Chapter

Introductory Chapter: Fine-Needle Aspiration Cytopathology as a Valuable Tool in the Pathologist's Toolbox

Sofia Khan, Rameen K. Walters and Hilal Arnouk

1. Introduction

Fine needle aspiration (FNA) is a widely used initial diagnostic procedure utilized to evaluate the cellular characteristics of bodily masses found superficially or lesions in the internal organs. The first report on the use of surgical instruments that resemble modern-day aspiration needles can be traced back to the most influential book of Arab Medieval Medicine, Kitab al-Tasrif (The Method of Medicine), written by Albucasis, the court physician to the caliph of the Andalusia, who was first to describe the therapeutic punctures of the thyroid gland. Albucasis called the thyroid tumor "Elephant of the throat" and described it as a large tumor that has the color of the body and commonly occurs in women.

Subsequent medical advancements in the nineteenth century allowed for the use of FNA for the diagnosis and treatment of palpable masses or lesions [1]. The application has since been extended to different organs, the technique was refined, imaging modalities were utilized, and the procedure gained worldwide acceptance during the twentieth century [2–5]. Today, FNA is a standardized technique, allowing for a rapid and reliable method of evaluating masses ranging from the thyroid, lymph nodes, and even the bones. It is a minimally invasive, relatively painless, rapid, and inexpensive procedure.

2. The fine needle aspiration procedure

To prepare the sample for cytologic interpretation, options include a conventional preparation or liquid-based preparation. Conventional smears involve directly applying aspirated material on the glass slide, followed by air drying or alcohol fixation and subsequent staining [6]. Liquid-based preparation, an automated method of preparing cell samples, involves collecting the aspirate sample in a liquid fixative, removing obstructing debris and cells, and placing the cells in a monolayer for examination [7]. There are several factors that make liquid-based preparation a superior method compared to conventional preparation. The automated process allows for well-distributed cells for examination without artifacts or obstruction from red and white blood cells. Liquid-based preparations also allow for ancillary tests for additional information on cellular characteristics (**Figure 1**).

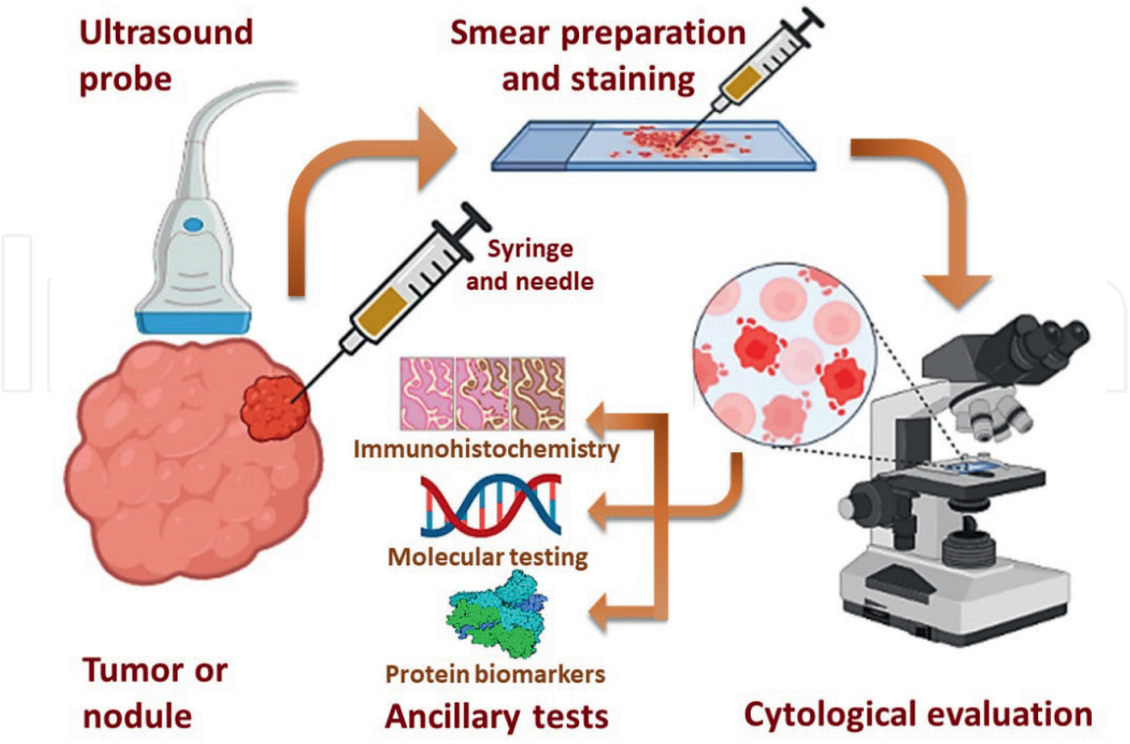

Figure 1.
Schematic showing the procedures for acquiring fine needle aspiration, smear preparation, cytological evaluation, and ancillary tests to increase the diagnostic accuracy of FNA cytopathology. This original figure was created by the authors using Microsoft Office PowerPoint and BioRender software.

Ancillary tests can be performed on aspirated samples which include immunohistochemistry, molecular testing, and flow cytometry amongst others. Choosing an ancillary test is dependent on the type of specimen, the quality of the specimen, and type of cellular information needed. For example, FNA with an immunohistochemistry test is used to diagnose melanoma using S-100 staining [8]. Flow cytometry is particularly useful for lymphoid tissue to identify cell surface markers or white blood cell clonality [9].

The fine needle aspiration has evolved significantly, from a simple diagnostic tool to a sophisticated procedure incorporating various imaging modalities and molecular techniques. Looking to the future, FNA continues to evolve and improve in various ways. One complication to FNA involves internal bleeding, and if performed on the thyroid, can lead to swelling and constriction of the airway space. To reduce the risk of bleeding during fine needle aspiration biopsy of thyroid nodules rich in blood supply, a method uses microwave or radiofrequency energy to block the blood supply to the target lesion. Color Doppler flow imaging identifies the arteries to that supply oxygen and nutrients to the target nodule where the ablation antenna or electrode is inserted to the site of color signals of arteries to coagulate them until the blood signals related to the nodule disappear. This method reduces the risk of bleeding during FNAB, while maintaining the natural state of the cellular specimen obtained [10].

The "9+X" needle passage puncture modality for FNA sampling of thyroid nodules involves dividing the target nodule into nine partitions and taking cellular components from each partition using a fine needle, which only requires one entry and exit of the patient's body. An extra FANB sampling may be conducted if there are special ultrasonic features present in the target lesion. This modality improves the standardization and rigor of thyroid nodule FNA by increasing the comprehensiveness,

adequacy, and quality of the sampling sites while minimizing mechanical injury. The multi-puncture technique, which involves multiple needle entries and exits, increases the risk of bleeding and pseudo-aneurysm formation. The "9+X" needle passage puncture mode is recommended as the first choice for large thyroid nodules to obtain sufficient and high-quality specimens [11].

Indeed, a vast amount of information can be derived from tissue acquisition by FNA, which influences patient management. Here we describe the most common organ systems where FNA is utilized as well as the advantages of FNA in comparison to other diagnostic tools where applicable.

3. Salivary glands lesions

According to the American Society of Clinical Oncology, providers should perform a tissue biopsy, either fine needle aspiration or core needle biopsy, to differentiate between salivary gland cancers and benign salivary gland lesions. Diagnosing salivary gland lesions is especially challenging for the pathologist due to the variety of benign and malignant salivary gland tumors that exist [12]. Several classification systems for salivary gland tumors were proposed, with the most recent one being the Milan system for reporting salivary gland cytopathology, reported to be a useful tool in risk stratification (**Figure 2**) [13].

The sensitivity and specificity of FNA in identifying benign versus malignant parotid lesions was found to be as high as 78% and 98%, respectively, in prospective cohort studies [14]. The same study found 13.3% of all FNAs performed to be non-diagnostic. The reported sensitivity and specificity for core-needle biopsy (CNB) to detect malignancy in the parotid gland is 94% and 98% respectively, with non-diagnostic samples reported to be 3.6% [15]. CNB has many advantages over FNA: larger sample sizes, perseverance of histological architecture, and ability to determine tumor type and grading, more suitable for immunohistochemical tests compared to FNA, and higher accuracy rate in identifying salivary gland tumor subtypes than FNA [16].

DIAGNOSTIC CATEGORY	ROM
I. Non-diagnostic	25%
II. Non-neoplastic	10%
III. Atypia of Undetermined Significance (AUS)	20%
IV. Neoplasm	
IVA. Neoplasm: Benign	<5%
IVB. Neoplasm: Salivary Gland Neoplasm of Uncertain Malignant Potential (SUMP)	35%
V. Suspicious for Malignancy	60%
VI. Malignant	90%

Figure 2.
Diagnostic categories and risk of malignancy (ROM) according to the Milan System for Reporting Salivary Gland Cytopathology (MSRSGC).

4. Thyroid gland lesions

The American Thyroid Association Management Guidelines recommends that thyroid sonography should be performed on all patients with thyroid nodules >1 cm to determine if the nodule has malignant or benign characteristics [17]. One study has found FNA to have a sensitivity of 90% and specificity of 79% in detecting malignancy [18]. FNA poses a significant limitation due to its high rate of non-diagnostic results, which may be mitigated with a subsequent CNB [19]. To standardize and classify the cytological findings in thyroid FNA samples, the Bethesda System for Reporting Thyroid Cytopathology is utilized to determine the risk of malignancy (**Figure 3**).

FNA can be useful in determining the histological types of thyroid cancer: differentiated histology includes papillary or follicular thyroid cancer, undifferentiated thyroid cancer include anaplastic thyroid cancer, and medullary thyroid cancer.

DIAGNOSTIC CATEGORY	ROM (%)
Non-diagnostic or Unsatisfactory	1-4
Benign	0-3
Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance	~5-15
Follicular Neoplasm or Suspicious for a Follicular Neoplasm	15-30
Suspicious for Malignancy	60-75
Malignant	97-99

Figure 3.
Diagnostic categories and risk of malignancy (ROM) according to the Bethesda System for Reporting Thyroid Cytopathology (TBSRTC).

5. Breast lesions

Fine needle aspiration is highly utilized in the setting of a new breast mass and is highly dependent on findings on imaging. The sensitivity and specificity of FNA is 93.4% and 97.5%, respectively, in diagnosing carcinoma in one study [20]. The inadequate biopsy rate is found to be higher in FNA compared to CNB [21]. Lack of adequate aspiration and inability to determine characteristics of the tissue itself is a recurring issue with FNA of the breast as well as other organ systems, which can be mitigated by CNB. A relatively new alternative to CNB is Vacuum Assisted Biopsy (VAB), with the main difference being the use of a vacuum to gather a larger sample and requiring insertion of the needle only once. VAB is shown to provide accurate diagnostic information [22], which may replace the use of CNB or FNA altogether. FNA can have therapeutic utility by removing fluid from cystic breast masses; the aspirated fluid can also be evaluated for malignant characteristics. To standardize FNA cytological findings of the breast, the International Academy of Cytology published the IAC Yokohama Reporting System; however, only about 17% of survey respondents were aware of the IAC Yokohama Reporting System [23] (**Figure 4**).

DIAGNOSTIC CATEGORY	RISK OF MALIGNANCY (%)
Insufficient	2.6–4.8
Benign	1.4–2.3
Atypical	13–15.7
Suspicious	84.6–97.1
Malignant	99–100

Figure 4.
Diagnostic categories and risk of malignancy (ROM) according to the International Academy of Cytology Yokohama System for Reporting Breast Fine Needle Aspiration Biopsy Cytopathology.

FNA can aid in classifying fibroadenoma, invasive ductal carcinoma and mastitis, while presenting a challenge in classifying lobular carcinomas, metaplastic carcinomas, papillary carcinomas, and fibrocystic changes [24].

6. Lymph nodes lesions

Malignancy can either originate in the lymph node or malignant cells can be seeded in the lymph node from a nearby primary tumor. For example, the first sign for head and neck cancer can first present as a suspicious cervical lymph node. Alternatively, lymphomas such as Hodgkin's and Non-Hodgkin's lymphoma can begin in a cervical lymph node itself. According to the American Academy of Otolaryngology and American Academy of Family Physicians, FNA is recommended for masses suspicious of malignancy. One study reported the sensitivity and specificity of FNA in identifying malignancy to be 97 and 98%, respectively [25]. Several options for ancillary tests can be performed for lymphomas, including immunocytochemistry, flow cytometry, polymerase chain reaction, fluorescent in situ hybridization, southern blot technique. These tests provide further information regarding cell-surface markers, cell clonality, and chromosomal translocation to help determine the diagnosis [26, 27].

7. Conclusion

In conclusion, the fine needle aspiration biopsy can be a valuable tool to establish diagnoses for several benign and malignant lesions, especially when obtaining surgical excision biopsies is not feasible. The application of FNA can even extend to internal organs, bones, bacterial infections, and more. Subsequent ancillary tests increase the diagnostic yield, providing a wealth of information on cellular characteristics that ultimately helps classify and diagnose tumors. In comparison to CNB and excisional biopsies, FNA is relatively painless, rapid, inexpensive, and results have quick turnaround times. While the advantages of FNA are plentiful, there are notable drawbacks. Inadequate sample sizes and indeterminate samples obtained by FNA are especially problematic, raising concern over FNA's position as the initial diagnostic step in suspicious masses.

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