Cytology

DIAGNOSTIC CYTOLOGY

2-Fine-Needle Aspiration Cytology / Biopsy (FNAC/FNAB)

is now a widely accepted diagnostic procedure, which has largely replaced open biopsy.

- This method is applicable to lesions that are easily palpable, for example swellings in Thyroid, Breast, superficial Lymph node etc.
- Imaging techniques, mainly ultra-sonography and computed tomography, offer an opportunity for guided FNAC of deeper structures.

The three pre-requisites for a meaningful diagnosis on FNAC are:

1. Proper technique - procedure, preparation of smears, fixation, staining.

2. Microscopic evaluation of smears.

3. Correlation of morphology with the clinical picture

(history, clinical features, radiological and laboratory findings).

The Technique:

Attention to technique is necessary to optimize the yield of the sample, making its interpretation easier and more reliable. Expertise regarding the technique comes from constant practice and correlation of the smear technique with the results (feedback).

Common Problems with FNA

- ✓Few or no cells obtained
- ✓Some lesions do not exfoliate cells well.
- ✓The needle may miss the site of the lesion
- ✓Timid collection
- ✓Inadequate negative pressure
- ✓Blood contamination
- ✓Using too large needle gauge
- ✓Prolonged aspiration
- ✓Failure to blot if doing imprint

Common Problems with Preparation

- Poorly prepared slides due to thick or high cell numbers
- Allowing material to dry on slide before squash prep or other smear technique.
- If a large amount of material is present, spread between two slides
- May have to do 4-5 slides form the same site in order to get valuable diagnostic sample

Equipment:

The success or failure of the aspiration procedure depends to some extent on the organization of the set up. Some institutions set aside appropriately equipped areas dedicated to the procedure.

Otherwise, the materials can be arranged on movable carts or even in portable containers. Thus FNA can be performed as an outpatient procedure or at the patient's bedside.

Needles: Standard disposable 22-24 gauge 1-1½-inch needles are used for plain FNAC.

Syringes: Standard disposable plastic syringes of 10ml are used. Syringe should be of good quality and should produce good negative pressure. 5cc syringes can be used for vascular organs like thyroid.

Pistol handle:

FAILURE TO OBTAIN A REPRESENTATIVE SAMPLE

- Needle has missed the target tangentially
- Needle in central cystic/necrotic/hemorrhagic area devoid of diagnostic cells.
- Needle in dominant benign mass missing a small adjacent malignant lesions.
- Fibrotic/desmoplastic target tissue giving a scant cell yield.

Equipment

Slides/coverslips: Plain glass slides of good quality are used. Slides should be clean, dry, transparent and grease free.

Fixative: 95% ethyl alcohol is recommended. Fixative is kept ready in Coplin jars.

Other supplies: Test tubes, pencil for marking, alcohol, swabs for skin, watchglass, saline, adhesive dressing, gloves etc. are needed.

All the materials required are assembled in advance before starting the procedure. This is extremely important as delay in fixation can make interpretation of smears difficult.

Aspiration Procedure

Steps to be followed before performing the aspiration

1. Relevant history and clinical details, radiological findings, provisional diagnosis etc. must be entered in the requisition form. Site of FNA must be clearly stated.

2. Lesion to be aspirated is palpated and its suitability for aspiration assessed. The appropriate needle is selected accordingly.

3. The procedure must be clearly explained to the patient and consent and co-operation ensured. Patient may be anxious which needs to be allayed. Ignoring this simple but crucial step can result in failure.

4. Before starting the procedure, ensure that all the required equipment, instruments and supplies are available.

5. All universal precautions should be followed during the procedure.

Steps to be followed in the actual performance of the aspiration:

Positioning the patient: Any comfortable position can be chosen depending on the convenience to palpate the lesion and the comfort of the patient. FNA is usually carried out with the patient lying supine on an examination couch.

Immobilization of the lesion: Skin is cleansed firmly with an alcohol swab (as used for routine injection). Local anesthetic may not be necessary.

The lesion is fixed between the thumb and index finger of the left hand, with the skin stretched. Try to avoid significant muscle mass eg. sternocleidomastoid, while fixing the lesion because it is not only painful, but also muscle tends to plug the needle tip, preventing further material from entering the needle.

Penetrating the lesion: Fixing the lesion with one hand, grasp the syringe with the needle attached (with or without syringe holder) by the dominant hand and introduce through the skin into the lesion, carefully and swiftly. The angle and depth of entry varies with the type of lesion. For small lesions, aspiration of central portion is indicated. For larger lesions that may have necrosis, cystic change or hemorrhage in the center, aspiration may be done from the periphery. If pus or necrotic material alone is aspirated from larger lesions, FNA can be repeated immediately from the periphery. With experience, a change in tissue consistency will be felt as the needle enters the lesion. If the needle goes tangentially missing a small slippery lesion or if penetrates beyond the lesion, representative material will not be obtained.

Note: If the site of FNA is located near the thoracic cage e.g. axillary or supraclavicular swelings, aspiration is better performed in a plane parallel to the thoracic cage to avoid pneumothrax. In thyroid FNA, patient should be instructed not to swallow or talk when the needle is inside the nodule

Creation of a vacuum and obtaining the material: Suction is applied after entering the lesion and while maintaining the suction, needle is moved vigorously back and forth in a sawing or cutting motion, changing the direction a few times, ensuring that the needle is inside the mass throughout; the whole procedure taking only 4-8 seconds.

Do not rotate the needle or pump the plunger in the syringe in and out. Purpose of suction is to pull the tissue against the cutting edge of the needle and to pull the dislodged tissue fragments and cells into the lumen of the needle. Material is procured by cutting motion of the needle and not by suction. This is evident in the non-aspiration technique in which the needle alone is moved back and forth in the lesion and withdrawn. Admixture with blood is less with this technique and is useful in thyroid aspiration.

When the needle is moved in different directions, it samples a much wider area than a core biopsy (FNA is thus more representative than a core biopsy). The to and fro movements and changing the direction of the needle, while it is still inside the lesion are the two crucial steps in procuring an adequate representative sample.

Movement of the needle is adjusted according to the type of lesion. A sclerotic lesion will require more force than a soft tumor. A cyst will almost aspirate by itself. When fluid is aspirated, its color, consistency and amount should be recorded in the requisition form, which allows the lesion to be recognized as cystic. Fluid can be sent in a bottle for centrifugation and preparation of smear. In cystic lesions, especially of breast and salivary gland, a large cyst may obscure a small malignant tumor. Hence cysts should be completely aspirated (fluid is sent for centrifugation) and residual lump if any, should be re-aspirated and labeled separately.

Release of vacuum and withdrawal of the needle: When material is seen in the hub of the needle, procedure is discontinued. Before withdrawing the needle, suction is released and needle pulled straight out. The piston is just allowed to slowly fall back by itself (never push). Failure to release negative pressure within the lesion will cause the aspirated material to enter the syringe, which is difficult to recover. In desperate situations, syringe and the needle can be rinsed with saline or fixative and then centrifuged to prepare a smear. Immediately after withdrawing the needle, firm local pressure is applied at the site for sometime, preferably by an assistant. This is to prevent bruising or haematoma formation especially in thyroid, breast etc.

Note: If a cork of tissue is obtained during FNA or if the sample clots quickly, entrapping the cells, the clot or tissue can be fixed in formalin and processed as for histology.