Fine-needle aspiration biopsy of lymph nodes

Fine-needle aspiration biopsy (FNAB), when performed by trained operators, and for the correct indications, is a safe and minimally invasive procedure, with an excellent diagnostic yield.

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Definition, history and epidemiology

Arguably the best definition of FNAB is in a textbook of cytology by de May and reads 'FNA biopsy can be defined as the removal of a *sample* of cells, using a *fine* needle, from a *suspicious* mass for *diagnostic* purposes'.[1] Fine-needle aspiration biopsy (FNAB), when performed by trained operators and for the correct indications, is a safe and minimally invasive procedure, with an excellent diagnostic yield.[2] It has been used since the early 20th century to diagnose infectious and neoplastic disease.[3] Although the first large-scale report on needle aspiration biopsies came from Memorial Hospital in New York in 1930, it was primarily used by the Scandinavians, who published widely on its utility in the literature in the 1960s. From here its use spread gradually through Europe until it found its way back to the United States in the 1980s.[3] Globally, FNAB is now used as a first-line diagnostic procedure for triage of palpable masses, particularly peripheral lymphadenopathy.

However, the indications for its use appear to differ between developed and developing countries. In a review of MEDLINE looking at publications on FNAB between 1996 and 2002 there were 5 609 articles published on FNAB. Those from developed countries concentrated more on breast and pancreatic disease while the developing countries emphasised small round cell tumours and infectious disease, reflecting the diagnostic needs and healthcare priorities in these countries.[3]

This has become ever more apparent with the dual human immunodeficiency virus (HIV) and tuberculosis (TB) pandemics, where the impact is felt most keenly in the developing world. The 2008 estimates from the World Health Organization (WHO) showed 9.4 million new cases of active TB worldwide,[4] with 80% of all cases occurring in 22 high-burden countries (HBC). South Africa ranks in the top five of these HBC. In addition, of the new cases of TB, 1.4 million are associated with HIV and 78% of these occur in Africa.[4] It has been estimated that one-third of HIV-infected patients have coexistent TB and 8 - 10% of these will develop clinical disease annually.5 In 2008 extrapulmonary TB accounted for approximately one-fifth of all cases of TB notified,[6] and in endemic areas such as South Africa TB lymphadenitis is the commonest form of extrapulmonary TB. This is particularly relevant in any discussion of FNAB of lymph nodes.

Indications for FNAB of lymph nodes

This is dependent on factors related to the *patient,* the *lymph node* and on the *population* in general. Children commonly have persistently enlarged lymph nodes and careful examination may reveal local causes such as traction folliculitis.[7] Although FNAB may be used in persistent reactive lymphadenopathy (LAD) less than 1 - 2 cm to reassure the patient, this is not an optimal indication for use. It should preferably be confined to those cases with a strong clinical suspicion of a specific infection such as TB or neoplasia, unless the alternative is surgical biopsy. In adults, enlarged lymph nodes are more likely to be malignant than in children, although in South Africa TB is still the commonest diagnosis in persistent adult LAD.[8] The immune status of the patient will influence the diagnosis, as patients with HIV are at risk for infections or a lymphoma associated with HIV. A solitary enlarged lymph node is more likely to be malignant, while TB presents with matted lymph nodes. Lymph nodes in the posterior cervical triangle and supraclavicular nodes are more suspicious for malignancy. The diseases that are prevalent in the population are probably the single most important indication for FNAB of lymph nodes and this may vary not only within countries (developed v. developing world) but may relate to a specific hospital or clinic serviced by a clinician. A clinician performing FNAB in a breast clinic will certainly see almost exclusively primary or recurrent breast carcinoma in aspirates of lymph nodes, while an oncologist in haemato-oncology practice will aspirate lymphomas.

In SA, cytotechnologists are permitted by the scope of practice of their professional boards to perform FNAB under medical supervision, as are registered professional nurses.

The indications for FNAB of lymph nodes are summarised in Table I.

Table I. Indications for FNAB of lymph nodes

- Confirm suspected reactive hyperplasia
  - Infective
  - Autoimmune (e.g. SLE)
  - Drug reactions
- Diagnose a specific infection
  - Viral (e.g. CMV)
  - Bacterial (e.g. TB)
  - Parasitic (e.g. toxoplasmosis)
  - Fungal (e.g. cryptococcus)
- Diagnose a neoplastic infiltration
  - Primary lymphoma (e.g. HL or NHL)
  - Confirm transformation of a LG to a HG lymphoma (e.g. FL to DLBCL)
  - Metastatic tumour
    - Known primary
    - Unknown primary

The site of the lymph node may also indicate the primary lesion in metastatic tumours, as lymphatic drainage from visceral organs is to a certain extent predictable (Table II).[9] However, haematogeneously derived tumours may also be found in lymph nodes. Immunocytochemistry or labelling of the malignant cells with specific antibodies to confirm their origin may minimise costly investigations to localise the primary and expedite management of these patients.

It should preferably be confined to those cases with a strong clinical suspicion of a specific infection such as TB or neoplasia, unless the alternative is surgical biopsy.

Contraindications to FNAB of superficial lymph nodes

There are few, if any, true contraindications to FNAB of superficial lymph nodes apart from an uncooperative patient. In patients with a bleeding diathesis, FNAB is the safest modality to acquire a tissue diagnosis and pressure to the puncture site will reduce, if not prevent, haematoma formation. Rarely a carotid body tumour may mimic a lymph node and theoretically there is a risk of release of vasoactive substances but this is more likely in phaeochromocytomas. Patients with respiratory compromise and small axillary or supraclavicular lymph nodes should be aspirated with care (if at all), as a pneumothorax could prove fatal in these patients.

Table II. Common site of primary tumours in lymph node metastases

- Inguinal nodes
  - Melanoma
  - Anal carcinoma
- Axillary nodes
  - Breast carcinoma
  - Lung carcinoma – small cell and non-small cell
- Cervical nodes
  - Lung carcinoma – small cell and non-small cell
  - Melanoma
  - Squamous carcinoma of the oropharynx
  - Nasopharyngeal carcinoma
- Submandibular nodes
  - Squamous carcinoma of the oral cavity
- Supraclavicular nodes
  - Right
  - Oesophageal carcinoma
    - Lung carcinoma – small cell and non-small cell
    - Breast carcinoma
  - Left
    - Breast carcinoma
    - Lung carcinoma – small cell and non-small cell
    - Gastric carcinoma (Virchow's node)

Who should perform the FNAB?

This will depend on the regulatory practices and the needs of the countries where FNAB is utilised. According to the latest code of practice published by the British Society for Clinical Cytology in 2009 'responsibility for taking FNAs should lie in the hands of individuals who have a sufficient FNA workload to gain and maintain the necessary expertise and who are subject to clinical audit'.[10] In South Africa FNABs are performed by clinicians both in family practice, in primary health care clinics and regional and tertiary hospitals, as well as by pathologists. It has often been said that the best result is obtained when the aspirator is the same person who examines the smear, and therefore cytopathologists should do FNABs. Considering the extreme shortage of these in our South African situation this is neither possible nor necessary, and this statement probably arose as a result of the poor history that often accompanies the specimen. The better the clinical information the more specific and useful the diagnosis is likely to be. The age, gender, site and size of node, clinical symptoms, retroviral status, CD4 count (if available) and previous medical or surgical history are all valuable clues to the correct diagnosis.

In SA, cytotechnologists are permitted by the scope of practice of their professional boards to perform FNAB under medical supervision, as are registered professional nurses. There are a number of nurses who run FNAB clinics within tertiary and regional hospitals with excellent results and provide invaluable support to clinicians within the hospital as well as the region.

How to perform the perfect FNAB

The materials required for FNAB and the procedure are listed in Tables III and IV. The method is depicted in Figs 1 and 2. Like many minimally invasive procedures, when

clearly initially demonstrated and the correct equipment used, FNAB is a safe, virtually painless and well-tolerated procedure with a high sensitivity and specificity for neoplastic and infectious diseases. It is ideally suited for triage of patients in primary health care clinics as it requires minimal infrastructure. Equally so in tertiary hospitals, where it may assist in the management of patients referred for specialist care.

Table III. Materials required for FNAB

- Cutting needles (bevelled tip) (sterile)
  - Never larger than 22 g (black)
  - Children, all thyroid aspirates – 23 g (blue)
  - Small children, skin nodules – 26 g (brown)
- 10 cc syringes (sterile)
- Glass slides (cytology slides with ground glass edges)
- Alcohol-based commercial spray fixative or 95% alcohol
- Plastic or cardboard slide holders for transportation of slides
- Pencil for labelling slides
- Disinfectant for skin and cotton swab
- Non-sterile gloves
- Material for ancillary studies, e.g. TB transport medium, flow cytometry medium

Ancillary investigations

Taking into consideration all of the above, enlarged lymph nodes are still very common and the majority are benign. FNAB therefore plays an important role in triaging the reactive lymphadenopathies which can be followed up, from the infectious diseases which can be treated, and the malignancies which can be appropriately referred and/or managed. This has enormous benefits for the patient and the clinician as well as cost savings for the health system as a whole (Table V). These benefits, however, assume that an adequate well-fixed aspirate has been sent to a cytopathology laboratory experienced in examination of FNAB.

Mycobacterial culture and NAATs

In order to achieve the above, cytomorphology may be sufficient in some cases, but for infectious diseases such as TB, ideally either culture or one of the newer molecular techniques for speciation and sensitivity in this era of drug-resistant disease is required. FNAB is ideally suited to obtain material for both of the above. After preparation of the slides, the residual material in the needle may be rinsed directly into TB culture medium or TB transport medium and sent to the microbiology laboratory for culture. If these are not available the needle may be rinsed in sterile saline but the yield will not be as good. If pus is aspirated from the node, 1 - 2 drops *only* should be placed in the transport medium.

Table IV. FNAB procedure

Consent
- As for any procedure written consent is advisable

Anaesthesia
- No local anaesthetic is needed as superficial FNAB is minimally painful when performed correctly. The exceptions would be placing the needle in the SCM muscle, brachial plexus, and inflamed salivary glands
- Light sedation in order to provide amnesia to allay fear may be advisable in children between the ages of approximately 6 m - 6 y if facilities are available for post-procedure observation.
- Topical anaesthetic (Emla cream) combined with oral analgesics such as paracetamol may be effective if sedation is not a safe option
- In older children discussing the procedure and the alternatives and allowing the caregiver to remain with the child during the procedure will usually allow the child to tolerate the procedure very well

Position
- If at all possible all aspirates should be performed with the patient lying down
- For thyroid aspirates the neck should be extended by placing a pillow under the shoulders. This is particularly useful for retrosternal thyroids
- An exception to this rule is patients with small supraclavicular nodes, where it is safer to aspirate patients in the sitting position

Procedure
- Always perform a minimum of 2 needle passes, unless fluid or purulent material is aspirated with the first pass; always use a sterile needle and syringe for each pass
- Each pass yields 2 slides – 1 air dried for DiffQuik staining (cytoplasmic stain) and 1 spray fixed for Papanicolaou staining (nuclear stain), therefore the average aspirate should always yield 4 slides
- Stabilise the node with one hand and introduce the needle, positioning the tip so as to be able to access the entire node; maintain constant 1 - 2 cc suction throughout the aspirate (Fig. 1)
- Aspirate using a cutting motion until material appears in the hub of the NEEDLE; release suction before withdrawing needle
- Place cotton wool on insertion site and ask patient or accompanying person to apply pressure
- Remove needle from syringe, introduce 5 - 10 cc air into syringe, re-attach needle and, holding needle onto syringe, use air to express material in needle onto glass slide, 1 cm from the frosted end; touch needle tip on glass slide during above, in order to prevent loss of material
- Place second slide face down on the first and, maintaining gentle pressure, pull 2 slides apart (Fig. 2)
- Spray-fix bottom slide with fixative from distance of about 30 cm until wet
- Repeat above procedure

TB culture
- Rinse needle and syringe in TB culture/transport medium

Cysts
- Always empty cyst – send ALL fluid to lab
- If mass remains, aspirate residual mass
- If no mass remains, aspirate the bed of the cyst

Bloody aspirates
- If only blood aspirated, remove needle, apply pressure for 1 min, repeat aspirate using new needle and syringe; try smaller needle; if still bloody, try aspirating using no suction at all
- If still bloody, consider vascular lesion, e.g. Kaposi's sarcoma, haemangioma
- Make slides from bloody material aspirated and inform pathologist on request form that on multiple passes of a lymph node only blood was aspirated and clinically this could be Kaposi's sarcoma

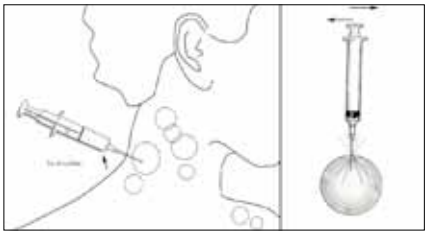

Fig. 1. Insert needle into node and aspirate with minimum of suction in a fan-like fashion, keeping needle in the lesion.

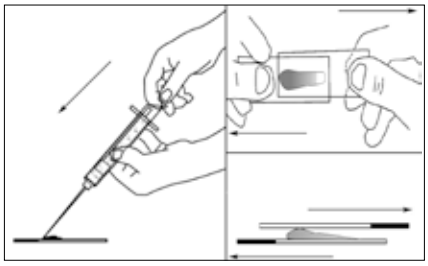

Fig. 2. Express material onto glass slide. Place second slide parallel to first, allow material to spread between slides and pull gently apart, keeping slides together at all times.

Table V. Advantages of FNAB

Patient advantages
- No hospitalisation – outpatient procedure
- No sutures or scars
- Minimal pain (avoid muscle)
- Less morbidity
- Inexpensive
- Quick procedure (average less than 10 min)

Clinician advantages
- Outpatient procedure
- No scarring to interfere with subsequent imaging
- No seeding of tumour to interfere with surgical planes
- Ancillary material available for microbiology etc.
- Rapid results
- Minimal infrastructure required

Healthcare system advantages
- Optimal use of scarce resources and funds
- Triage of patients at primary and regional level
- Improved turnaround time
- Improved patient compliance

This is to prevent a false positive culture or inactivation of the molecular test due to inhibitors in the pus. Aspirated pus may also be placed in a sterile tube and submitted to the microbiology laboratory. Alternatively, if available, the residual material in the same manner may be rinsed in the TB transport medium and sent for the Xpert® MTB/RIF test, which is able to simultaneously detect *Mycobacterium tuberculosis* complex and rifampicin resistance.[11]

Microbiological culture and sensitivity testing (other than mycobacterial)

It is rare that pyogenic abscesses arising in a lymph node require FNAB for diagnosis, as this is usually made on clinical grounds and treated appropriately. However, chronic inflammation due to unusual organisms such as *Actinomycoses* in the head and neck may be referred for FNAB. In these cases after smears are made, material aspirated may be ideally rinsed or expressed in a blood culture bottle containing nutrient medium to keep the organisms viable for cultures and sensitivity testing.

Fungal culture

If a fungal organism is suspected clinically or has been suggested on a previous FNAB, material aspirated should be expressed onto slides in the manner described above and the residue rinsed into sterile saline and submitted as soon as possible to the microbiology laboratory.

In adults, enlarged lymph nodes are more likely to be malignant than in children, although in South Africa TB is still the commonest diagnosis made in persistent adult LAD.

Immunocytochemistry

Immunocytochemistry detects specific antigens on cells and is used to identify or confirm tumours, e.g. carcinoma versus melanoma, to predict therapy, e.g. oestrogen and progesterone receptors in breast carcinoma and to identify specific infectious agents such as CMV. Numerous methods for the preparation of the slides have been proposed in the literature, but the one used in our laboratory is the application of the immunocytochemistry to the de-stained Papanicolaou-stained slides (which are alcohol fixed). The value of this method is that the cells of interest are identified and a preliminary diagnosis is made prior to immunocytochemistry guiding the selection of the antibody panel. This antibody panel will be dependent on the differential diagnosis, and an example of a panel which may be applied to a malignant tumour of unknown lineage and origin is shown in Table VI.

Flow cytometry

The WHO classification of lymphomas which was widely accepted globally in 1999 enabled primary diagnosis of lymphomas on FNAB for the first time. This was because architecture was no longer as important for the diagnosis of lymphomas but on the basis of the morphology of the cells, the clinical presentation of the patient and the specific immunophenotyping and genetic abnormality of the malignant cells.[12] This immunophenotyping can be performed using either immunocytochemistry (labelling of the cells on the slide) or flow cytometry, which is immunophenotyping of the cells in a fluid medium flowing in single file past a laser light source. In flow cytometry the antibodies are attached to a fluorescein label and the fluorescence is picked up by a light detection system, analysed by a computer and displayed graphically as histograms (Fig. 3).This is less objective than immunocytochemistry, which is read manually and is therefore subject to human interpretation. B-cell

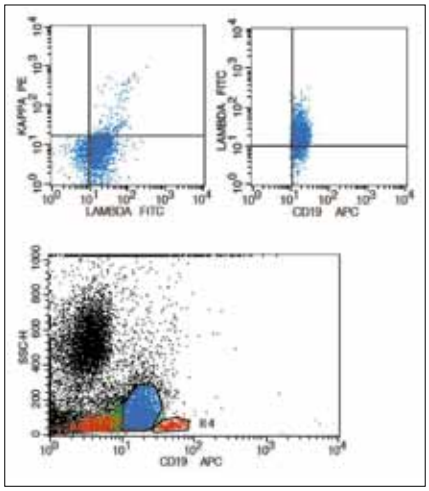

Fig. 3. Flow cytometry of a B cell non-Hodgkin's lymphoma showing CD19 positive staining (B cell marker) and clonality for Lambda light chains.

non-Hodgkin's lymphomas have specific immunophenotypic profiles which in most cases will allow for specific subtyping using flow cytometry if a good aspirate is obtained (Table VII).This requires a tertiary centre where these facilities are available. The advantages are a rapid diagnosis and referral to oncology. In many instances these patients do not require surgery for confirmation of the diagnosis. An exception is follicular lymphoma where grading of the lymphoma has prognostic and therapeutic implications and is controversial on cytology. Most T-cell non-Hodgkin's lymphomas and Hodgkin's lymphoma may be suggested as a diagnosis on FNAB and require excision biopsy for confirmation.

Table VI. Immunocytochemistry profile for tumour in a cervical lymph node

	CK7	CK20	Pan keratin	CD45	S100	TTF1
Melanoma	-	-	-	-	+	-
NHL	-	-	-	+	-	-
Breast carcinoma	+	-	+	-	-	-
Bronchogenic carcinoma	+	-	+	-	-	+
Gastric carcinoma	+	+	+	-	-	-
Renal cell carcinoma	-	-	+	-	-	-
Nasopharyngeal carcinoma	-	-	+	-	+/-	-

No local anaesthetic is needed, as superficial FNAB is minimally painful when performed correctly. The exceptions would be placing the needle in the SCM muscle, brachial plexus, and inflamed salivary glands.

Molecular genetic studies

Both the polymerase chain reaction (PCR) and fluorescence *in situ* hybridisation (FISH) identify tiny regions of DNA of interest to establish clonality for confirmation of malignancy in a lymphoid population, or to look for characteristic translocations, deletions and gene amplifications in the DNA which are specific for certain subtypes of lymphomas. PCR is used more frequently to confirm T-cell clonality, while FISH is applied in sophisticated centres to B-cell lymphomas. It is frequently used to confirm Burkitt NHL lymphoma, which is usually associated with a chromosomal translocation involving the C-myc proto-oncogene on chromosome 8.[13]

Conclusion

FNAB of lymph nodes is a simple and safe first-line diagnostic modality which is ideally suited for use in the public and private sector, in primary health care[14] and in academic centres. It is of particular value in resource-limited countries with the increased burden placed by the dual epidemics of TB and HIV on their healthcare resources. Although FNAB is traditionally performed by specialised medical doctors, it may safely be performed by junior medical staff and well-trained professional nurses under supervision.[15] However, for all aspirates success is determined by the correct technique and therefore prior training is necessary to ensure satisfactory adequacy and consequently diagnostically useful results.

References available at www.cmej.org.za

IN A NUTSHELL

- FNAB is a safe and minimally invasive outpatient procedure, triaging reactive from neoplastic lymph nodes which require further management/referral, and diagnosing infectious lesions that require treatment.
- Indications for FNAB are dependent on the *patient*, the *lymph node* and on the diseases prevalent in the *population.*
- FNAB has an excellent diagnostic yield when performed by trained and experienced aspirators.
- Professional nurses with appropriate training and experience can provide an excellent FNAB service under supervision.
- Good clinical information is essential for accurate assessment of the case by the cytopathologists.
- Adherence to the correct technique is essential. All steps recommended are specifically designed to allow optimal collection and preservation of cellular material in a manner that enables the cytopathologist to make the correct diagnosis.
- FNAB allows easy access to material for ancillary investigations such as TB culture, flow cytometry and immunohistochemistry. These are simple and relatively inexpensive modalities for the diagnosis of infective (e.g. TB) and neoplastic conditions (e.g. NHL and metastatic tumours).