FINE-NEEDLE ASPIRATION OF THE THYROID GLAND

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ABSTRACT

Thyroid nodules are common in clinical practice and the majority is benign with the risk of malignancy varying from 7 to 15%. Clinical evaluation includes careful history and physical examination, laboratory tests, neck ultrasound (US), and a fine-needle aspiration (FNA). Thyroid FNA or biopsy is an accurate test for determining malignancy in a nodule and is an integral part of current thyroid nodule evaluation. Results are superior when FNA is performed with ultrasound-guidance (USFNA). Herein, we describe techniques used for US-guided FNA.

FNA results are classified as diagnostic (satisfactory) or nondiagnostic (unsatisfactory). Unsatisfactory smears (5-10%) result from hypocellular specimens usually caused by cystic fluid, bloody smears, or suboptimal preparation. Diagnostic smears are conventionally classified into benign, indeterminate, or malignant. A benign cytology is negative for malignancy, and includes cysts, colloid nodule, or Hashimoto thyroiditis. Malignant (or suspicious for malignancy) cytology is usually positive for malignancy on histology, and includes primary thyroid tumors or, less frequently, nonthyroidal metastatic cancers. Papillary thyroid carcinoma (PTC) is the most common malignancy, characterized by increased cellularity, sheets of cells, and typical nuclear abnormalities.

Indeterminate or suspicious specimens include atypical changes, Hürthle (oncocytic) cells or follicular neoplasms, typically with absent or scant colloid, hypercellularity, and sometimes a microfollicular arrangement. The Bethesda Cytologic Classification has a 6-category classification. Overall, the indeterminate category Bethesda III category has a risk of malignancy of 6-18% if Non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) is not considered cancer. Advances in molecular testing can help further separate benign from malignant nodules with an indeterminate cytology.

INTRODUCTION

Thyroid nodules are common in clinical practice with a prevalence of up to 60%. The majority is benign and the risk of malignancy is between 7 to 15% (1).

Fine-needle aspiration biopsy (FNAB) of the thyroid gland is an accurate diagnostic test used routinely in the initial evaluation of nodular thyroid disease (2-6). Epidemiologic studies suggest that nodular thyroid disease is a common clinical problem, with a

prevalence of 4% to 7% in the adult population in North America and an annual incidence of 0.1%, which translates into approximately 300,000 new nodules in the United States (3).

In patients with a single palpable nodule, additional nodules can be detected in about 20-48% by ultrasonography (7).

A survey of clinical members of the American Thyroid Association revealed that most endocrinologists (96%) perform FNA for diagnosis of thyroid nodules (8). In addition, FNA with ultrasonographic guidance (US-FNA) is used routinely in follow-up surveillance of patients with thyroid cancer. Therefore, the importance of FNA biopsy in thyroid practice cannot be overemphasized.

This chapter describes biopsy techniques, cytologic diagnosis, complications, FNA results, diagnostic pitfalls, and other information that may be useful to clinicians who manage patients with nodular thyroid disease.

DEFINITIONS/HISTORY

The diagnosis of thyroid nodules by needle biopsy was first described by Martin and Ellis (9) in 1930, who used an 18-gauge–needle aspiration technique. Subsequently, cutting needle biopsy with Silverman or Tru-Cut needles was used for tissue examination. None of these techniques gained wide acceptance because of fear of malignant implants in the needle track, false-negative results, and serious complications. However, Scandinavian investigators introduced small-needle aspiration biopsy of the thyroid in the 1960s, and this technique came into widespread use in North America in the 1980s (10).

For FNA biopsy, most use fine or thin (22- to 27-gauge) needles, most commonly 25 or 27-gauge needles. As the name indicates, the biopsy technique uses aspiration to obtain cells or fluid from a mass. In contrast to percutaneous large-needle biopsy, which obtains tissue specimens and requires histologic fixation, aspiration biopsy offers cytological examination of the specimen. Another technique, fine-needle non-aspiration (FNNA) biopsy, also referred to as capillary technique, avoids aspiration but permits to obtain representative cytologic samples. Although the FNA technique appears simple, considerable time and experience are required to acquire and maintain skillful biopsy technique. Debate continues about who is best qualified to perform FNA biopsy, but the best results are obtained if the person performing the biopsy has appropriate technique and volume. In the opinion of the authors, individuals performing biopsies should have appropriate knowledge of thyroid pathologies in order to relate the findings to the clinical context.

EQUIPMENT

The basic equipment needed to perform FNA biopsy is simple and relatively inexpensive (2, 4, 11) The following items are necessary to have but many are not essential (Table 1):

Table 1. FNA biopsy equipment – Figure 1

1.	Disposable 3-10-mL plastic syringes
2.	Disposable 25- or 27-gauge needles, 1.5 inches long [shorter needles can be used for more superficial nodules]
3.	Alcohol prep sponges
4.	Sterile gauze
5.	Gloves
6.	Probe cover
7.	Sterile gel
8.	Lidocaine—1% for those who prefer biopsy with local anesthesia
9.	Glass slides, with 1 end frosted on 1 side, 1-mm thin (Gold Seal, Erie Scientific Company)
10.	Alcohol swabs and bandaid for post procedure
	Additional tools: For sample procesing
11.	Containers for (cystic) fluid collection and transportation to the cytology laboratory
12.	Laboratory slips with the patient's name, clinic number, biopsy sites, and other relevant information to be transferred to the cytology laboratory
13.	Alcohol bottles for immediate wet fixation of smears
14.	Rarely used nowadays: A syringe holder or syringe pistol—most commonly used is the Cameco syringe pistol (Belpro Medical). The pencil-grip syringe holder is another syringe-holding device (Tao and Tao Technology, Incorporated).-

THE PATIENT

The thyroid gland should be palpated carefully and the nodule(s) to be biopsied identified under sonographic guidance. The consent process is important. It should fully explain the procedure including risks and benefits, and address all questions raised by the patient. The procedure can be done with local anesthesia or without. Per nodule, typically 3 informative passes are obtained, in particular if rapid on-site evaluation (ROSE) by a cytopathologist is not available. In experienced hands, no serious complications are expected. Mild pain, minor bleeding or infection can occur but are unlikely to happen. Very rarely, transient hoarseness due to recurrent laryngeal nerve injury has been reported. With the appropriate caution, the procedure can usually be performed in patients with blood thinners.

The procedure is performed in the outpatient setting with the patient lying on an adjustable examination bed or chair. The procedure can be performed by a single person, but the presence of a nurse or clinical assistant is encouraged, if available. The patient may be seated or supine; we prefer the supine position. The patient is placed supine with the neck hyperextended to expose the thyroid; for support, a pillow is placed under the shoulders (Fig. 2 A). The patient is asked not to swallow, talk, or move during the procedure. It is best to talk to the patient and keep them informed of the progress of the biopsy. Once completed, firm pressure is maintained on the biopsy site(s) then the area is cleaned and a Band-Aid applied. It is best to observe patients for a few minutes for any side effects. This time can be used to counsel on after-procedure care instructions and potential results, if available including written instructions. Subsequently, the patient can be discharged.
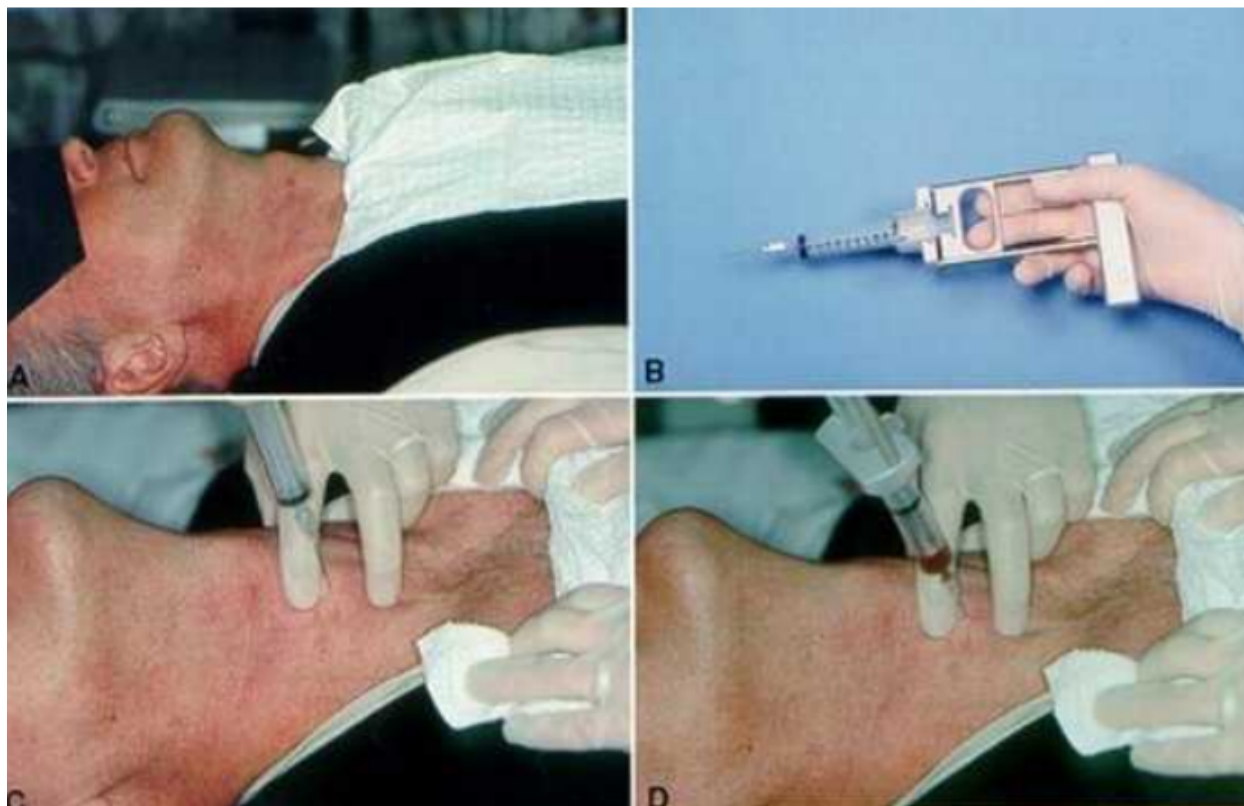
Figure 2. A) Patient position during fine-needle aspiration (FNA). A supine position and a pillow under the patient's shoulder allow hyperextension of the neck and maximal exposure. B) Syringe is placed in syringe holder. C) The nodule is identified and stabilized with operator's the nonaspirating hand. The operator stands on the side of the patient opposite that of the thyroid nodule. Current Occupational Safety and Health Administration regulations require the use of gloves because of concern about bloodborne diseases. D) With a quick motion, the needle passes through the skin and enters the nodule. Immediate mild suction follows. As soon as aspirate appears, suction is released and the needle is withdrawn.

THE TECHNIQUES

FNA Biopsy

Numerous reports, reviews, and even textbooks provide detailed descriptions of various FNA biopsy techniques (11-15). Although most reports agree on the principles of the technique, variations have been described to improve results. It is important to position the patient correctly, identify and locate the mass, provide adequate light during the biopsy, and have a clinical assistant available for help if needed. The physician performing the biopsy should be positioned at the patient's side, preferably contralateral to the lesion. The nodule(s) to be aspirated is identified, and the overlying skin is cleansed with alcohol. The use of povidone-iodine or sterile technique is not necessary but encouraged.

A retrospective study across multiple sites of the Mayo Clinic compared ultrasound-guided thyroid FNA using no anesthetic, subcutaneous injectable anesthetic, and topical anesthetic to compare the degree of pain/discomfort (16). The study found patient discomfort associated with FNA was comparable during and after the procedure regardless of the use of anesthetic or the type. However, others reported that FNA-associated pain is frequent and that the use of local anesthesia is beneficial (17)

In the method illustrated here, a 10-mL plastic syringe is attached to a Cameco syringe holder and held in the right hand by a right-handed operator (Fig. 2 B). Two fingers of the free (left) hand firmly grasp the nodule while the other hand holds a pistol-grip syringe holder (Fig. 2 C). The needle is then inserted through the skin and into the nodule. Once the needle tip is in the nodule, gentle suction is applied while the needle is moved in and out within the nodule vertically (Fig. 2 D). This maneuver allows the dislodging of cellular material and easy suction into the needle. During this period of 5 to 10 seconds, suction is maintained, and as soon as fluid or aspirate appears in the hub of the needle, the suction is released, and the needle is withdrawn. Of note, many operators do not use the handle; in this case, simply using a syringe with needle may suffice using the same maneuver.

Nowadays, ultrasound evaluation permits to identify cystic and partially cystic lesions. The appearance of fluid suggests that the nodule is cystic; suction is maintained, and as much fluid as possible is aspirated. It is important to release the syringe plunger and remove the vacuum before withdrawing the needle; this allows the aspirate to remain in the needle and not be sucked into the syringe. Next, the needle is detached from the syringe (Fig. 3 A), and 5 mL of air is drawn into the syringe (Fig. 3 B). The needle is reattached to the syringe, and with the bevel facing down, 1 drop of aspirated material is ejected onto each of several glass slides (Fig. 3 C). It is important that all slides be labeled and placed in order on a nearby table before the aspiration begins.

Smears are prepared by using a second glass slide in a manner similar to that of making blood smears (Fig. 3 D). The slides for wet fixation should be placed immediately in 95% alcohol for staining with the Papanicolaou stain. For Giemsa staining, air-dried smears are necessary, and prepared slides are left unfixed and transported to the laboratory.

Rapid on-site evaluation (ROSE) of cytology slides to evaluate for adequacy by cytopathologists may lower non-diagnostic rates and the number of necessary needle passes (18). This may, however, add more time to complete the procedure.
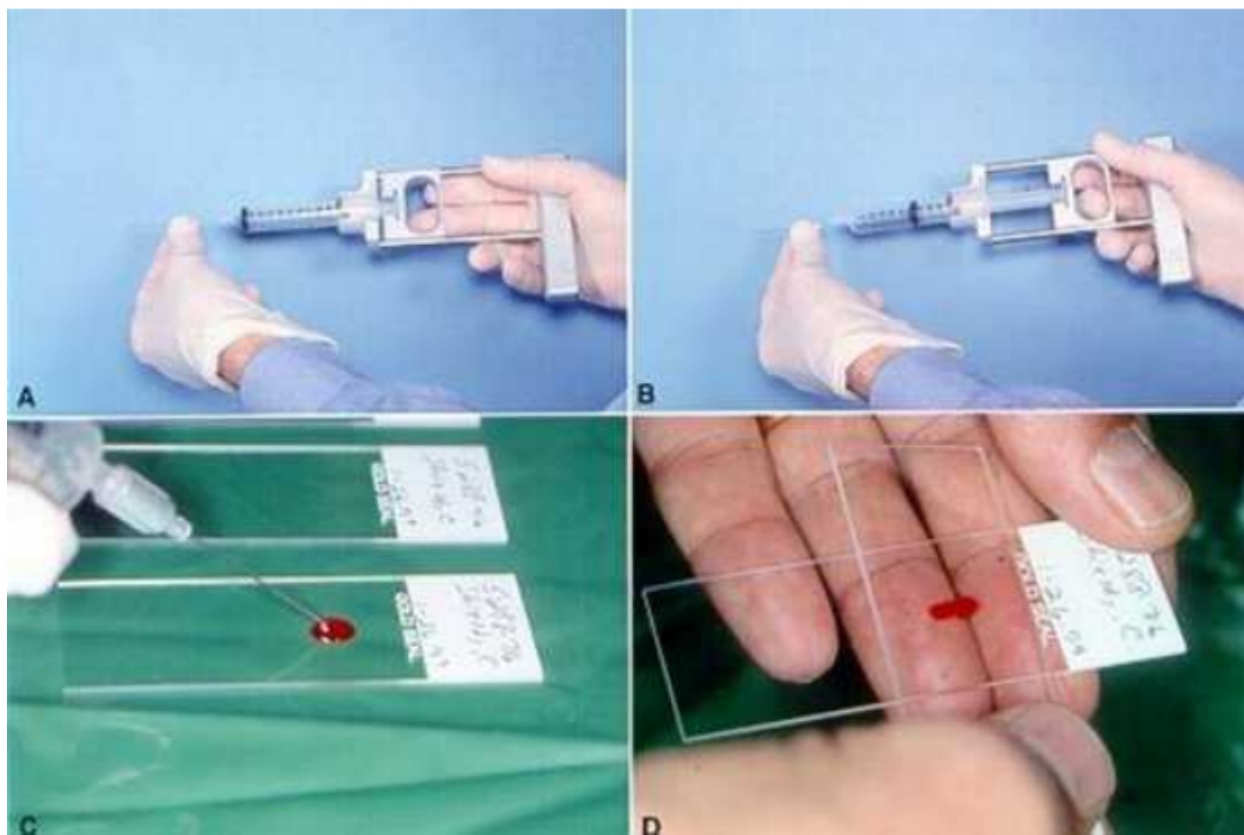
Figure 3. A) The needle is removed quickly from the syringe. B) Five ml of air is aspirated into the syringe, and the needle is placed back on the syringe. C) With the needle bevel facing down, 1 drop of aspirated material is expelled onto each of several glass slides. Slides are labeled and placed on the table before aspiration, ready for use. D) With a second slide, smears are prepared in a manner similar to that for blood smears. Slides are then immediately wet fixed by placing them in an alcohol bottle.

Usually, 3 to 6 aspirations are made (13, 14), some authors suggest at least 6 (19). Additional passes might be needed for molecular testing if decisions were made to obtain those. However, needle washouts typically provide sufficient materials for molecular analyses. Preferably, the aspirates should be obtained from the peripheral areas and different parts of the nodule in a sequential manner to ensure representative sampling (13). For larger nodules, the deep center of the mass should be avoided because it is more likely to contain degeneration and fluid, decreasing the chance of a diagnostic specimen. For cystic lesions, the fluid should be completely aspirated and FNA attempted on residual solid tissue if present. Aspirated fluid should be placed in a plastic cup and saved for cytology evaluation. For each pass, one should use a new needle and syringe.

The needle can be inserted in parallel or perpendicular way to the ultrasound probe. In the parallel approach, the US image can see the entire needle path. The perpendicular approach is generally simpler and the needle travels shorter distance; however, only needle tip can be seen in the nodule. Both techniques are equally effective.

Generally, minor procedures such as thyroid FNA, can be safely performed while patients on antiplatelets/anticoagulation(20). Special attention should be made to use a smaller gauge needle (27-gauge needle), minimize the number of passes, and monitor the patient few minutes for signs of bleeding. In certain situations, INR might need to be less than 2-2.5 at the day of the procedure per certain operator preferences. Overall, the risk of stopping anticoagulation for the purpose of FNA procedure must always be weighed against the risk of complication related to discontinuing the medication for few days. The overall data suggest low risk of bleeding when doing the biopsy on anticoagulation and antiplatelet therapy with hematoma only occurred in 0.89% (21).

FNNA Biopsy

The FNNA, also referred to as fine-needle capillary (FNC) sampling technique, has been described by several authors (2, 22). This technique is thought to minimize trauma to thyroid tissue and to reduce blood contamination. For this technique, patient preparation is similar to that for FNA. However, no syringe or suction is necessary. The hub of a 27-gauge needle is held in a pencil-grip fashion, and the needle is gently inserted into the nodule and then moved in and out for 5 to 10 seconds. Aspirate flows into the needle through capillary action, and as soon as aspirate appears in the hub, the needle is withdrawn and attached to a syringe with air inside. Next, the plunger is used to expel the material onto glass slides. The procedure is repeated several times, and the slides are prepared as described above for FNA.

Other techniques such as Core-needle biopsy (23) or Large-needle aspiration biopsy (24) are used less commonly as first-line procedure. They can be associated with a higher risk of complications such as pain and bleeding, but they have some utility with

repeatedly non-diagnostic FNA or when lymphoma, which requires additional analyses such as flow cytometry, is suspected.

Post Biopsy Procedure

After the biopsy has been completed, firm pressure is applied to the biopsy site(s) with a 4×4-inch gauze pad. Once bleeding has stopped, an adhesive bandage is placed on the puncture site(s), and the patient is observed for a few minutes. If there are no problems, the patient is allowed to leave.

COMPLICATIONS

Thyroid FNA biopsy, particularly under US-FNA, is very safe. No serious complications such as tumor seeding, nerve damage, tissue trauma, or vascular injury have been reported (11-15). Needle puncture may cause slight discomfort and possible skin ecchymosis at the aspiration site(s). However, even a minor hematoma is uncommon. Patient use of anticoagulants or salicylates does not preclude FNA biopsy. Needle track implantation of thyroid carcinoma is extremely rare and appears to an exceptional complication (25). Post aspiration hemorrhage within a cystic lesion is uncommon.

CYTOLOGIC DIAGNOSIS

Aspirates from normal glands often have scant thyroid follicular cells and colloid. Wet-fixed smears are usually prepared with a modified Papanicolaou stain, which shows nuclear details. Air-dried smears are often prepared with a Romanovsky stain. May-Grünwald-Giemsa is a modified Romanovsky staining procedure that is sometimes used in thyroid cytologic preparations. The six Bethesda Classification System categories of cytology results

include: Non-diagnostic (unsatisfactory), Benign, Atypia or undetermined significance (or follicular lesion of undetermined significance), Follicular neoplasm or suspicious for follicular neoplasm, suspicious for malignancy, and malignant (Table 6).

Benign Cytology

Aspirates obtained from multinodular goiters, a benign microfollicular adenoma, or normal thyroid are referred to as colloid nodules and show loosely cohesive sheaths of follicular epithelium, colloid, blood, and rare macrophages. Colloid nodules are the most common cytology and contain an abundance of colloid with sparse follicular cells. There is considerable variation in the number of cells, as well as the type and amount of colloid present (Fig. 4).
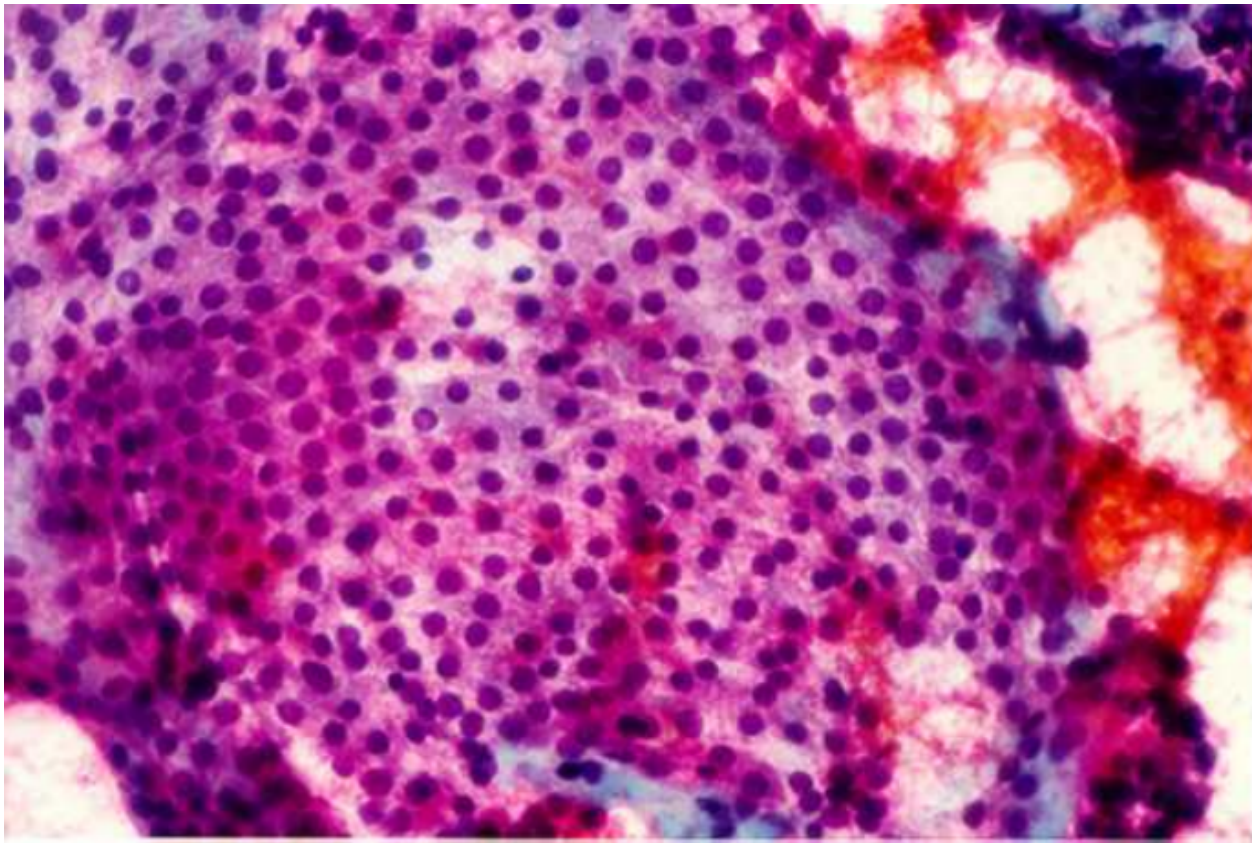
Figure 4. Colloid nodule. Sheath of normal thyroid epithelium shows uniform nuclei and pale cytoplasm (Papanicolaou, ×100).

Another benign diagnosis is Hashimoto thyroiditis. It has typically a characteristic pattern on FNA smears, showing hypercellularity with lymphocytes, Hürthle cells, and minimal or no colloid (Fig. 5).
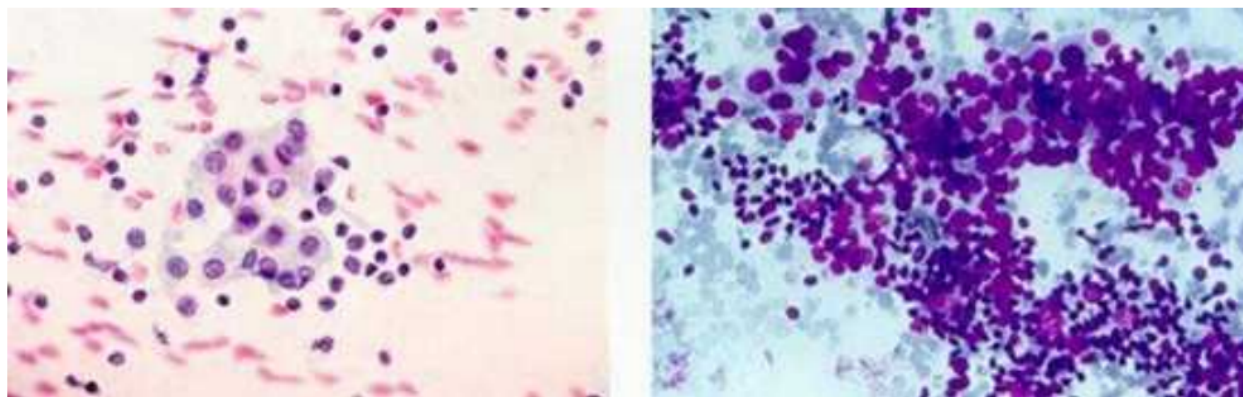
Figure 5. Hashimoto thyroiditis. A) Group of Hürthle cells with large cytoplasm and prominent nuclei, surrounded by a teratogeneous population of lymphocytes (Papanicolaou, ×60). B) Hypercellular aspirate with lymphocytes and Hürthle cells (May-Grünwald-Giemsa, ×250).

Subacute (granulomatous) thyroiditis is a rare condition with a benign aspirate. Typically, the smear shows multinucleated giant cells, epithelioid histiocytes, and scattered inflammatory cells.

Malignant Cytology

Papillary carcinoma, the most common thyroid malignancy, is readily diagnosed by FNA. Typically, cytology shows large irregular nuclei, and nuclear grooves. Psammoma bodies may or may not be present, but if present, they are highly suggestive of papillary thyroid carcinoma (Fig. 6).
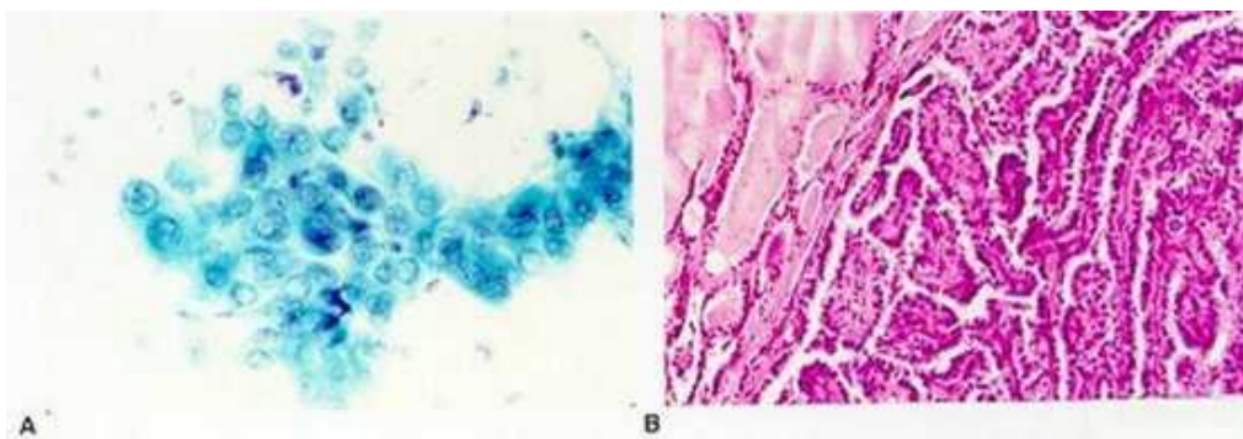
Figure 6. Papillary thyroid carcinoma. A) Follicular cells with large irregular nuclei, nuclear grooving, and pale chromatin (Papanicolaou, ×400). B) Histologic preparation showing typical papillary configurations (hematoxylin-eosin, ×50).

Medullary thyroid carcinoma accounts for 5% to 10% of thyroid cancers and may present as a thyroid nodule or neck mass. Typically, aspirates from a medullary thyroid carcinoma are hypercellular, composed of

large, poorly cohesive cells, and predominantly spindle shaped. Amyloid is often, but not invariably, present, and there is no colloid (Fig. 7).
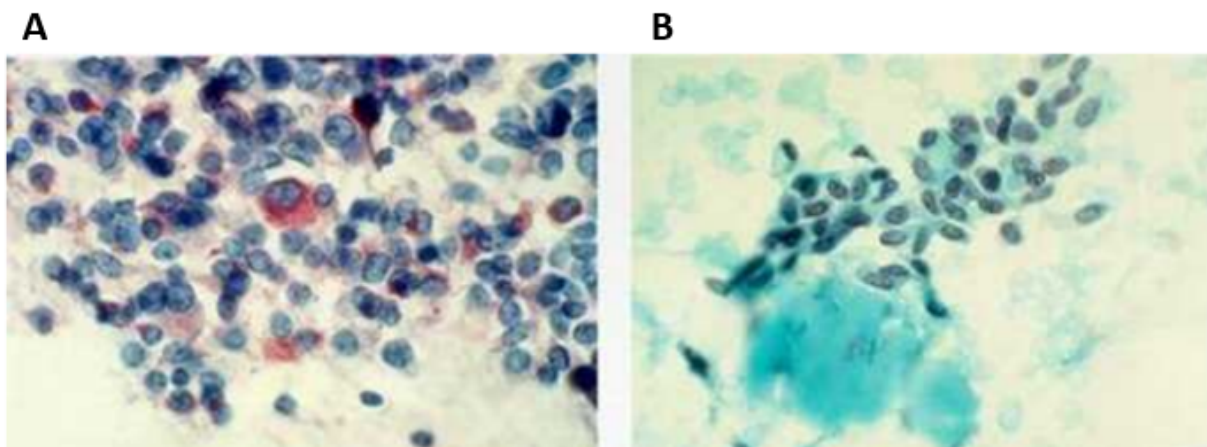

Figure 7. Medullary thyroid carcinoma. A) Cellular specimen staining positively for calcitonin by immunohistochemistry (×100). B) Loosely cohesive fragments of spindle-shaped cells. Amyloid is present as amorphous blue material intimately associated with neoplastic cells (Papanicolaou, ×400).

High-grade carcinoma can be diagnosed cytologically, but distinguishing between primary and metastatic cancer is not easy.

FNA RESULTS

The accumulated experience of the past 4 decades has confirmed the reliability and usefulness of FNA as a diagnostic test (2-4, 11-15, 26-31). The role of FNA biopsy in the evaluation of thyroid nodules is now firmly established, and FNA has become the initial test because it is both safe and cost effective. In most clinics, FNA has become a standard test, performed most often by an endocrinologist.

Diagnostic Cytology

An adequate specimen of good technical quality is considered diagnostic or satisfactory and may be benign, suspicious, or malignant. A benign (negative) cytologic diagnosis is reported for 50% to 90% of the specimens (average, 70%) (13, 15, 27, 32), 10-30% of FNA cytologic specimens may be suspicious for malignancy (indeterminate) (average, 20%) (32, 33). A malignant (positive) cytologic diagnosis is made in about 1% to 10% of cases (average, 5%). For example, Caruso and Mazzaferri (33) reported the following results from 9 series that included more than 9,000 patients: benign, 74%; malignant, 4%; inadequate, 11%; and suspicious, 11%. We reviewed more than 18,000 specimens from 7 large series and obtained similar cytologic results: benign, 69%; malignant, 4%; suspicious, 10%; and nondiagnostic, 17% (32).

False-Negative Rates

False-negative results reflects missed malignancy. False-negative rates generally occur in 1.5% to 11.5% (average, <5%) (19, 30, 33, 34). The false-negative rate is defined as the percentage of patients with a benign cytology in whom malignant lesions are later confirmed on thyroidectomy. The frequency of false-negative cytologic diagnosis depends on the number of patients who subsequently have surgery and histologic review. In most retrospective series, less

than 10% of patients with a benign cytologic diagnosis subsequently undergo thyroid surgery, suggesting that false-negative rates should be interpreted with some skepticism (33, 35). Despite this note of caution, most authorities agree that the true false-negative rate is less than 5% if all patients undego thyroid surgery. False-negative rates are lower in centers experienced with the procedure and with cytologic interpretation by expert cytopathologists.

False-Positive Rates

False-positive rates vary from 0% to 8% (average, 3%) (30, 32, 33). A false-positive diagnosis indicates that a patient with a malignant FNA result was found on histologic examination to have benign lesions.

Causes of False Diagnoses

Interpretive or sampling errors account for false diagnoses (14, 32, 34). Hashimoto thyroiditis is probably the most common cause of a false-positive cytology. Misclassification of follicular and Hürthle cell adenomas as papillary carcinomas accounts for other errors. FNA biopsy of thyroid lymphomas may yield lymphocytes that can be interpreted as Hashimoto thyroiditis, accounting for a false-negative diagnosis. Inadequate or improper sampling accounts for some false-negative errors. For example, nodules smaller than 1 cm may be too small for accurate needle placement, and nodules larger than 4 cm may be too large to allow proper sampling from all areas, thereby increasing the likelihood of misdiagnosis. Finally, the cytopathologist should establish and observe criteria to exclude a diagnosis of malignancy (2, 4, 11, 26).

The Problem of Cellular Tumors

Hypercellular specimens from follicular or Hürthle cell lesions may have features suggestive of, but not diagnostic for malignancy (3, 11, 14, 27). Thus, the cytopathologist labels these suspicious for malignancy because cytologic features neither confirm nor rule out malignancy. Histologic examination is necessary for definitive diagnosis. Hypercellularity may be seen with nonneoplastic lesions, and Hürthle cell changes may be seen in patients with lymphocytic thyroiditis. The diagnosis of follicular neoplasm is indicative of an underlying malignancy in 14% of cases and Hürthle cell neoplasm in 15% (29, 32). Many pathologists maintain that benign and malignant follicular tumors cannot be distinguished on the basis of aspirated cells only, and the lesion must be removed for histopathologic examination (4, 14, 35). However, Kini et al (36) believes that follicular adenomas and follicular carcinomas usually can be differentiated on the basis of nuclear size.

Several authors have discussed the problem of follicular neoplasm. In a study of 149 patients with the cytologic diagnosis of follicular neoplasm, Tuttle et al. (37) reported that risk of malignancy was higher in men, solitary nodules, and nodules larger than 4 cm. In a study of 219 patients with follicular neoplasm, Schlinkert et al. (38) showed that nodules are more likely malignant in younger patients, in men, if the nodule is solitary, and if it is larger than 4 cm. Baloch et al. (39) studied 184 cases of follicular neoplasm and reported that risk factors for malignancy included male sex, older age (>40 years), and larger nodules (>3 cm). Overall, they found that 70% of these lesions are benign.

Molecular studies are now routinely used with thyroid nodule evaluation and biopsy to help further asses the likelihood of malignancy in thyroid nodules in nodules with undetermined cytology with reasonable sensitivity and specificity [see section below].

Nondiagnostic Cytology

Inadequate specimens are labeled nondiagnostic or

unsatisfactory and account for 2% to 20% of specimens (average, 10%) (32, 33). Several factors influence nondiagnostic rates for FNA results, including the skill of the operator, vascularity of the nodule, criteria used to judge adequacy of the specimen, and the cystic component of the nodule (40-43). Overall, a satisfactory smear contains at least 6 clusters of well-preserved cells, with each group consisting of at least 10 to 15 cells. Reaspiration with US-FNA yields satisfactory specimens in more than 50% of cases with a nondiagnostic initial FNA (19, 35). Chow et al. found a 7% malignancy rate in 153 patients with initial nondiagnostic smears (44). Among 27 patients treated surgically, 37% had cancer. Reaspiration with US-FNA was diagnostic in 66% and 56% without US; overall, 62% of reaspirations were diagnostic.

For patients with non-diagnostic FNA biopsy, FNA can be repeated few weeks later. A repeat ultrasound-guided FNA will yield a diagnostic cytology specimen in 75% of solid nodules and 50% of cystic nodules (45).

If a patient has two nondiagnostic FNAs, ultrasound-guided core-needle biopsy can be considered as it has higher diagnostic yield compared to further repeating FNA (46, 47). However, the prevalence of cancer in non-diagnostic results is lower than in the general population. Nodules with repeatedly non-diagnostic FNA results typically have a low risk of malignancy and can be monitored if suspicious sonographic features are lacking (48).

Diagnostic Accuracy

Analysis of the data reveals that the sensitivity of FNA ranges from 65% to 98% (mean 83%), and specificity ranges from 72% to 100% (mean 92%) (13, 30, 33). The predictive value of a positive or suspicious cytologic result is approximately 50%. The overall accuracy for cytologic diagnosis approaches 95% (Table 2).

Table 2. Summary Data From Literature Survey on Thyroid FNA*

Feature	Mean	Range	Definition
Sensitivity (%)	83	65-98	Likelihood that a patient who has disease has a positive test result
Specificity (%)	92	72-100	Likelihood that a patient without disease has a negative test result
Positive predictive value (%)	75	50-96	Fraction of patients who have a positive test who have disease
False-negative rate (%)	5	1-11	FNA negative; histology positive for cancer
FNA, fine-needle aspiration.			
*From Gharib et al. (4). Used with permission.			

FNA Guidelines

Several guidelines and reviews have been published to help improve the adequacy and accuracy of cytology specimens (4, 49-51).

FNA biopsy should be performed by individuals who have had training in both thyroid ultrasound examination and thyroid biopsy. Thyroid FNA in the

hands of experienced operators achieves high diagnostic accuracy. Aspirates should be obtained from different portions of the nodule, preferably peripheral areas, in an organized and sequential manner. It is essential to ensure that an adequate number of follicular cells are present. A cytopathologist, preferably one with experience in thyroid cytology, should review and interpret the slides. If re-aspiration yields insufficient material, a core biopsy is the next step. In the event that the final result is still insufficient, surgical excision is warranted for nodules with suspicious features.

Several reports have offered suggestions to minimize false-negative rates (3, 4, 19, 32). In a review on thyroid FNA, Belfiore and La Rosa suggested acquiring biopsy expertise, avoid suboptimal sampling, and repeat FNA during follow up specially if sonographically suspicious to reduce false-negative results (11).

To minimize false-negative results, we follow the steps summarized in Table 3.

Table 3. Steps to Improve Accuracy of FNA and Lead to Better Nodule Management*

Step	Explanation
Biopsy performed by expert in thyroid pathologies	Offers better thyroid examination; accumulates experience with FNA
Experienced cytopathologist reviews slides	Improves cytologic interpretation
Caution with small (<1 cm) or large (>4 cm) nodules	Increased chance of misdiagnosis; US FNA improves accuracy
3-6 aspirates from different nodule sites	Improves cytologic sampling
Rebiopsy if cytology is nondiagnostic	About 50% will be diagnostic on reaspiration
Nondiagnostic cytology is not negative	Risk of cancer is low but not ruled out
Aspirates with no follicular cells are nondiagnostic	These should not be considered negative for malignancy
Excise nodules yielding suspicious cytology	20-40% chance of malignancy
Excise clinically suspicious, cytologically benign nodules	Clinical impression overrides FNA diagnosis
FNA, fine-needle aspiration; US-FNA, FNA with ultrasonographic guidance. **Modified from Gharib (2). By permission of Mayo Foundation for Medical Education and Research.*	

US-FNA BIOPSY

Published thyroid guidelines and reviews state that thyroid US should not be used as a screening test in the general population (3, 4). However, US is recommended for all patients with a single palpable nodule or a multinodular goiter or in a patient suspected of having a nodule (3) (Box 1). US machines are safe, easy to use, relatively inexpensive, have high resolution, and are widely available. It is important to note that US results are quite operator dependent.

THYROID ULTRASOUND AND SONOGRAPHIC RISK STRATIFICATION SYSTEMS

Thyroid US is the imaging modality of choice for the evaluation of thyroid nodule(s). High-resolution neck US is safe and provides excellent imaging results with no significant cost, and can be done in the outpatient settings. Sonographic features used to characterize thyroid nodule risk of malignancy focus on five major

sonographic criteria: echogenicity, composition, shape, echogenic foci, and margins (Table 4). Vascularity is debatable and therefor, not currently included in those criteria. US can also identify concerning lymph nodes. Features such as hypoechogenicity, irregular/infiltrative border microcalcification and a taller-than-wide shape in the transverse view, individually or in combination, are suggestive of malignancy especially if cervical lymph node involvement is seen. The diagnostic sensitivity and specificity of each of these features is variable and none of them alone can reliably distinguish malignant lesion from otherwise (52).

Professional organizations such as the American Thyroid Association (51) and the American College of Radiology (50) developed ultrasound based risk stratification systems based on the above criteria and assigned a value or points to each of those when increasing the risk of malignancy, and assigning cutoff size when deciding FNA and to avoid unnecessary over diagnosis. There are several other Thyroid Imaging Reporting and Data Systems (TIRADS) and current efforts aim at developing an international TIRADS to unify lexicons and definitions and ultimately unify recommendations.

Neck ultrasound also plays a predominant role in the follow-up of thyroid nodules and thyroid cancer, including active surveillance of small papillary thyroid carcinomas.

Box 1. Indications for Thyroid Ultrasound Examination*

| Palpable solitary nodule |
| Palpable multinodular goiter |
| Suspicion of nodule(s) in patient with difficult neck palpation |
| Prior history of neck radiation |
| Family history of medullary thyroid carcinoma, multiple endocrine neoplasia type 2, or papillary thyroid carcinoma |
| Unexplained cervical adenopathy |
| Preoperative thyroidectomy for cancer; long-term postoperative surveillance |
| *Data from Gharib et al. (4). |

An ever-increasing number of practicing endocrinologists are training to use US in routine practice. US is now used to supplement physical examination, a thyroid mass is present, a nodule needs careful measurement, or an impalpable thyroid lesion is suspected. As a result of this widespread use, many small (<1.5 cm) thyroid incidentalomas are detected, creating what has been referred to as a "thyroid nodule epidemic" (3, 7, 53). Such a finding has been an unintended consequence of thyroid US use and has created a management dilemma for the clinicians.

Table 4. Sonographic characteristics of thyroid nodules:

1	Echogenicity: hypo- iso,or hyperechoic
2	Calcifications: micro- or macrocalcifications
3	Margins: well-defined/smooth or irregular
4	Vascularity: high or low
5	Shape: taller than wide
6	Composition: Solid, Cystic, Mixed

The overall predictive value of US for malignancy based on a systematic review and meta-analysis of multiple studies is summarized in Table 5 (54). Although no single US feature is diagnostic for malignancy, the specificity is highest for taller-than-wide and microcalcifications, and lowest for echogenicity. The presence of at least 2 suspicious US criteria reliably identifies 85% to 93% of thyroid malignancies (3).

Table 5. Value of US Features Predicting Thyroid Malignancy*

US Feature	Sensitivity, %	Specificity, %
Microcalcifications	39.5	87.8
Hypoechogenicity	62.7	62.3
Irregular margins	50.5	83.1
Solid	72.7	53.2
Intranodular vascularity	45.9	78.0
Taller than wide	26.7	96.6
Adapted and modified from Remonti et al. (54)		

Sensitivity, positive predictive value, and negative predictive value increase significantly with US-FNA (3, 55-58). Nowadays, FNA should be exclusively performed with US-guidance. US-FNA permits precise needle placement in a nodule, thereby increasing both the rate of satisfactory aspirates and the diagnostic accuracy (3, 55-58).

FNA PITFALLS

The experience as well as the expertise of the

cytopathologist is critical in avoiding pitfalls. Determining the adequacy of an aspirate, cellular atypia, performing and interpreting of immunostains, and differentiation of lymphocytic thyroiditis from lymphoma are but a few of the challenges. Larger nodules are more likely to yield false-negative results. To improve sampling, aspirates should be obtained from multiple sites of the nodule rather than repeatedly from a single spot. The absence of malignant cells in an otherwise acellular specimen does not exclude malignancy. It is good practice to biopsy all suspicious nodules in a multinodular gland (3, 4).

REBIOPSY

Opinions on indications for reaspiration are divided, some favoring (11, 59), others not favoring (4, 60) routine rebiopsy. Lucas et al. (60) reported no advantage in routine rebiopsy, whereas Chehade et al. (59) found that repeated biopsy may decrease the rate of false-negative FNA from an average of 5.2% to less than 1.3%. Thyroid nodule guidelines from the American Association of Clinical Endocrinologists and the Associazione Medici Endocrinologi (4) did not suggest routine rebiopsy of FNA-benign nodules. The American Thyroid Association also recommends against routine rebiopsy (51). Repeat FNA for nodules that grow during serial sonographic follow-up can be done although nodule growth can be defined variably due to interobserver variation. Minimally significant change in nodule size of 50% increase in volume may warrant repeat FNA (61). Additional indications for re-biopsy are a nondiagnostic initial FNA, indeterminate cytology, and sonographically suspicious nodules with a benign cytology. In such instances, the current ATA guidelines recommend repeat biopsy within 1 year.

Box 2. The Bethesda System for Reporting Thyroid Cytopathology: Recommended Diagnostic Categories

I. **Nondiagnostic or unsatisfactory** • Cyst fluid only • Virtually acellular specimen • Other (obscuring blood, clotting artifact, etc.) II. **Benign** • Consistent with a benign follicular nodule (includes adenomatoid nodule, colloid nodule, etc.) • Consistent with lymphocytic thyroiditis (Hashimoto) in the proper clinical context • Consistent with granulomatous thyroiditis (subacute) • Other III. **Atypia of undetermined significance or follicular lesion of undetermined significance** IV. **Follicular neoplasm or suspicious for a follicular neoplasm** • Specify if Hürthle cell type (oncocytic) V. **Suspicious for malignancy** • Suspicious for papillary carcinoma • Suspicious for medullary carcinoma • Suspicious for metastatic carcinoma • Suspicious for lymphoma • Other

VI. **Malignant**
- Papillary thyroid carcinoma   - Poorly differentiated carcinoma   - Medullary thyroid carcinoma   - Undifferentiated carcinoma (anaplastic)   - Squamous cell carcinoma   - Carcinoma with mixed features (specify)   - Metastatic carcinoma   - Non-Hodgkin lymphoma   - Other   *Adapted with permission from Cibas and Ali (62).*

Table 6. The Bethesda System for Reporting Thyroid Cytopathology: Risk of Malignancy and Recommended Clinical Management

Diagnostic Category	Risk of Malignancy (%)**	Usual Management
Nondiagnostic or unsatisfactory	5-10	Repeat FNA with ultrasound guidance
Benign	0-3	Clinical follow-up
Atypia of undetermined significance or follicular lesion of undetermined significance	6-18	Repeat FNA, molecular testing, or lobectomy
Follicular neoplasm or suspicious for a follicular neoplasm	10-40	Molecular testing, Lobectomy
Suspicious for malignancy	45-60	Near total thyroidectomy or lobectomy
Malignant	94-96	Near total thyroidectomy or lobectomy
FNA-fine needle aspiration *Adapted with permission from Cibas and Ali (62)*		

** When NIFTP is not considered cancer

THE UTILITY OF MOLECULAR TESTING IN THYROUD FNA/BIOPSY

Molecular testing is particularly useful in indeterminate cytology such as Bethesda class III or class IV that are expected to have a malignancy risk of 18 and 40% respectively (Table 6).

The use of molecular markers in indeterminate thyroid nodule cytology has lowered the need for diagnostic lobectomy in those cases. Repeat biopsy of Bethesda class III and IV can still be done to obtain more definitive diagnosis but the cytological assessment of a 2nd FNA only provide definitive diagnosis in about 40% (63).

Molecular testing of the thyroid FNA samples has significantly evolved since its introduction at the beginning of the 21st century. Multiple methods were developed for this approach and the tests are based

on 3 main pathways: testing for somatic mutations, gene expression profiles, and microRNA (MiRNA) classifiers.

The most widely used molecular tests, ThyroSeq and Afirma assays have reasonable positive and negative predictive values suitable for using them when ruling-in and ruling-out thyroid cancer. Thyroseq V3 and Afirma GSC perform best to rule out malignancy (64)

Afirma molecular testing initially started as a microarray analysis of mRNA expression, currently a Genomic Sequencing Classifier (GSC) relying on RNA sequencing approach with a sensitivity of 96%, specificity of 68%, and positive predictive value of 47% (65).

The current version of ThyroSeq (version 3) is based on targeted next-generation sequencing analysis of 112 cancer related genes for point mutations, gene fusions, copy number alterations, and abnormal gene expression. This test has a reported sensitivity of 94% and a specificity of 82%, with a negative predictive value of 97%, and positive predictive value of 66% (66).

Other molecular tests may include a combination of more than one method such as those based on miRNA classification (ThyraMIR) and next-generation sequencing mutational analysis (ThyGeNEXT) which have excellent positive and negative predictive values (67).

When obtaining molecular testing, an additional dedicated needle pass can be useful. However, it is also possible to rinse the needles of all passes in the preservation fluid or cells from cytology slides. Furthermore, original cytology slides can be used in select cases such as in ThyGeNEXT/ThyraMIR (68).

THE USE OF US-GUIDED FNA WITH MINIMALLY INVASIVE THYROID PROCEDURES

Ultrasound-guided minimally invasive interventional techniques have been widely recognized and increasingly used over the last two decades. Those techniques include percutaneous ethanol injection (PEI) and thermal ablations such as laser and radiofrequency ablation. They can be used to treat both benign and malignant thyroid lesions. This includes the treatment of small papillary carcinomas, especially in patients who do not wish to pursue active surveillance, and in patients who are poor surgical candidates (69). Multiple international consensus statements were published to guide the use of these techniques with rapidly evolving indications (70-73).

Pre-procedural evaluation involves performing neck ultrasound, thyroid function status and requiring two benign cytology for most solid nodules, one benign cytology for autonomously functioning thyroid nodules with low-risk thyroid ultrasound features (69).

PEI is used for predominantly cystic benign thyroid nodule. It was introduced in the early 1990s as successful treatment for benign thyroid nodules (74-76). With success rate defined as volume reduction of more than 50% with symptom control, PEI can reduce nodule volume from 50% to 98% (77, 78). Long-term outcomes of cystic nodule treated with PEI are excellent with minimum side effects and proven benefit. The procedure can be done in the outpatient setting under local anesthesia (69). Radiofrequency ablation and laser ablation can be also effectively used in the outpatient setting under local anesthesia for treating benign thyroid nodules, in particular autonomous nodules with a volume reduction range 50% to 85%, usually with better outcomes in smaller nodules (<10 mL) (69). Thermal ablation can also be performed in selective cases of thyroid malignancy such as low risk papillary thyroid microcarcinomas (79, 80). Complications include local pain, dysphonia, skin irritation, hematoma and rarely nodule rupture (69).

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