H. Thyroid Fine Needle Aspiration (FNA) and Cytology

The prevalence of palpable thyroid nodules in adults increases with age (average 4 -7% for the United States population) with thyroid nodules being more common in women than men *(439-441)*. In adults, 95% of these nodules are benign. In contrast, although rare (0.22% to 1.8%) patients with thyroid nodules who are under 21 years of age have a higher incidence of malignancy (33% versus 5%, children versus adults, respectively) *(442-445)*. The methods currently used for assessing thyroid nodules include, fine needle aspiration (FNA), thyroid scan and ultrasound. Practice guidelines suggest that an initial FNA is more diagnostically useful and cost effective than other forms of investigation *(446)*. Despite such guidelines, a recent United States study reported that in 1996, FNA was only used as the initial procedure in 53% of thyroid nodule cases *(447)*. Despite the fact that isotopically "cold" thyroid nodules are considered suspicious for carcinoma, most benign thyroid nodules (cysts, colloid nodules, benign follicular lesions, hyperplastic nodules and nodules of Hashimoto's thyroiditis) also present as "cold" nodules. In addition, "warm" or iso-functioning nodules that do not result in a completely suppressed TSH and thus surrounding normal thyroid tissue is not suppressed, can be malignant. Logistic regression analysis indicates that adequate cytologic material significantly increases with the size of the nodule *(448)*. Although ultrasound can be used to detect non-palpable nodules, ultrasound cannot differentiate between benign and malignant lesions. In general, ultrasound is typically used for evaluating complex cystic masses and nodules that are difficult to palpate *(449)*. Ultrasound is also used to determine the size of nodules and to monitor nodule growth, as well as verify the presence of non-palpable nodules that have been incidentally detected by other imaging procedures. Ultrasound-guided FNA should be used for hypoechoic nodules and when aspiration cytology fails to yield adequate cellular material *(450,451)*.

1. Indications for FNA

All solitary or dominant nodules ≥ 1cm in diameter should be evaluated by FNA. FNA is preferred to thyroid scanning or ultrasonography as the initial diagnostic test for evaluating patients with thyroid nodules *(452)*. Since FNA became popular in the 1970s, the number of thyroid surgical procedures has decreased by 50% whereas the percent yield of cancers for patients undergoing surgery for thyroid nodules has increased from 10-15% to 20-50% *(453)*. The frequency of false negative FNA reports is related to the skill of the operator and the experience of the cytopathologist *(454)*. False negative rates appear to be less than 2 % *(455)*.

Guideline 56. Use of Fine Needle Aspiration (FNA) of the Thyroid

- FNA is recommended for all palpable solitary or dominant nodules, independent of size.
- FNA is preferred over thyroid scan or ultrasonography as the initial diagnostic test for thyroid nodules. However, a previous ultrasound may aid the physician performing the aspiration.
- When TSH is suppressed or the patient is thyrotoxic, a nuclear scan maybe indicated before FNA. However, the result of the scan should not exclude the necessity for FNA.
- "Hot" nodules detected by nuclear scan are less likely to be malignant than "cold" nodules.

2. Factors Suggesting a Higher Risk for Thyroid Cancer

A number of factors are associated with an increased risk for thyroid carcinoma *(456-458*). These are:

- Age, < 20 or > 40 years
- Nodule size > 2cm diameter
- Regional adenopathy
- Presence of distant metastases
- Prior head or neck irradiation
- Rapidly growing lesion
- Development of hoarseness, progressive dysphagia, or shortness of breath
- Family history of papillary thyroid cancer
- Family history of medullary cancer or MEN Type 2

Some of these risk factors are included in tumor risk-assessment protocols. The TNM classification protocol (tumor size, presence of lymph nodes, distant metastases) and age is the general tumor risk assessment algorithm. A number of thyroid-specific staging protocols have been developed *(12)*. These protocols are used to provide objective information necessary for establishing an appropriate treatment plan for the projected outcome. Although the TNM classification protocol is in general use, it can be misleading when applied to thyroid tumors. Specifically, with non-thyroid cancers, the presence of lymph node metastases is a heavily weighted factor that negatively impacts on mortality. In contrast, differentiated thyroid cancers often arise in young patients in whom the presence of lymph node metastases may or may not have a minimal effect on mortality, but increase the risk of recurrence.

Guideline 57. For Physicians

- It is important that the endocrinologist, surgeon, nuclear medicine physician and cytopathologist act in concert to integrate the staging information into a long-term treatment plan and thereby ensure continuity of care.
- Preferably, the physicians responsible for the long-term management of the patient should review the slides with the cytopathologist and understand the cytopathologic interpretation to establish meaningful treatment strategies for the patient.

3. Factors Suggesting a Lower Risk for Thyroid Cancer

FNA may be deferred in low-risk patients with the following characteristics:

- Autonomous "hot" nodules (serum TSH < 0.1 mIU/L).
- Incidental nodules < 1 cm, detected by ultrasound.
- Pregnant patients presenting with a solitary nodule. FNA of nodules detected during pregnancy can be deferred until after delivery without increasing the risk of morbidity from DTC *(459)*. If it is necessary to surgically remove a nodule during pregnancy, surgery during the 2nd.trimester minimizes the risk to the fetus.
- Multinodular thyroid glands with nodules < 1 cm.
- Fluctuating or soft nodules.
- Hashimotos' thyroiditis. Indications include firm, "rubbery" gland on physical examination without dominent nodules and an associated elevation in TPOAb.

4. Follow-up of Patients with Deferred FNA

The follow-up frequency (i.e. every 6 to 24 months) should be appropriate for the degree of diagnostic certainty that the nodule is benign. The efficacy of L-T4 therapy to suppress TSH can be variable. The goal of follow-up is to identify patients with undiagnosed or subsequent malignancy and to specifically recognize any progressive enlargement that could result in local compressive complications and cosmetic concerns by monitoring nodule size preferably with ultrasound. If ultrasound is not available, a careful physical examination should be made. This may be accomplished by:

- Placing a tape over the nodule and outlining the borders with a pen, then pasting the tape into the patient's chart.
- Using a ruler to record the nodule diameter in two dimensions
- Palpating for enlarged adjacent lymph nodes
- Diagnosing any associated clinical or mild (subclinical) thyroid dysfunction by periodic serum TSH and TPOAb measurements.
- Evaluating patients for signs of undiagnosed or subsequent malignancy such as:
  - progressive nodule or goiter enlargement
  - rising serum Tg level.
  - local compression and invasive symptoms (i.e. dysphagia, dyspnea, cough, pain)

- hoarseness
- tracheal deviation
- regional lymphadenopathy

5. Guidelines for Who Should Perform FNA

Experience with aspiration cytology is essential. If the cytologist or ultrasonographer performs the FNA, there must be an exchange of appropriate information with the clinician *(460)*. Physicians performing FNA should be able to request a review of the slides with the cytopathologist and understand the cytology results in order to recommend appropriate therapy based on the tissue diagnosis. Ideally, the physician performing the FNA should also be the physician responsible for the long-term management of the patient in order to assure continuity of care.

Guideline 58. Selection of Physicians to Perform FNA

Thyroid gland aspirations should be performed by physicians who:

- Are skilled in the technique and perform thyroid aspirations frequently.
- Can understand the interpretation of the cytology results.
- Are able to recommend appropriate therapy depending on the results of the aspiration.

6. Technical Aspects of Performing FNA

It is recommended that aspirin or other agents that affect coagulation be discontinued for several days before the procedure. FNA is typically performed using 22 to 25 gauge needles and 10 or 20 ml syringes that may, or may not be attached to a "pistol-grip" device. Aspiration should be as minimally traumatic as possible. Some physicians favor administering topical local anesthetic (1% lidocaine) while others do not. It is recommended that a minimum of two passes be made into various portions of the nodule to decrease sampling error. Slides are typically fixed in Papanicolaou's fixative and stained. It is imperative to fix immediately and avoid drying and drying artifacts to preserve nuclear detail. It is also useful to use a rapid stain, such as Diff-Quik and examine the slides at the time of aspiration to assess adequacy of specimen for cytologic evaluation. Other slides may be air-dried for alcohol fixation and subsequent staining (excellent for detecting colloid). Any additional material can be combined with material rinsed from the needle and spun down to form a cell block which can then be embedded in agar. Cell-blocks can provide histologic information and be used for special staining studies. It is important to adequately protect the slides for transport to the laboratory. Slides should be submitted to the cytopathologist with clinical details together with the nodule size, location and consistency.

Firm nodules are usually suspicious for carcinoma whereas fluctuant or soft nodules suggest a benign process. When cyst fluid is aspirated the volume, color and presence of blood should be recorded together with a record of any residual mass left after aspiration. If there is a residual mass after cyst aspiration it should be re-aspirated. Clear, colorless fluid suggests a parathyroid cyst, whereas yellow fluid is more typical of a cyst of thyroid follicular origin. After aspiration, local pressure should be applied to the site of the aspiration for 10-15 minutes to minimize the likelihood of swelling. The patient can be discharged with a small bandage over the aspiration site with instructions to apply ice should discomfort occur later.

Often the FNA cytology information can be augmented by submitting the material for flow cytometry or immunoperoxidase staining [Section-3 H8]. Any thyroid tissue in a lateral neck node is thyroid cancer (99%) unless proven otherwise!

7. Cytologic Evaluation

If a cytopathologist experienced with the thyroid is not available locally, it may be essential that the slides be sent to an outside expert for review. In the future, electronic review of cytopathology specimens will become increasingly available as tele-cytopathology technology develops.

Guideline 59. Selection of the Cytopathologist

- The cytopathologist should have an interest and experience in reading thyroid cytology. If an experienced cytopathologist is not available locally, the slides should be sent for review by a cytopathologist with thyroid expertise outside the institution.
- Cytopathologists should be willing to review the slides with the patient's physician on request.

8. Special Tissue Stains

Special tissue stains can be helpful in the following situations:

- When there is a mass of questionable malignancy or thyroid origin - Use specific antibody stains for Tg, TPO (MoAb 47) Galectin-3 and CEA *(461-466)*.
- For questionable lymphoma, use B-cell immunotyping
- Undifferentiated/anaplastic thyroid cancer - stains for vimentin, P53, keratin
- Questionable medullary thyroid cancer - stains for calcitonin, neuron-specific enolase, chromogranin and/or somatostatin.

9. Diagnostic Categories

Some cytopathologists believe that there must be at least six clusters of follicular cells of 10 to 20 cells each on two different slides in order to accurately report a thyroid lesion as benign *(466-468)*. A cytologic diagnosis of malignancy can be made from fewer cells, provided that the characteristic cytologic features of malignancy are present.

Guideline 60. Cytopathologic Characteristics

Thyroid cytology interpretation can be difficult and challenging. The amount of tissue contained on the slides may depend on the method of aspiration (ultrasound versus manual).
The evaluation should assess:

- The presence or absence of follicles (microfollicles versus variable-sized follicles)
- Cell size (uniform versus variable)
- Staining characteristics of the cells
- Tissue polarity (cell block only)
- Presence of nuclear grooves and/or nuclear clearing
- Presence of nucleoli
- Presence and type of colloid (watery and free versus thick and viscous)
- Monotonous population of either Follicular or Hurthle cells
- Presence of lymphocytes

(a) Benign Lesions (~ 70% of cases)

Clinical presentations that suggest a benign condition *(but not necessarily exclude FNA)*

- Sudden onset of pain or tenderness suggests hemorrhage into a benign adenoma or cyst, or subacute granulomatous thyroiditis, respectively. (However, hemorrhage into a cancer can also present with sudden pain).
- Symptoms suggesting hyperthyroidism or autoimmune thyroiditis (Hashimoto's).
- Family history of benign nodular disease, Hashimoto's thyroiditis or other autoimmune disease.

- Smooth, soft easily mobile nodule.
- Multi-nodularity (no dominant nodule).
- Mid-line nodule over hyoid bone that moves up and down with protrusion of tongue is likely to be a thyroglossal duct cyst.

• ***Cytologic and/or laboratory analyses that suggest a benign condition*** *include:*

- presence of abundant watery colloid
- foamy macrophages
- cyst or cyst degeneration of a solid nodule
- hyperplastic nodule
- abnormal serum TSH
- lymphocytes and/or high TPOAb (suggests Hashimoto's thyroiditis or rarely lymphoma)

Guideline 61. For Laboratories & Physicians

- In addition to routine cytology, the laboratory should provide access to special immunoperoxidase staining for CT, Tg, TPO or Galectin-3 for special cases. (Send out to a different laboratory if necessary).
- Laboratories should archive all slides and tissue blocks "in trust" for the patient and make materials available for a second opinion when requested.
- Cytopathology laboratories should use standardized reporting of FNAs. The simplest approach uses four diagnostic categories: (1) Benign, (2) Malignant, (3) Indeterminate/Suspicious, and (4) Unsatisfactory/Inadequate. This should help achieve meaningful comparisons among different laboratories regarding outcomes.
- Cytopathology laboratories should share their analysis of FNA results with clinicians by citing their rates for true and false positives and negatives

Guideline 62. Follow-up of Patients with Benign Disease

- Some advocate performing a second FNA several months later to confirm the test.
- Others do not recommend a repeat FNA if the first yielded adequate tissue, provided that the nodule was less than 2 cm and has been stable in size during a year of follow up. In this case, follow-up with an annual physical examination and measurement of the nodule size, preferably with ultrasound is recommended. If ultrasound is not available, changes in nodule size may be detected by measurements made by a tape and/or ruler.
- It is recommended that enlarging lesions or any clinically suspicious nodules should be re-aspirated.

Benign conditions *include, but are not be limited to, the following:*

- simple goiter
- multinodular goiter
- colloid nodule*
- colloid cyst*
- simple cyst*
- degenerating colloid nodule
- Hashimoto's thyroiditis
- hyperplastic nodule

**often have inadequate cytologic specimen due to lack of follicular cells*

(b) Malignant Lesions (~ 5-10% of cases)

There are differences of opinion regarding the optimal degree of surgery for thyroid malignancies. In most

centers in the United States, near-total or total thyroidectomy, performed by an experienced surgeon, is the favored opinion. In Europe, other opinions exist *(469)*. The risk of complications is lower when a surgeon is selected who performs thyroid operations frequently.

(i) Papillary Carcinoma (~ 80% of malignancies)

This classification includes mixed papillary and follicular and variants such as the tall cell variant and the sclerosing variant (a histological diagnosis)

Cytologic/Histologic. Two or more of the features below suggest a papillary malignancy:

- nuclear inclusions, "cleared-out", "ground glass" or "orphan annie" nuclei.
- nuclear "grooves" (not just a few)
- overlapping nuclei
- psammoma bodies (rare)
- papillary projections with fibrovascular core
- "ropey" colloid

(ii) Follicular or Hurthle Cell Neoplasms (~20% of malignancies)

Lesions in this diagnostic category display cytologic evidence that may be compatible with malignancy but are not diagnostic *(457,470)*. Factors suggesting malignancy include male gender, nodule size ≥3 cm and age >40 years *(470)*. Definitive diagnosis requires histologic examination of the nodule to demonstrate the presence of capsular or vascular invasion. Re-aspiration is usually discouraged as it rarely provides useful information. There are currently no genetic, histologic or biochemical tests that are routinely used to differentiate between benign and malignant lesions in this category. Appropriate markers would need to be shown to distinguish between benign and malignant neoplasms in FNA specimens by multiple investigators. A number of studies suggest that TPO expression, measured by the monoclonal antibody MoAb 47, improves the specificity of correctly diagnosing histologically benign lesions over FNAB cytology alone (83 versus 55%, TPOAb immunodetection versus cytology alone, respectively) *(461,462)*. More recently, Galectin-3, a beta-galactoside binding protein has been found to be highly and diffusely expressed in all thyroid malignancies of follicular cell origin (including papillary, follicular, hurthle and anaplastic carcinomas) but minimally in benign conditions *(463-466,471)*. Most surgeons believe that an intra-operative frozen section offers minimal value in differentiating malignant from benign lesions when patients have follicular or Hurthle cell neoplasms *(472)*. Sometimes a staged lobectomy is performed followed by a completion thyroidectomy within 4 to 12 weeks if capsular or vascular invasion in the histologic specimen indicates malignancy. A recent study found that the prognosis for patients with Hurthle cell carcinoma is predicted by well-defined histomorphologic characteristics *(473)*.

Cytologic/Histologic. Features suggesting a Follicular or Hurthle malignancy include:

- minimal amounts of free colloid
- high density cell population of either follicular or Hurthle cells
- microfollicles

Cytology. These lesions may be reported as:

- "Hurthle cell neoplasm"
- "Suspicious for follicular neoplasm"
- "Follicular neoplasm/lesion"
- "Indeterminate" or "non-diagnostic"

(iii) Medullary Carcinoma (1-5% of thyroid malignancies)

This type of thyroid cancer should be suspected when patients have a family history of medullary cancer or multiple endocrine neoplasia (MEN) Type 2 [Section-3 F].

Cytologic/Histologic Features suggesting this type of malignancy include:

- spindle-type cells with eccentric nuclei
- positive calcitonin stain
- presence of amyloid
- intranuclear inclusions (common)

(iv) Anaplastic Carcinoma (< 1% of thyroid malignancies)

This type of thyroid cancer usually only occurs in elderly patients who present with a rapidly growing thyroid mass. Such patients may have had a previous indolent thyroid mass present for many years. It is necessary to differentiate between anaplastic carcinoma for which there is very limited therapy and thyroid lymphoma for which treatments are available.

Cytologic/Histologic Features that suggest this malignancy include:

- extreme cellular pleomorphism
- multinucleated cells
- giant cells

(v) Thyroid Lymphoma (rare)

Suggested by rapid growth of a mass in an elderly patient, often with Hashimoto's thyroiditis.

Cytologic/Histologic Features suggesting this malignancy include:

- monomorphic pattern of lymphoid cells
- positive B-cell immunotyping

10. Inadequate / Nondiagnostic FNA (~ 5 to 15 %)

A cytologic diagnosis cannot be reached if there is poor specimen handling and preparation or if inadequate cellular material was obtained at the time of FNA. The principal reasons for insufficient material for diagnosis may be inexperience on the part of the physician performing the procedure, insufficient number of aspirations done during the procedure, the size of the mass, or the presence of a cystic lesion. Adequate FNA specimens are defined as containing six groups of follicular cells of 10 to 20 cells each on two different slides*(467)*. When small nodules are of concern, the repeat FNA should be done with ultrasound guidance. FNA using ultrasound guidance reduces the incidence of inadequate specimens from 15-20% down to 3-4% in such patients *(215,450,451,474,475)*. Ultrasound guided FNA is also indicated for nodules <1.5 cm, cystic (complex) nodules to assure sampling of the solid component, posterior or high substernal nodules or any nodule difficult to palpate, especially in the obese, muscular or large frame patient *(215,450,451)*. FNA should be made on dominant nodules within a multinodular goiter using ultrasound guidance in order to focus the procedure on the more clinically suspicious nodule(s).

Guideline 63. Patients with Inadequate or Non-diagnostic FNA

- Repeat FNA for small nodules often yields adequate cellular material for a diagnosis. Preferably, the repeat FNA should be done with ultrasound guidance. FNA using ultrasound guidance reduces the incidence of inadequate specimens from 15-20% down to 3-4%.
- Ultrasound guided FNA is also indicated for nodules <1.5 cm, cystic (complex) nodules to assure sampling of the solid component, posterior or high substernal nodules or any nodule greater than 1.0 cm that is

difficult to palpate, especially in the obese, muscular or large frame patient. The principal (i.e dominant) nodule(s) in a multinodular goiter should be biopsied using ultrasound guidance.