breast cancer	Detect prognostic markers on breast carcinoma: oestrogen receptor, progesterone receptor, HER2 amplification Determine eligibility for therapy
Molecular markers	Lung adenocarcinoma	Detect EGFR mutations to determine eligibility for therapy

HER2 human epidermal growth factor receptor 2

EGFR epidermal growth factor receptor

* While immunostains can be done on smears, cell blocks are a more effective and productive strategy

Specimens are often mixed with agar to lock the cells into a gel, a cell block, which can then be processed and sectioned using histology (Table 1). Cell blocks can be processed from most cytology specimens if required but are commonly made from fluid and fine needle aspirates when adequate material is received.

Immunohistochemistry

Immunostains are not one test but a selection of possibly hundreds of antibodies or stains that detect specific cell markers. The amount of sample and cost limit their use to a small number on any occasion. A clear request establishing what question needs answering together with basic clinical information can make all the difference in the outcome of the investigation. For example, a history of colonic carcinoma or a known ovarian mass would enable targeting of the test to markers for those malignancies. The use of molecular markers to define eligibility for new anticancer drugs is increasing, putting pressure on fine needle aspiration as an adequate source of

tissue. This may shift the diagnostic process towards more invasive core biopsies processed as histology specimens. However, new needle designs may increase the tissue recovery and provide larger and more consistent fragments for cell blocks. These have been developed initially for endoscopic fine needle aspiration and currently await approval from the US Food and Drug Administration.

Conclusion

Cytology is the original minimally invasive diagnostic technology beginning with exfoliated cells, aspirated fluid and subsequently fine needle aspirations. As endoscopic and imaging technologies have advanced, the surgical diagnostic specimen is being replaced by small image-guided fine needle aspirations and core biopsy specimens which provide a challenge for pathologists to do more with less. ◀

Conflict of interest: none declared

REFERENCES

1. Australian Institute of Health and Welfare. Cervical screening in Australia 2007–2008: data report. Cat. no. CAN 50. Canberra: AIHW; 2010.
2. National Health and Medical Research Council. Screening to prevent cervical cancer: guidelines for the management of asymptomatic women with screen-detected abnormalities. Canberra: NHMRC; 2005.
3. Department of Health and Ageing. National Cervical Screening Program Resources. 2008. www.cancerscreening.gov.au/internet/screening/publishing.nsf/Content/Cervical-resources-menu [cited 2012 May 7]
4. DeMay R. The Art & Science of Cytopathology. Chicago: American Society for Clinical Pathology Press; 1996.
5. Schreiber G, McCrory DC. Performance characteristics of differing modalities for diagnosis of suspected lung cancer: summary of published evidence. *Chest* 2003;123:115S-28S.
6. Simsir A, Rapkiewicz A, Cangiarella F. Current Utilization of Breast FNA in a Cytology Practice. *Diagn Cytopathology* 2008;37:140-2.



SELF-TEST QUESTIONS

True or false?

9. Urine cytology is sensitive for detecting solid renal tumours.
10. Fine needle aspiration is as effective as core biopsies for diagnosing breast cancer.

Answers on page 103

Valediction

Dr Shanthi Kanagarajah

Dr Shanthi Kanagarajah joined the Editorial Executive Committee of *Australian Prescriber* in 1997. Despite a career which has seen her move between Newcastle, Wollongong, Melbourne, Sydney and Brisbane, she has always maintained her strong commitment to *Australian Prescriber*. Dr Kanagarajah firmly believes in the importance of independent information about therapeutics and helped to ensure editorial independence was maintained when the National Prescribing Service – now NPS, Better choices, Better health – took over the publication of the journal in 2002.

In view of Dr Kanagarajah's extensive experience with *Australian Prescriber*, it was appropriate that she concluded her time with the journal as the chair of the Editorial Executive Committee. The editorial team has appreciated Dr Kanagarajah's good humour in steering the Committee through many manuscripts. Dr Kanagarajah has a sound understanding of the matters which are important to practising clinicians and this has supported the continuing growth in the readership of the journal. She has particularly supported the journal's online development. Despite retiring from the Committee, Dr Kanagarajah will continue to contribute to the quality use of medicines in Australia.

