
Key Words: thyroid; fine needle aspiration; ancillary studies; immunohistochemistry; flow cytometry; molecular studies

The authors of this document comprised Committee V. The charge of this committee was to evaluate the utilization of ancillary studies in thyroid FNA. This area was further sub-divided into several discussion topics (http://thyroidfna.cancer.gov/pages/info/agenda/). This is a summary of the “Review and Conclusions” of the subject matter that this committee analyzed based on several parameters: literature reviews limited to English language publications dating back to 1995 using PubMed as the search engine with key words determined by the committee members; online forum discussions (http://thyroidfna.cancer.gov/forums/default.aspx), and formal interdisciplinary discussions held at the October 2007 conference. This is not a “standards of practice” guidelines, nor it is endorsed as such by the National Cancer Institute.

The following agenda was established to accomplish a comprehensive review of the current state of the science of the utilization of ancillary studies in thyroid FNA:

1. Indications for ancillary studies on thyroid FNAs
2. Specific ancillary studies to be performed for each indication
3. Sample preparation for each type of study

Indications for Ancillary Studies on Thyroid FNAs

The indications for ancillary studies on FNAs of the thyroid (Table I) are based largely upon the cytomorphic features of the FNA sample. Most of these ancillary studies rely on the identification of proteins specifically associated with various lesions and aim at the characterization of suspected malignancies involving the thyroid particularly medullary carcinoma, anaplastic carcinoma and lymphoma, or a metastatic carcinoma to the thyroid. Two other indications include the suspicion of a parathyroid...
lesion instead of a thyroid process and suspected cases of metastatic thyroid carcinoma to lymph node. A more controversial area involves the utilization of ancillary studies to reclassify an indeterminate/suspicious FNA into a benign or malignant category or to refine the risk of malignancy within this category. In addition, the clinical setting should also be factored into the decision of performing ancillary studies, particularly a family history of thyroid cancer, a history of other cancer, or a rapidly growing firm nodule.

Specific Ancillary Studies to be Performed for Each Indication

Ancillary studies (Table I) with the widest utility involve the detection of specific proteins using immunologic techniques, typically immunohistochemistry (IHC) on cell block preparations. IHC is commonly used for the characterization of suspected thyroid malignancies; however, little data are available comparing the sensitivity and specificity of IHC panels in large cohort studies. Immunocytochemistry on cytospins, direct smears or prefixed monolayer preparations may also be utilized, but protocols should be carefully validated for this type of specimen since reactivity may differ from histologic and cell block preparations.

The IHC panel for suspected medullary carcinoma cases should include calcitonin, thyroglobulin, carcinoembryonic antigen (CEA), and chromogranin. This panel allows for the distinction of medullary carcinoma from neoplasms derived from the follicular epithelium. In addition, clinicians should consider obtaining a serum calcitonin level since most patients with medullary carcinoma have serum calcitonin levels of $\geq 10$ pg/ml. This is particularly important if there is insufficient FNA sample for ancillary studies.1–8

IHC for suspected anaplastic carcinoma cases is not very useful since anaplastic carcinoma often lacks staining with thyroid transcription factor-1 (TTF-1) and thyroglobulin. However, positive immunoreactivity with IHC for pan-cytokeratin may be utilized to distinguish anaplastic carcinoma from sarcomas as anaplastic carcinomas often show pleomorphic cytomorphology and aggressive clinical presentation that may simulate a sarcoma. Furthermore, the clinical setting may also raise the possibility of a metastatic lesion.9–14

In cases of suspected lymphoma involving the thyroid gland, flow cytometric immunophenotyping is the standard method for the characterization of such malignancies. One challenging area is in the setting of Hashimoto’s thyroiditis where clonal B-cell lymphoid populations that have not yet or do not evolve into lymphoma may be detected.15 Not all cases of Hashimoto’s thyroiditis should be automatically sent for flow cytometric immunophenotyping. The indication for flow cytometric analysis should be based on cytomorphologic or clinical features that raise the suspicion of lymphoma. In addition, immunophenotyping results from thyroid FNA samples should be interpreted with caution since Hashimoto’s thyroiditis may yield $k/\lambda$ ratios that are skewed beyond normal values associated with reactive lymph nodes.16

IHC for TTF-1 should be the first step in suspected cases of metastatic carcinoma to the thyroid. This would

| Table I. Conclusions on the State of the Science of Ancillary Studies in Thyroid FNA Samples |
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| Indications | Ancillary study | Sample preparation |
| Suspected medullary carcinoma | • IHC panel (calcitonin, thyroglobulin, CEA, chromogranin); • Serum calcitonin | CB from FNA, preferably including at least one dedicated pass |
| Suspected anaplastic carcinoma | IHC for pan-cytokeratin | CB from FNA, preferably including at least one dedicated pass |
| Suspected lymphoma | Flow cytometric immunophenotyping | Live cells in supportive medium, preferably including at least one dedicated pass |
| Suspected metastatic carcinoma | IHC for TTF-1, expand panel if TTF-1 is negative | CB from FNA, preferably including at least one dedicated pass |
| Suspected parathyroid tissue | • IHC for TTF-1, PTH and chromogranin; • May consider PTH level on FNA sample | CB from FNA, preferably including at least one dedicated pass |
| Suspected metastatic thyroid carcinoma to lymph node | • IHC for TTF-1, thyroglobulin and calcitonin; • May consider thyroglobulin level on FNA sample | CB from FNA, preferably including at least one dedicated pass |
| Indeterminate/suspicious FNA | Insufficient evidence for either IHC or molecular techniques | No conclusion at this time |

IHC, Immunohistochemistry; CB, cell block; FNA, fine needle aspirate; CEA, carcinoembryonic antigen; TTF-1, thyroid transcription factor-1; PTH, parathyroid hormone.
limit the primary site to thyroid or lung. If necessary (negative TTF-1 staining), subsequent immunohistochemical characterization of the primary site should be guided by morphology and clinical setting. Although rare, the most common metastases to the thyroid arise from primary carcinomas of the kidney, lung, breast, colon, or malignant melanoma. The clinical history and presentation are important in determining the appropriate additional ancillary studies.

In cases of suspected parathyroid tissue, the IHC panel should contain TTF-1, parathyroid hormone (PTH), and chromogranin. This panel should distinguish thyroid tissue from parathyroid tissue. Morphologically, this distinction can be extremely difficult. Neither the IHC nor the cytomorphology should be utilized to distinguish normal from abnormal parathyroid tissue. Chemical detection of PTH levels in FNA samples has been utilized in isolated cases, and may be considered following careful assay validation.

The identification of metastatic thyroid carcinoma to a lymph node in patients with a known history of thyroid carcinoma may not require any ancillary studies. Nonetheless, in cases where IHC is deemed necessary the panel should include TTF-1, calcitonin, and thyroglobulin. A more challenging area is one of cases of a metastatic carcinoma of unknown primary site. For these cases, an IHC panel with TTF-1, calcitonin, and thyroglobulin may be useful in identifying a thyroid primary site. Studies have suggested that chemical assays for thyroglobulin on the FNA sample are useful in identifying metastatic papillary thyroid carcinoma when the cytomorphology is equivocal or nondiagnostic. Such approaches should be implemented with caution since clinical management of patients with benign or indeterminate lymph node FNAs containing detectable thyroglobulin remains undefined.

The management of patients with an indeterminate/suspicious thyroid FNA remains problematic, so ancillary studies that would permit reclassification into a benign or malignant category would benefit these patients and clarify their management. However, the utilization of either IHC or molecular techniques in this setting remains controversial. Several different molecular markers exist that have been associated with thyroid carcinomas. These include several proteins (galectin-3, Cytokeratin-19, HBME-1, thyroid peroxidase, and DAP IV), genetic mutations (BRAF, RAS), and chromosomal rearrangements (RET/PTC, PAX8/PPARG). The current focus has been on molecular markers that have proven efficacy for the stated indication, that is, the reclassification of indeterminate/suspicious FNAs based on the application of a molecular test to this type of sample. Of those, the detection of BRAF mutation appears particularly promising. This is because the V600E BRAF mutation is highly specific for papillary carcinoma, provides additional prognostic information, and can be reliably detected by various molecular techniques.

Sample Preparation for Each Type of Study (Table I)

Preanalytical sampling and processing protocols and variables are very important in the interpretation of results from any ancillary study. The relatively limited cellularity of thyroid FNAs raises challenges for any ancillary study. Also, the material obtained for cytomorphic analysis should not be compromised by the ancillary study.

For IHC analysis, most studies have utilized cell block preparations from a portion of the FNA sample. In some cases, entire passes are dedicated to the cell block preparation. Immunocytochemistry on cytospins, direct smears or prefixed monolayer preparations may also be considered, but laboratories should carefully validate their reagents and controls for these specimen types.

Flow cytometric immunophenotyping of suspected lymphoma cases requires live cells suspended in a supportive medium, preferably from at least one dedicated pass. Ancillary studies to detect genetic alterations may require dedicated passes and special processing protocols depending on the analyte (DNA or RNA) and the methodology (polymerase chain reaction, reverse transcriptase polymerase chain reaction, fluorescence in situ hybridization). Detection of RET/PTC rearrangements can be detected by reverse transcriptase polymerase chain reaction using RNA isolated from fixed FNA samples. Material from a dedicated pass requires extra effort for obtaining, but would allow reliable detection of all molecular alterations, including mutations and chromosomal rearrangements. Standardized protocols with clinical validation may be required before nucleic acid-based ancillary studies can be widely utilized as an adjunct test in thyroid FNA samples.

In summary, the most widely utilized ancillary method is IHC, performed most often on cell blocks, preferably including at least one dedicated pass. The IHC panel varies according to the suspected diagnosis based on the cytomorphic features of the FNA sample. Flow cytometric immunophenotyping on viable cells suspended in a supportive medium, also preferably including at least one dedicated pass, is the standard ancillary method in suspected cases of thyroid involvement by lymphoma. Although limited validation precludes their widespread use, these techniques are widely accepted as important tools in the management of thyroid neoplasms.
Clinical use in thyroid FNAs at this point in time, molecular studies on thyroid FNAs are likely to play an important role in the future.

References