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Fine Needle Aspiration Cytology [FNAC] – Review Article

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ABSTRACT

FNAC (Fine Needle Aspiration Cytology) as we know it today dates back to around 1950. FNAC being easy, safe, cost effective should be preferred as first line diagnostic method by all clinicians. Before any surgical intervention, FNAC reports direct surgeon about what treatment modality to be used. Surgical pathology has its own confirmatory role post- operatively but importance of FNAC is also well known by all clinicians. Therefore, there has to be a setting of dedicated FNAC clinic in the department of pathology. With the help of imaging modalities, FNAC has evolved as more accurate and specific method, while the use of ancillary techniques makes even this easy procedure as highly useful for diagnosis and prognosis of various lesions.

Key Words: FNAC, Preoperative, Diagnostic test

INTRODUCTION

The Origins of FNAC /Historical Aspects

In mid –nineteenth century, Kun [1] (1847) & Lebert[2] (1851) & Menetrier[3] (1886) used needles to obtain cells & tissue fragments to diagnose malignant tumours. Kun described this technique as "new instrument for diagnosis of tumours".

Leyden [4] (1883) employed the same method to isolate pneumonic microorganisms. Grieg and Gray who in 1904 diagnosed trypanosomiasis in cervical lymph node aspirates from patients with sleeping sickness in Uganda. Their findings were reported by a captain Bruce in a British Medical Journal memorandum in 1904.

In the mid -1920s there were attempts in New York and Chicago to employ large needle aspiration for a variety of sites ranging through lymph nodes, prostate and breast.

In the UK in 1927, Dudgeon & Patrick [5] suggested the needling of tumours as a means of rapid microscopic diagnosis.

Interest in the procedure was resurrected by Europeans in mid 1950s. It was in Europe that "FNAC" as the technique was usually called began to flourish in 1950s &1960s.

Soderstrom [6] &Franzen [7] in Sweden, Lopes Cardazo [8, 9] in Holland , Zajdela [8] in France & others became major proponents , studying thousands of cases each year.

FNAC soon established its place as a diagnostic routine to be used by team of pathologists & clinicians.

History of FNAC has been very well documented by Grunze and Spriggs,[10] and Naylor[11].

FNAC as an important clinical TOOL

FNAC is a simple, inexpensive, easily performed outpatient procedure which can provide a rapid diagnosis. It has been widely used in Europe for decades, mainly in Scandinavian countries [12-15] .

A technique which is safe , rapid , relatively pain free, cost effective & accurate is always a clinician's first choice and this is what FNAC is about .

It is eminently suitable as first line investigation for almost all superficial palpable swellings as well as many deep seated lesions. FNAC was initially conceived as a means to confirm a clinical suspicion of local recurrence or metastasis of known cancer without subjecting the patient to further surgical intervention.

FNAC is a time tested simple office procedure having a high degree of diagnostic accuracy & precision. The specificity & sensitivity of diagnostic precision lie in range of 60% & 80% respectively.

The acceptance both by surgeons & pathologists itself speaks

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of the tale of comfort which it allows.

The art of medicine is practiced within a community of caregivers who are perched on innumerable speciality branches and these braches intersect each other at various times.

Clinical consultations help to acquaint cytopathologist about probable diagnoses possible for any lesion. Often a major surgical biopsy can be avoided by performing a needle aspiration instead.

FNAC SURPRISES THE SURGEON MANY TIMES

Surgeons are always impressed by the help of FNAC to make diagnoses which affect treatment of patient in a wide manner. Many tumours being diagnosed high grade on FNAC make the surgeon to go for chemotherapy before surgical intervention.

Such a simple technique and so many wonders.

Benefits of FNAC are innumerable.

Cost effective

1. It has lower risk than surgical biopsy.
2. It is readily repeatable and useful for multifocal lesions.
3. Minimal physical and psychological discomfort for the patient.
4. Rapid reporting and bedside diagnosis of neoplastic, hyperplastic and inflammatory masses.
5. Active participation of patient in treatment planning and provides opportunity for fuller preoperative counselling.
6. Elimination of a two stage procedure
7. Therapeutic procedure for evacuation of cystic lesions.
8. Allows cases to be prioritized when there is a waiting time for surgery
9. Permits the diagnosis of some benign conditions for which there is no need for surgery
10. It is a rapid means of confirmation and recurrence of previously treated malignancy without surgery.

Technique of FNAC (Fig. 1)

Success of FNAC depends to a high degree on perfecting the technique of sampling & and preparation of samples .palpation skills learnt through practice & experience, judiciously complemented by radiological image guidance when appropriate are essential to obtain **representative samples.**

Choice of needles, the use or not of aspiration & the manipulation of needle within the target relative to type of tissue decide the **adequacy of sample.**

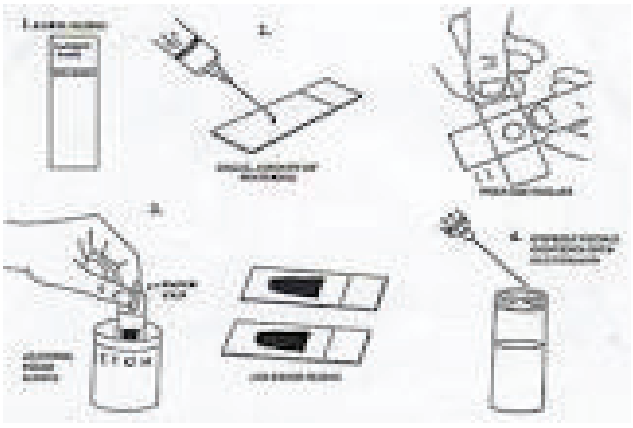

Figure 1: Schematic diagram showing steps of FNAC.

Finally, correct smearing, fixation and staining of samples is critical to assure optimal **preservation and presentation** of cells.

Specific staining defines and highlights specific features of aspirate smears .Comparisons between two commonly used methods are

1. Air drying followed by a Romanowsky type stain such as MGG.
2. Alcohol fixation followed by H&E staining.

Table 1

	Air dried MGG	Wet fixed Pap
Dependence on smearing technique	Strong	Moderate
Tissue fragments	Cells poorly seen due to heavily stained ground substance	Individual cells usually clearly seen
Cytoplasmic details	Well demonstrated	Poorly demonstrated
Nuclear details	Pattern different from familiar Pap stain	Excellently demonstrated
Nucleoli	Not always discernible	Well demonstrated

Usually, pathologists trained in gynaecological cytology prefer alcohol fixed –Pap stained smears while those trained in haematology choose air dried MGG stained smears.

Utility of special stains in diagnosing lesions

1. PAS /diastase or alcian blue for **mucins** - mucins can help in diagnosing mucin producing tumours of many anatomic sites (breast, gastrointestinal site, pancreas, ovary) and sometimes can be completely devoid of cells (e.g. pseudomyxomaperitonei or pure mucinous carcinoma), yielding a false negative diagnosis.
2. Prussian blue for **Iron** in hemosiderin containing lesions.
3. Masson Fontana for **melanin** in melanoma
4. Grimelius for **argyrophilic granules**

5. Congo red for **amyloid**
6. PAS for **glycogen** –extracellular glycogen production is appreciated in tumours like Ewing's sarcoma, rhabdomyosarcoma , and glycogen rich clear cell tumours).
7. Oil red O for **fat**
8. Fouchet's reagent counterstained with Sirius red for **bile pigments**
9. **Microorganisms** identified by Gram, PAS, ZN or Gomori silver stain.

Pitfalls or drawbacks of morphological diagnosis

As every other technique in this world, FNAC also has some complications and limitations. These have been enlisted below:

1. Instances of serious complications have been reported in relation to different sites & organs, such as major haemorrhage, septicaemia, vasovagal reaction, seeding of tumour, bile peritonitis, acute pancreatitis, pneumothorax etc[16].
2. Pre-operative FNAC may cause local tissue changes which could render subsequent histological diagnosis difficult .Such changes include hematoma, infarction, capsular pseudoinvasion, reparative reactions have been reported[17]
3. Results and accuracy are highly dependent on quality of samples and smears.
4. Many pathological processes are heterogeneous and tiny sample obtained from FNAC may not be representative.
5. Some lesions are recognised on basis of specific micro architectural pattern, which may not be represented in cytological preparations
6. Small FNA sample may not allow full armamentarium of ancillary techniques to be drawn upon.

Aspiration cytology requires highly skilled and trained personnel in both aspiration and assessment .Stewart commented in 1933 that "until the pathologist has familiarized himself with the various pitfalls, errors are certain to occur" and "it must not be inferred that the diagnosis is always a simple and that no errors have been made".

Extrinsic factors which may lead to diagnostic pitfalls are lack of or misleading clinical information, non-representative samples, contamination of samples by tissue adjacent to target lesion, artefacts due to poor processing of samples and too much reliance on and technical failure of ancillary tests.

Intrinsic factors which may lead to diagnostic pitfalls arise mainly due to deviations from general cytodiagnostic criteria which can occur in various benign and malignant tumours.

Pitfalls are an inseparable part of the practice of FNAC, but they can be minimised, if requisite diagnostic rigours are applied and care taken to correlate cytology with clinical an radiological findings. Judicious use of ancillary techniques also helps in reducing incidence of pitfalls.

Ancillary techniques

No wonder that technology is the fastest spreading tumour in pathology, but this tumour is having all gains.

Various new techniques have revolutionised FNAC since its history. The pathologist must always keep in mind to apply any of these appropriate ancillary diagnostic techniques to cytological preparations.

1. **Electron microscopy** – It is particularly useful in unusual lung or mediastinal lesions. Valuable information is obtained in recognizing neuroendocrine tumours, in specific diagnosis of melanoma, mesothelioma, and some carcinomas where immunocytochemistry often cannot provide such positive diagnostic features[18.19] .
2. **Immunocytochemistry** – It is the most important recent development in diagnostic cytology. Monoclonal antisera to various proteins and cell products are nowadays commercially available.
   Alcohol fixed smears are usually preferred over air dried smears. The avidin –biotin complex method is the most commonly used with both monoclonal and polyclonal primary antibodies. Diaminobenzidine is used as marker dye. Appropriate controls are crucial to achieve diagnostic accuracy[20].The results of immunocytochemistry should be interpreted with caution in relation of conventional cytomorphology and clinical data [21].

Role of immunocytochemistry (Table 2)

I.	Helps in objectively recognising the line of differentiation shown by cells
II.	Allows confident specific diagnosis even on relatively scanty material (eg medullary carcinoma thyroid)
III.	Immune markers are extremely useful in differentiation between anaplastic carcinoma, neuroendocrine tumours, malignant lymphoma , amelanotic melanoma, in search for a primary in metastatic malignancy & in histogenic typing of mesenchymal tumours.
IV.	Markers for B & T cells, immunoglobulins & light chains are very useful in typing of lymphoma.

3. **Image analysis –** there are 3 ways of image analysis

A. Morphometry - quantitative analysis of geometric features of structures such as tissues, cells, nuclei or nucleoli [22,23].

B. Object counting – quantitation of mitosis or measurement of proliferation fraction of a cell population using antibodies. It also makes it possible to quantitate apoptotic figure by TUNEL assay [24].

C. Cytometry – based on ability to detect a particular substance of interest by a specific dye & to measure the concentration of dye by assessing optical density [25].
Fluorochromes can also be used such as propidium iodide dyes [26-28].
Powerful computers also have automatic cell classification based on pattern recognition for diagnostic [29,30], prognostic [31,32]& predictive purpose [33].

Quantitation of nuclear immunostain of Estrogen and progesterone receptors [34], proliferation markers [35-37] can be done.

4. **Flow cytometry**
   Based on fundamental work showing that DNA content , measured by UV visible light in unstained cells , double during cell cycle [38], followed by improved detection of antigens using fluorescence methods [39] & development of apparatus capable of counting [40] & sizing blood cells [41].
5. **Molecular cytopathology**
   Application of molecular probes to cytologic samples of human malignancies has refined the diagnostic & prognostic armamentarium [42-45].

In situ hybridization – It is a newly developed and global approach to detect genetic changes in tumors.

For localization of specific nucleic acid within individual cells based on complementary binding of a nucleotide probe, labelled with non-isotopic reporter molecule, to a special target sequence of DNA or RNA [46].

Using probes to chromosome specific sequence, it is possible to detect aneuploidy in interphase nuclei [47-48] &losses, gains or amplification of chromosome regions with known prognostic value [49-50].

In situ amplification – based on PCR this allows recovery of large amount of DNA from minute quantities of starting material [51].

Various adaptations of PCR have been developed for cytological preparations [52] such as PCR in situ hybridisation ,in situ PCR, reverse transcriptase in situ PCR[53], methylation specific PCR [54],and primed in situ synthesis [55] . The most crucial steps in optimising in situ amplifications are fixation and preparation of cells.

IMAGING METHODS FOR GUIDANCE OF ASPIRATION CYTOLOGY

Nowadays, to make FNAC more accurate and precise, imaging modalities have been used for guiding the tract of needle. Various imaging modalities used are

1. **Fluoroscopy –**

Uses-

1. Quick alternative for radiologist not experienced in USG guidance.
2. Is most useful in guidance for small, very mobile lesions.
3. Efficient sampling options for cortical bony lesions.

2. **Ultrasound –**
   Only real time guidance which allows imaging in any plane & is only suitable guidance for biopsy of foetal tissues.
   Some parts of body [56] such as chest wall, musculoskeletal system, through neglected in past, have undergone an increase in interest for both diagnosis and interventional studies.
3. **CT scanning –** localization of needle tip with in a lesion is very accurate with CT. There are very few areas of body which cannot be biopsied under CT control & extremely small lesions can be sampled.
4. **MRI –** its sensitivity is generally greater than that of other imaging methods, particularly useful in brain, liver& breast [57].

CONCLUSION

Fine needle aspiration cytology has an utmost importance in the current era of surgical practice in the preoperative stage as it guides the clinician a lot in the treatment plan and mostly clear the pathological aspects of the disease avoid untoward complications related to disease and treatment for the sake of pathological diagnosis. Many times it avoids unnecessary surgical intervention

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