Gender: F Age: 74
Social Security Number:
Patient ID/Case Number: 000000

Date of Procedure: 1/31/2014
Date received: 1/31/2014

Medical Imaging
Doe, John MD
1 Main Street
New York, NY 10022
(000) 000-0000

CBLPATH
A Sonic Healthcare Company

Clinical Information: Right lower pole thyroid nodule. Prior FNA Bethesda III. Do ThyroSeq.
1-FNA, Thyroid, right lower nodule (3.7 x 2.6 x 2.8 cm isoechoic, hypervascular, solid, circumscribed, hard long-standing nodule with microcalcifications that increased in size).

ADDENDUM Cytology Report

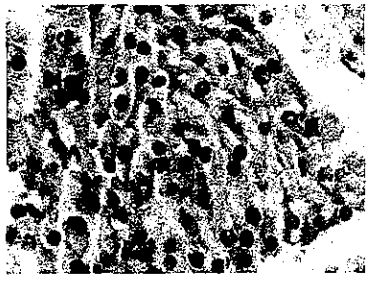
Diagnosis

Thyroid, right lower nodule: ***Hurthle cell nodule of undetermined significance (Bethesda Category III).***

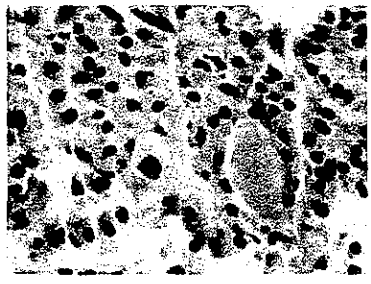
Microscopic Description: The Papanicolaou stained direct smears and the histopathologic section of the aspirated material (cell blocks) show a population of Hurthle cells with enlarged nuclei of varying in size, focally prominent nucleoli and abundant granular cytoplasm. The Hurthle cells are seen dispersed singly, in syncytial sheets and within fragments of hyperplastic epithelium in a hemorrhagic background with scant thin colloid.

Gross Description: Received labeled with the patient's full name: 6 direct smears submitted in ethanol for Papanicolaou staining (DOC); aspirated light turbid fluid submitted in ethanol for cell block preparation, designated "1A". blood clots submitted in formalin for cell block preparation, designated "1B". cds

PHOTOMICROGRAPH



Specimen 1: *Thyroid, right lower nodule*
Cell Block: Uniform Hurthle cells



Specimen 1: *Thyroid, right lower nodule*
Cell Block: Uniform Hurthle cells focally forming a microfollicle

Specimen 1: *Thyroid, right lower nodule*
PAP: Hurthle cells with nuclear size variation

Comments

The Thyroid Cancer Mutation Panel (Thyroseq) is pending and the findings will be reported separately and as an addendum.

Addendum

UPMC Presbyterian

Department of Pathology
200 Lothrop Street
Pittsburgh, PA 15213

(412) 647-3720
(412) 647-6251

Pathology Report

The following statement applies to all immunohistochemistry, insitu hybridization (ISH & FISH), molecular anatomic pathology, and immunofluorescence testing:

The testing was developed and its performance characteristics determined by the University of Pittsburgh, Department of Pathology, as required by the CLIA '88 regulations. The testing has not been cleared or approved for the specific use by the U.S. Food and Drug Administration, but the FDA has determined such approval is not necessary for clinical use. Immunohistochemical stains (where applicable) are performed with appropriate positive and negative control reactions. Immunohistochemistry assays have not been validated on decalcified tissues. Results should be interpreted with caution given the raised possibility of false negativity on decalcified specimens.

ESTROGEN/PROGESTERONE RECEPTORS (ER/PR) TEST DETAILS: Although the use of ER/PR is primarily for differential diagnostic purposes rather than therapeutic ones, we utilize the therapeutic criteria for positive and negative immunohistochemical results below: The test for the presence of hormone receptor protein is performed by the immunoperoxidase method according to the ASCO-CAP Guidelines. A positive Estrogen or Progesterone receptor tumor shows nuclear immunostaining in greater than or equal to 1% of the tumor cells (i.e. and H-score of 1 or higher). The ER and PR Histologic Score (H-Score, or HS) is calculated as the sum of intensity of staining times the proportion of cells staining and has a dynamic range of 0 to 300. The semiquantitation immunostaining raw data used to calculate the H-score is also shown above in the report. Generally, the H-score correlates to percentage of positive cells. Estrogen receptor antibody SP1, an IVD, is performed using the IVIEW detection on the Benchmark XT (Ventana, Tucson, AZ). Progesterone receptor antibody 1E2, an IVD, is performed using the IVIEW detection on the Benchmark XT, (Ventana, Tucson, AZ).

HER2 IMMUNOHISTOCHEMISTRY TEST DETAILS: 4B5 antibody clone is used as part of FDA approved Pathway on the Benchmark XT (Ventana, Tucson, AZ) and interpreted as follows: Score 0 (negative) = No staining is observed or membrane staining is observed in less than 10% of the tumor cells. Score 1+ (negative) = A faint/barely perceptible membrane staining is detected in more than 10% of the tumor cells. The cells are only stained in part of their membrane. Score 2+ (equivocal) = A weak to moderate complete membrane staining is observed in more than 10% of the tumor cells. This score requires reflex testing by FISH. Score 3+ (positive) = A strong complete membrane staining is observed in more than 30% of the tumor cells.

This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 ("CLIA") as qualified to perform high-complexity clinical testing. Pursuant to the requirements of CLIA, ASR's used in this laboratory have been established and verified for accuracy and precision. Additional information about this type of test is available upon request.

PATIENT HISTORY:

The patient is a 74 year old female.
OSS F14NY1-0030410/C14NY1-0026810 1B, 1/16/2014, FROM CBLPATH
PRE-OP DIAGNOSIS: Thyroid, Right Lower Nodule, FNA
POST-OP DIAGNOSIS: Same
PROCEDURE: FNA
clt

HISTO TISSUE SUMMARY/SLIDES REVIEWED:

Part 1: SIX (6) UNSTAINED SLIDES RECEIVED
Taken: 1/23/2014 15:10 Received: 1/23/2014 15:10

Stain/cnt	Block
IOUTTHYSEQ x 1	A
UPMC-MAP x 1	A
UPMC-MAP x 1	A
UPMC-MAP x 1	A
UPMC-MAP x 1	A
UPMC-MAP x 1	A
UPMC-MAP x 1	A

BC1
ICD-9 Diagnosis Codes: 000.

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Pathology Report

Finding *BRAF, RET/PTC* or *PAX8/PPARg* mutations in thyroid FNA samples correlates with ~99% probability of cancer and finding *RAS* mutation correlates with malignancy in 74-85% of cases (1-3). Additionally, in a limited analysis, the presence of TSHR mutations correlated with a 71% risk of malignancy (4). Mutations in the TP53, PIK3CA, CTNNB1, and AKT1 genes are found more commonly in advanced thyroid cancers and rarely in benign nodules. Mutations of the RET gene are typically present in sporadic and familial forms of medullary thyroid carcinoma.

Based on our prospective study at UPMC, risk of cancer in thyroid nodules negative for the most common mutations (BRAF, RAS, RET/PTC, PAX8/PPARg) is 6% in nodules with atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS) cytology (including the 2.3% risk of invasive cancer and 0.5% risk of cancer with extrathyroidal spread in this group), 14% in nodules with follicular neoplasm/suspicious for follicular neoplasm (FN/SFN) cytology, and 28% in nodules with suspicious for malignant cells (SMC) cytology (3). Based on the recently published study, the use of this expanded NGS-based mutation panel results in a higher rate of cancer detection in thyroid nodules and is expected to further decrease the risk of cancer in nodules negative for mutations (4).

In addition, *BRAF* V600E (T1799A) mutation has been associated with more aggressive behavior of papillary carcinoma (5) and may be used for risk stratification of patients with papillary thyroid cancer (6). In a similar way, the finding of multiple mutations and/or TP53 mutations may predict more aggressive tumor behavior (7). However, the results of molecular testing should be interpreted in combination with clinical information, imaging, and cytology.

The percent of mutant allele reflects the abundance of mutation within the targeted region. The finding of low frequency of the mutant allele (<10%) may confer a lower risk of malignancy than the risk quoted for a high frequency of mutant allele (>10%) indicated above.

1. Nikiforov YE et al. Molecular testing for mutations in improving the fine-needle aspiration diagnosis of thyroid nodules. J Clin Endocrinol Metab. 2009 Jun;94(6):2092-8.
2. Cantara S, et al. Impact of proto-oncogene mutation detection in cytological specimens from thyroid nodules improves the diagnostic accuracy of cytology. J Clin Endocrinol Metab 2010;95:1365-1389.
3. Nikiforov YE, et al. Impact of Mutational Testing on the Diagnosis and Management of Patients with Cytologically Indeterminate Thyroid Nodules: A Prospective Analysis of 1056 FNA Samples. J Clin Endocrinol Metab, 2011 95(11):3390-7.
4. Nikiforova MN, et al. Targated next-generation sequencing panel (ThyroSeq) for detection of mutations in thyroid cancer. J Clin Endocrinol Metab. 2013 98(11):E1852-60.
5. Elisei R, et al. BRAF V600E mutation and outcome of patients with papillary thyroid carcinoma: a 15-year median follow-up study. J Clin Endocrinol Metab 2008, 93:3943-3949.
6. Xing M, et al. BRAF mutation testing of thyroid fine-needle aspitation biopsy specimens for preoperative risk stratification in papillary thyroid cancer. J Clin Oncology 2010, 27:2977-82.
7. Ricarte-Filho et al. Mutational profile of advanced primary and metastatic radioactive iodine-refractory thyroid cancers reveals distinct pathogenetic roles for BRAF, PIK3CA, and AKT1. Cancer Res. 2009 Jun 1;69(11):4885-93.
8. Gupta N et al. RAS mutations in thyroid FNA specimens are highly predictive of predominantly low-risk follicular-pattern cancers. J Clin Endocrinol Metab. 2013 May;98(5):E914-22.

METHODOLOGY:
Extraction of DNA and RNA from either thyroid FNA samples collected in the nucleic acid preservative solution, frozen samples, or fixed samples was performed using standard laboratory procedure. If required, manual microdissection was performed from unstained slides under the microscope with H&E guidance. Specimens with a minimum of 50% of tumor cells or at least 300 tumor cells in a microdissection target were accepted for analysis. DNA and RNA quantity and quality was evaluated by spectrophotometric (NanoDrop) and by fluorimentric (Qubit) analysis.

Genomic DNA was used for multiplex PCR amplification of 34 amplicons, which target 284 mutations in 12 key cancer genes (AKT1, BRAF, CTNNB1, GNAS, HRAS, KRAS, NRAS, PIK3CA, PTEN, RET, TP53, TSHR), with the custom designed ThyroSeq Kit. Amplicons were barcoded, purified and ligated with specific adapters. A final check of DNA quantity and quality was performed using the Agilent 2200 TapeStation. The Ion One Touch 2 and One Touch ES were used to prepare and enrich templates and enable testing via Ion Sphere Particles on a semi conductor chip. Next generation bidirectional sequencing was performed on the Ion Torrent Personal Genome Machine and analyzed with the Torrent Suite Software v3.4.2. The full list of mutations/sequence variants can be found at www.path.upmc/divisions/map. Sequence variant details can be obtained from the Catalogue of Somatic Mutations in Cancer (COSMIC) database with the corresponding COSMIC ID http://www.sanger.ac.uk/genetics /CGP/cosmic/. DNA sequences used as references (hg19, GRCh37 Genome Reference Consortium Human Reference 37 (GCA_000001405.1))