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Review Article

Fine Needle Aspirate; a Vital Technique in the Characterization of Masses and Lesions

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Abstract

Fine needle aspiration is a cost effective, non-invasive, less painful, rapid reporting bedside diagnostic procedure for obtaining small amount of sample from palpable and non-palpable masses or lesions in the body using a needle. The objective of FNA is to provide information on the nature of the sampled tissue in order to focus appropriate diagnostic and therapeutic decisions, all at minimal risk to the patient. Its sensitivity in distinguishing benign from malignant tumours varies from 58% to 96% with specificity usually between 90 to 100%. In the characterization of lesions and masses using FNA, the concept of "triple test", that is the combination of physical examination, imaging findings and cytologic examination, is recommended. The triple test is positive if any of the three components is positive, and negative if all the components are negative. The triple test has sensitivity (true positive rate) of 99.6%, and a specificity of 93%. More so, a combination of FNA and Cell-block technique revealed sensitivity of 94% and specificity of 98%. However, a minimum number of epithelial cells (5–10 cells/group) for FNA made diagnosis have been advocated, and samples containing fewer than the specified minimum be considered non-diagnostic. Despite the reported specificity and sensitivity of FNA, proper training of personnel and adherence to recommendations are quite important in producing reliable result.

ABBREVIATIONS

FNAC: Fine Needle Aspirate Cytology; CT: Computed Tomography; EUS-FNA: Endoscopic Ultrasound Guided Fine Needle Aspiration; DCIS: Ductal Carcinoma *in Situ*; MRI: Magnetic Resonance Image; IHC: Immuno Histochemistry; IPMN: Intraductal Papillary Mucinous Neoplasm

INTRODUCTION

FNA can be viewed as a coordinated sequence of diagnostic events: a) collection of pertinent clinical data, b) needle sampling of the abnormality, c) specimen preparation and staining, d) interpretation, and e) communication and reporting. The objective of FNA is to provide the referring physician information on the nature of the sampled tissue in order to focus appropriate diagnostic and therapeutic decisions, all at minimal risk to the patient [1]. FNAC are employable as initial diagnostic procedure for palpable breast lesions for the following reasons: cost effectiveness, it has lower risk than surgical biopsy, it is readily repeatable and useful for multifocal lesions, minimal physical and psychological discomfort for the patient, rapid reporting and bedside diagnosis of neoplastic, hyperplastic, and inflammatory masses, active participation of the patient in treatment planning and provides opportunity for fuller preoperative counseling, elimination of a two stage procedure, therapeutic procedure for the evacuation of cystic lesions, allows cases to be prioritized when there is a waiting time for surgery, permits the diagnosis of some benign conditions for which there is no need for surgery, renders unnecessary the need for excision biopsy in advanced disease, elderly patients, or in cases where the treatment is non-surgical (e.g. in neoadjuvant chemotherapy), it is a rapid means of confirmation of recurrence of previously treated malignancy without surgery [2-5]. Fine-needle aspiration cytology (FNAC) is recommended as the initial diagnostic test for such patients, because of its simplicity and reliability [6].

FNAC is both diagnostic and therapeutic in a cystic swelling [7]. Fine needle aspiration cytology is helpful for the diagnosis of salivary gland tumours where it can differentiate between a malignant and a benign tumour with over 90% accuracy [8]. FNAC is particularly helpful in the work-up of cervical masses and nodules because biopsy of cervical adenopathy should be

avoided unless all other diagnostic modalities have failed to establish a diagnosis [9]. Fine needle aspiration cytology does not give the same architectural detail as histology but it can provide cells from the entire lesion as many passes through the lesion can be made while aspirating [10]. FNA cytology results should always be interpreted in the context of the triple test [11,12]. Triple assessment, consisting of clinical evaluation, mammography or ultrasound and fine-needle aspiration cytology (FNAC), allows a precise initial diagnosis and reduces the risk of such misdiagnosis [13]. The aims of the triple test are to [14]: maximise the diagnostic accuracy in breast disease, maximise the preoperative diagnosis of cancer, minimise the proportion of excision biopsies for diagnostic purposes, minimise the proportion of benign excision biopsies for diagnostic purposes. It comprises the following components: clinical breast examination and medical history, imaging – mammography and/or ultrasound, non-excision biopsy – FNA cytology and/or core biopsy. The triple test is positive if any of the three components is positive, and negative if all the components are negative. The triple test has sensitivity (true positive rate) of 99.6%, and a specificity of 93% [15]. This review paper aims to give an overview of FNA importance and better approaches towards achieving accurate FNA in diagnostic Cytopathology.

Sensitivity and Specificity of FNA

FNAC provides predictive diagnosis of benign or malignant neoplasm, or even in some case specific tumour type [16]. Also, if the lesion is benign and the patient is elderly, risk of surgery can be avoided. In case of recurrences of malignancy a cytological diagnosis can help in the administration of palliative treatment [17]. Studies have shows that the sensitivity of FNAC in distinguishing benign from malignant salivary tumours varies from 58% to 98%, with specificity usually above 90% [18-21]. The sensitivity (84%) and specificity (93%) of repeat FNAC in distinguishing benign from malignant tumours was higher to initial FNAC (70% and 95%, respectively) reported by Brennan et al. [22]. Repeat FNAC may provide a cytological diagnosis in cases where the initial diagnosis is not clear; although cytology should be used in conjunction with other investigations of salivary tumours, including image-guided biopsy examination where appropriate [22]. According to the reports by Seningen et al. [23], the specificity of FNA cytology (percentage of cases correctly identified as negative by FNA [true negatives] among all cases identified as negative by excisional biopsy in the study) ranged from 81.2% to 100% [23]. The latter report is supported by Cheung et al. [24] who reported a specificity and positive predictive value of 100% for FNA with relatively low sensitivity in a diagnosis of thyroid carcinoma.

A study conducted by Zubaida et al. [16] on 100 cases of Soft tissue lesions by Fine Needle Cytology (FNAC) and subsequent correlation by Histopathological examination revealed that the accuracy for benign soft tissue masses was 94.38% and in 100% malignant soft tissue lesions. The discordance of 5.62% in the benign soft tissue masses was due to aspiration of inadequate material and loss of architectural pattern. A smear may be inadequate or unsatisfactory for a variety of reasons, including 1) acellularity/hypocellularity, 2) poor fixation, 3) poor preparation (crush artifact), 4) poor staining, 5) excessive blood obscuring cellular details, or 6) excessive necrosis or debris. Other factors that may adversely affect specimen adequacy include irreparably broken slides, inadequate patient identification, inadequate clinical data, and lack of identification of the type and source of specimen [1]. The different accuracy, specificity and sensitivity reported in different shows that individual factor (skill and knowledge of clinician or pathologist) and method of diagnosis are major key players in FNA made diagnosis.

According to the reports of Teague et al. [25], an image analysis system (Xcyt) was used to categorize 56 breast FNAs diagnosed as ''indeterminate'' and the computer diagnosis compared with the surgical biopsy. Based on the analysis of three nuclear features of (area, texture, and smoothness), the Xcyt system computed a benign or malignant diagnosis and a corresponding probability of malignancy for each case. Probabilities of malignancy for the respective cases ranged from 0.0–1.0. Benign cases were defined as those having probabilities of malignancy <0.3; those with probabilities above this limit were considered malignant. Using these criteria, the computer identified 33 cases as benign and 23 cases as malignant. When compared with the surgical biopsy, 42 of the cases (75%) were correctly classified with a sensitivity and specificity of 73.7% and 75.7%, respectively. There were only 5 false-negative cases with a false-negative rate of 13.5% and a predictive value of a negative test of 84.8% [25]. Hence, it is opined that the use of image analysis in inconclusive diagnoses on FNAs of breast masses, is a valuable tool in the further classification of such lesions, thereby providing a more appropriate triage for surgical biopsy. The reliability of FNA in separating benign from malignant breast lesions has been established. However, the ability to distinguish proliferative lesions with and without atypia and Ductile Carcinoma in situ (DCIS) by FNA is more limited [26-28]. FNAs have low cellularity [29-31]. Those laboratories requiring a minimum number of cells, the quantitative requirements closely followed the published recommendation of at least six clusters of cells with a minimum of 5–10 cells/group [30].

The past decade has seen a decline in breast FNAC in favor of more aggressive core biopsy techniques. Core biopsy is a technique in which a probe is inserted into the breast to take a small sample of breast tissue from an area of concern. Some pathologists prefer the Histologic evaluation of core biopsies because they can be analyzed relatively quickly and easily, and they allow immunohistochemistry (IHC) to be applied. Combining FNAC with core biopsies has been shown to increase diagnostic accuracy [32]. Cell blocks are prepared from residual material obtained from FNA after smears are prepared and are useful adjuncts for establishing a more definitive cytopathological diagnosis. Additional studies (like immunocytochemistry-ICC) can be performed easily on cell block. A combination of FNA and Cell-block technique revealed sensitivity of 94%, specificity of 98%, positive predictive value of 94%, negative predictive value of 98%, false positive rate of 1.15%, false negative rate of 6% and total accuracy of 98% [33]. Combined use of FNAC smear and cell-block can be useful for establishing a more definitive cytopathologic diagnosis. It is suggested to perform cell-block for each case of breast FNAC, to decrease the pitfalls and to improve the diagnosis and management of breast lumps [33].

Equipment/apparatus used during FNA

Needles: Needle gauge is based on external diameter. Fine needles should be 23 gauge (external diameter 0.6 mm) or less (external diameter 0.7 mm). It is important to use smaller needles for the following reasons: i) it is less painful ii) it causes less bleeding and iii) the risk, albeit rare, of tumour seeding is considerably reduced [34]. Thicker needles (G18, external diameter 1.2 mm, or wider) carry an ever-increasing risk of complications including significant haemorrhage [34,35].

Syringes and syringe holders

The Swedish-designed syringe holder (Cameco AB, Taby, Sweden) is suitable, although alternatives are now available. Either a 10 ml or a 20 ml sterile disposable plastic syringe can be used, depending on personal preference [36].

Slides, fixative and collection fluid

Clean slides with frosted ends are required if direct smears are to be prepared at the time an aspirate is taken. Direct smears can be either wet-fixed by alcohol spray or, preferably, by immersion in 95% alcohol, or rapidly air-dried. There are two main types of transport media: fixation fluid that kills organisms and cells, and non-fixative / culture fluid that keeps the material viable until it can be processed. If fixed cell preparations are required then an alcohol-based fixative is satisfactory. If only cell blocks are to be prepared, then 10% buffered formalin is satisfactory. Hank's physiological saline and sterile normal saline, which is universally available in the hospital environment, can also be used for transporting samples, providing processing is not unduly delayed [36].

Local anaesthetic

A small volume of 2% lignocaine is generally sufficient for local anaesthetic. Application of anaesthetic cream such as Emla cream (AstraZeneca, London, UK) at the proposed puncture site is helpful in children and needle-phobic patients. Ethylene spray for skin anaesthesia and needle-free commercial kits for application of local anaesthetic can also be used [36].

Methods used in Fine Needle Aspiration Cytology

There are two methods used for fine needle aspiration cytology include:

i) Suction fine needle aspiration cytology techniques: In this method, the needle is passed into the lesion and negative pressure is applied, usually by virture of a syringe attached to the needle, and often with the help of a syringe holder. This method is particular useful when draining a liquid from the lesion (eg cyst fluid, ascites or pleural fluid)

ii)The capillary method: In this method the FNAC is performed without the aid of suction, with a needle alone, the needle is passed into the lesion and multiple fast jabbing movements in and out of the lesion as well as in different directions are performed, once the material is seen in the hub of the needle, there is usually sufficient materials for collection.

PROCEDURE

Procedures for fine needle aspiration can be grouped into:

a) Technique for Superficial fine needle aspiration Under Direct Visualization: Fine needle aspiration biopsy is a safe and efficient method of obtaining cells for diagnostic cytologic evaluation of palpable superficial masses from breast, thyroid, salivary glands, lymph nodes, cysts and metastatic tumors, utilizing a 20 cc syringe, 22 gauge needle and optional syringe holder.

b) Technique for Image Guided fine needle aspiration of Deep Lesions: Non-palpable, deep lesions may be accessed by guiding the 22 gauge needle through a trajectory to its target under the guidance of ultrasound, fluoroscopy or computed tomography. The radiologist uses scans such as CT (computed tomography) and/or ultrasound) to locate the sampling area [37].

Ultrasound-guided FNA

Endoscopic ultrasound guided fine needle aspiration (EUS-FNA) is a valuable and safe tool to obtain cellular material for cytological examination [38]. In experienced hands, EUS-FNA or core biopsy of lesions and lymph nodes above and below the diaphragm has been demonstrated to be extremely safe when compared with other tissue sampling modalities, with a risk profile similar to that of conventional endoscopy [39]. The ultrasound transducer on the distal tip of the echo endoscope permits needle advancement into the lesion under real-time guidance. EUS-FNA and core biopsies are performed after Doppler assessment to avoid puncturing intervening blood vessels [40].

EUS-FNA cytology is an excellent method for procurement of diagnostic samples from the pancreas, with a diagnostic accuracy of more than 90% for pancreatic adenocarcinoma [41]. It is currently used for the preoperative diagnosis of pancreatic cysts and for small neoplasms, but the introduction of gastric or duodenal epithelium and mucin into the specimen during the procedure (e.g. gastrointestinal contamination) has created diagnostic challenges, particularly in the area of mucinous cysts of the pancreas [42]. EUS-FNA allows placement of the needle inside the dilated ductal system, at several levels if necessary in order to distinguish IPMNs from other causes of duct dilation such as obstruction or chronic pancreatitis. There are several reports in literature on EUS diagnosis of Intraductal papillary mucinous neoplasm (IPMN) [43-46]. It is believe that close cooperation between an experienced endoscopist and cytopathologist may result in an accurate diagnosis of IPMN, based on EUS-guided FNA cytology. The study carried out by Salla et al. [47] showed that EUS-guided FNA cytology emerges as a valuable and accurate method in the pre-operative diagnosis of IPMNs. They also stated that EUS-FNA coupled with immunocytochemistry plays a vital role in determining the biological behavior of these tumors. US is readily available and provides a rapid, safe and inexpensive means of guiding FNAs and is increasingly used for breast, thyroid and head & neck aspirates [48-53].

Computed tomography (CT) guided FNA

Lesions less than 1 cm in diameter located deeply within the body can be reached with precision using this technique. Magnetic resonance image (MRI) guided FNA MRI is rarely used to guide sampling because patient access is limited by the scanner; special coils giving reasonable operator access, may be used to guide breast aspirates, if the abnormality is only visible using this modality, using non-magnetic needles [36].

Post-Operative Care and Complications

As with any surgical procedure, complications are possible, but major complications due to thin needle aspiration biopsies are fairly uncommon, and when complications do occur, they are generally mild. The kind and severity of complications depend on the organs from which a biopsy is taken or the organs gone through to obtain cells. After the procedure, mild analgesics are used to control post-operative pain. Since sterility is maintained throughout the procedure, infection is rare. But should an infection occur, it will be treated with antibiotics. Bleeding is the most common complication of this procedure. A slight bruise may also appear. If a lung or kidney biopsy has been performed, it is very common to see a small amount of blood in sputum or urine after the procedure. Only a small amount of bleeding should occur. During the observation period after the procedure, bleeding should decrease over time. If more bleeding occurs, this will be monitored until it subsides. Rarely, major surgery will be necessary to stop the bleeding [54]. Other complications depend upon the body part on which the biopsy takes place: i. Lung biopsies are frequently complicated by pneumothorax (collapsed lung). A small percentage of patients will develop a pneumothorax serious enough to require hospitalization and placement of a chest tube for treatment. ii. For biopsies of the liver, bile leakages may occur, but these are quite rare. iii. Pancreatitis (inflammation of the pancreas) may occur after biopsies in the area around the pancreas. iv. In biopsies in the area of the breast, bleeding and bruising may occur, less frequently also infection (rarely) or (very rarely, and only if performed near the chest wall) pneumothorax. v. Deaths have been reported from needle aspiration biopsies, but such outcomes are extremely rare.

vi. Haemorrhage: serious haemorrhages have only been reported after FNA of deep structures such as the lung, liver, and kidney. Bleeding from CT scan-guided FNA of retroperitoneal adenopathy is reported at less than 1% despite the proximity to great vessels [54].

Aspirations of ovarian malignancies are not recommended, unless the poor condition of patients precludes surgery or the lesion is a recurrence or metastasis of a previously diagnosed and treated cancer [55,56]. Aspiration of a clinically and radiologically benign ovarian cyst by an experienced clinician is considered reasonable, although this practice is not universally accepted because of the fear of rupturing a malignant cyst [57].

FNA limitations

The limitations of FNA can either be technical, related to the nature of the lesion itself or intrinsic (theses are limitations that are specific to FNA regardless of technique or lesion type). These pitfalls are discussed below.

Technical limitations: sometimes, poor technique can mislead the unwary pathologist into making a false-positive diagnosis. Excessive application of force while spreading the smear can lead to crushing and nuclear distortion and dissociation (i.e. crushing artefacts), which can result in the false impression of hyperchromasia. Also, delay in fixation of the smear for Papanicolaou staining can result in cellular enlargement; comparison with air-dried Giemsa stained smears can be helpful in avoiding such false-positive diagnoses. Finally, poor quality staining can cause artefactual changes in the nature of the chromatin pattern.

Limitations related to the lesion itself: some lesions share similar features on FNA and are difficult to differentiate from each other. Certain types of lesions can lead to false-negative diagnoses. For example, it is difficult to fix the small mobile lesion by hand, and thus it may be missed. Also, it is difficult to aspirate fibrous lesions, and samples are often hypocellular and haemorrhagic. The smears may show only stromal fragments. In a proportion of cases, further investigation with imaging modalities and core biopsies may be necessary [58]. The case of necrotic and vascular lesions, the smears may not contain any viable cells or may be haemorrhagic. Finally, smears from lobular carcinoma can be hypocellular and cells may not show significant pleomorphism. Their resemblance to lymphocytes may result in false-negative diagnosis. Cytology of tubular carcinoma can resemble many benign conditions, including adenoma, microglandularadenosis and fibroadenoma [59].

Intrinsic limitations: There are a number of limitations that are intrinsic to FNA cytology. First, identification of benign fibroadenoma or frankly malignant phyllodestumour may not be difficult, but distinguishing between cellular fibroadenoma and a phyllodestumour can cause problems. However, stromal cellularity and the presence of a number of long spindle cells may be helpful in some cases [60], secondly the cytological appearances of papillary lesions, which range from benign papilloma to invasive papillary carcinoma, can be similar. In addition, benign papillomas can harbour areas of ductal carcinoma *in situ*. Third, it can sometimes be difficult to distinguish between a mucocele-like lesion and mucinous carcinoma on cytology. The presence of high cellularity, single or small three-dimensional groups of tumour cells, and cytological atypia should raise suspicion of carcinoma [61]. In the absence of architectural information, the distinction between ductal carcinoma *in situ* (DCIS) and invasive carcinoma may be difficult cytologically [62]. Others include: Sampling is scanty and histological architecture is lost thereby rendering impossible diagnosis based on histology, Inflammatory, metaplastic or degenerative lesions may mimic malignancy, Diagnosis is indefinite in some conditions such as follicular adenoma vs. carcinoma of the thyroid, Samples taken may not be representative of the lesion, Difficulty of cytological diagnosis in some conditions e.g. lymphomas [62].

FNA contrasted with Core Biopsy

FNA cytology and core biopsy were originally used to diagnose palpable breast lesions. Both methods have a high degree of sensitivity and specificity. The use of core biopsy has increased, especially in the evaluation of lesions that are associated with high inadequacy rates with FNA cytology – such as mammographically detected lesions that are very small, suspected radial scars or micro calcifications [63]. Both the sensitivity and specificity of core biopsy for the diagnosis of impalpable lesions are usually reported to be at least 90% [64]. In a multidisciplinary breast setting it has been shown that ultrasound-guided core biopsy has a sensitivity of 82% and specificity and positive predictive value (PPV) for malignancy of 100% [65]. In general, core biopsy has been shown to be superior for the confirmation of benign lesions, as the rate of samples reported as unsatisfactory is less than for FNA cytology (12.5% versus 34.2%) [11].

Rosen [66] reports that core biopsy is accurate for the diagnosis of most breast lesions, but fails to identify 6–12% of mammographically detected micro calcifications and under-diagnoses ductal carcinoma in situ (DCIS). However, in recent years there has been an increase in the use of core biopsies to facilitate a preoperative diagnosis [67]. There are two principal explanations for this trend. One is the increased rate of inadequate specimens in impalpable lesions, sampled by FNA cytology. The other is the lack of expertise among pathologists in the interpretation of fine needle aspirates. The first explanation may be due to lack of technical skill or the nature of the lesion. The experience and skill of the operators and pathologists and the nature of the lesion will affect the choice of biopsy technique. FNA cytology and core biopsy are complementary procedures [63,68]. Pinder and associates [68] and Masood [63] have stated there is insufficient evidence to decide if one method is better than another. These authors recommend the use of the appropriate combination of FNA cytology and/or core biopsy as the best approach for the diagnosis of breast lesions at different settings [63,68].

Current National Accreditation Standards for Breast Screen Services [69] specify that: a) at least 75% of cancers are diagnosed without the need for diagnostic excisional biopsy; b) the rate of FNA cytology specimens reported as inadequate/insufficient is less than 25%; c) the false negative rate for FNA cytology procedures is less than 6%; d) the rate of core biopsy specimens reported as false negative or inadequate is less than 15%; e) the false positive rate for FNA cytology procedures and core biopsy is less than 1%; f) the false positive rate for core biopsy is less than 0.5% [14].

Generally, the advantages of FNA cytology are: the possible availability of results within a few hours, few complications and good patient acceptability [11]. In addition, with careful selection of suitable lesions, and when performed and examined by experienced operators and cytologists, FNA cytology is highly specific for the detection of malignant cells. There is some evidence that compared core biopsy with FNA cytology, core biopsy has higher sensitivity and specificity and a lower rate of samples reported as unsatisfactory [11,63,67], particularly for image-detected lesions. Most importantly, core biopsy but not FNA cytology enables invasive cancer to be differentiated from DCIS, but it is still difficult to distinguish atypical ductal hyperplasia from low grade in situ carcinoma [70]. However, core biopsy requires local anaesthesia and may result in more discomfort post-procedure, and its results usually take longer to be obtained. Disposables and equipment required to perform FNA are less expensive than for core biopsy. FNA cytology or core biopsy of a palpable lesion may require image-guided localisation, regardless of which sampling technique is selected. The use of image guidance for either FNA cytology or core biopsy increases the likelihood of obtaining a representative sample from the lesion [14].

CONCLUSION

The FNA remains an indispensible tool in diagnostic Cytopathology despite some of its limitations. Its accuracy, sensitivity or specificity is a function of the technique applied during sample collection and the skill of the Aspirator. Hence, constant training of personnel involved in FNA and review of FNA approach may reduce the limitations in using FNA in diagnostic communities. It is technically advantageous to come combine certain procedure such as FNA and Core biopsy or cell block rather than depending on a single procedure. Furthermore, the triple test is a holistic approach to offering better diagnoses, characterization and management of palpable and non-palpable masses.

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