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An academic community hospital experience using commercially available molecular testing in the management of indeterminate thyroid nodules

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Introduction Molecular thyroid testing is increasingly being used to further stratify risk of malignancy in cytologically indeterminate thyroid nodules. We report our experience using three commercially available tests in a community hospital setting.

Materials and methods All molecular test reports (Afirma, ThyroSeqV2, and ThyGENX/ThyraMIR) on thyroid nodules from Einstein Medical Center, Philadelphia, between April 2014 to March 2017 were compared with follow-up surgical results as part of a quality assurance exercise. Slides and records of disparities were reviewed.

Results Ninety-five thyroid nodules with molecular testing were identified with surgical follow up available on 19. No benign Afirma results had surgical follow-up. All 7 suspicious Afirma results had surgery, with 3 being benign on follow-up. Ten ThyroseqV2 tested nodules had follow-up surgery and included 2 papillary carcinomas following a completely negative result and another papillary carcinoma following over expression of the NIS gene reported as likely benign. One case with a TP53 mutation was benign on follow-up total thyroidectomy. Follow-up on 1 NRAS point mutation by ThyGenX/ThyraMIR was confirmed malignant although the microRNA portion of the test was negative.

Conclusions Quality assurance review refined our utilization practices as we better appreciated the limitations of molecular testing and use relative to other factors in managing indeterminate thyroid nodules.

Introduction

The introduction of fine-needle aspiration biopsy (FNAB) of thyroid nodules allows for a definitive diagnosis of benign versus malignant neoplasm in 60% to 80% of thyroid

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nodules, resulting in a significant decrease in the number of thyroidectomies being performed for benign nodules.[1] Nonetheless, atypical/indeterminate thyroid nodules still present significant diagnostic and management challenges. In The Bethesda System for Reporting Thyroid Cytopatholgy (TBSRTC), atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS) category III carries a 5% to 15% risk of malignancy.[2] and follicular neoplasm/suspicious for follicular neoplasm (FN/SFN) category IV carries a 15% to 30% risk of malignancy.[3] Therefore, molecular diagnostic testing is increasingly being used as an adjunct to further stratify risk in the management of cytologically indeterminate thyroid nodules.

To date, the most common molecular tests for thyroid commercially available include the Afirma Gene Expression Classifier (Veracyte, Inc., South San Francisco, Calif.), ThyroSeqV2 (commercially offered by CBL PATH, Rye Brook, N.Y., with test performed and interpreted at the University of Pittsburgh Medical Center), and ThyGenX/ThyraMIR (Interpace Diagnostics Group, Inc. Parsippany, N.J.).[4] The Afirma Gene Expression Classifier utilizes a proprietary RNA expression classifier based on microarray technology that is used as a "rule-out" test to identify nodules that are likely benign. ThyroSeqV2 is a next-generation sequencing-based gene mutation and fusion test that can be used as both a "rule-out" and "rule-in" test to identify nodules that are likely benign or malignant. ThyGenX/ThyraMIR is the combination of both an oncogene panel using next-generation sequencing as well as a miRNA classifier to "rule-out" and "rule-in" malignancy. We performed a retrospective quality assurance review at Einstein Medical Center, Philadelphia, an urban academic community hospital, to determine if our real world experience using all 3 of these reference tests matched our high expectations for their usefulness in managing indeterminate thyroid nodules.

Material and methods

All thyroid aspiration biopsies sent to reference laboratories for molecular testing from the Einstein Medical Center Philadelphia (EMCP) cytology laboratory during April 2014 to March 2017 were prospectively recorded in a manual log book that also included the corresponding surgical pathology follow-up as it became available. This log book was the source of the cases for retrospective quality assurance review. Discordance between molecular testing and histologic follow-up for nodules (false negative and false positive/suspicious) was investigated with further review of corresponding electronic medical records, cytology, and histology.

All cytology cases were signed out by either one of 2 board certified cytopathologists (NAY or CM) in the EMCP cytology department. Because the positive and negative predictive value of molecular testing can be influenced by the prevalence of disease in the group being tested, reports were analyzed to determine the percentage of all nodules that had an AUS/FLUS diagnosis and the follow-up malignancy rate with comparison to what is reported in the medical literature.

All thyroid FNABs were obtained under radiologic ultrasound guidance without rapid onsite evaluation. The radiologist performed on average 3 separate passes for routine cytology providing air-dried smears for Diff-Quick and fixed smears in 95% alcohol for Papanicolaou staining for each pass. The remaining contents of the syringes were flushed into buffered saline solution for preparation of a cell block. Separate passes for molecular testing were submitted in proprietary collection media provided by the corresponding reference laboratory.

Business agreements were signed with 3 separate molecular testing companies at different points during the period of this analysis. The first molecular test made available was the Afirma Gene Expression Classifier from April 2014 to November 2016, followed by the ThyroSeqV2 from March 2015 to October 2016, and then by the ThyGenX/ThyraMIR from November 2016 through the end of the study period.

The referring endocrinologist placed an order to the interventional radiologist to co-collect for Afirma during the thyroid FNAB, usually following a previous TBSRTC category III or IV diagnosis. Two separate passes from the procedure were placed in a vial containing the company's proprietary collection medium. The vial was placed directly in a −80°C freezer upon receipt of the specimen in the cytology laboratory and discarded if unused after 2 months.

Our laboratory then performed our own internal cytology assessment of the specimen, sending the vial to Veracyte for reflex testing following only an AUS/FLUS or SFN diagnosis because testing was validated only for these categories.

The cytotechnologists identified on the Afirma test requisition form the referring clinician who would be receiving the separate molecular report. Afirma reports out results for their gene expression classifier (GEC) and for a test to identify the presence of medullary thyroid cancer (MTC). Eventually BRAF testing became another option for testing but was not performed automatically and had to be specifically requested on the requisition form.

When the laboratory phased in ThyroSeqV2, the ordering, collection, and storage requirements were similar to the Afirma process, although only 1 separate pass was needed for Thyroseq as per manufacturer's instructions (instead of the 2 passes required for Afirma) and suspicious for malignancy (category V) could be tested. When the laboratory subsequently phased in ThyGenX/ThyraMIR, the only change from Thyroseq was that now samples could be stored at room temperature, facilitating a new process of co-collection on all thyroid aspirates by the interventional radiologist. Because an individual patient may not want the extra expense of molecular testing, especially if it would