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Review Article

Molecular Testing in Thyroid Evaluation of Nodules

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Abstract:

The high prevalence of thyroid nodules are due to increased availability of neck ultrasound . FNAC plays a central role to see benign and malignant nodules. About 20% of FNAC results come back as indeterminate nodule (Thy 3A/3F, Thy 4) for which diagnostic hemithyroidectomy or total thyroidectomy is done but ony 10-30% cases ultimately come back as malignant nodules after biopsy leading to unnecessary operation of 70% cases. . In these context the recent availability of molecular tests has helped to supplement malignancy risk assessment in lieu of proceeding directly with a strategy of either surveillance or diagnostic surgery. This paper aims to review and compare three main commercially available molecular cytology platforms using in Europe, and USA, Afirma GSC, Thyroseq GC, and ThyGeNEXT + ThyraMIR. Between these tests, with considerations for factors such as cost and processing time. Thyroseq GC is most commonly used provides detailed genomic information and specific management recommendations. ThyGeNEXT + ThyraMIR, though less studied, presents promising results, particularly in miRNA analysis for weak driver mutations. While molecular testing has primarily served diagnostic purposes, advancements in understanding genetic alterations now offer therapeutic implications.

Key words; *Thyroid cancer, Fine needle aspiration, indeterminate nodules, molecular cytology, mutation.*

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Introduction:

Thyroid nodules are highly prevalent and detectable in up to 25% of the general population. [1] Ultrasound scan is the first line of investigation followed by FNAC which plays a central role to see benign or malignant status of the thyroid .[2,3] .About 20% of the FNAC results come back as an indeterminate result, which includes atypia or follicular lesion of indeterminate significance (Bethesda III) (AUS/FLUS) (Thy 3A/3F) and follicular neoplasm/suspicious for follicular or oncocytic (formerly Hurthle) neoplasm (Bethesda IV)(Thy4)[4] .For these Thy 3A/3F or Thy4 our treatment guideline was to proceed diagnostic hemithyroidectomy or total thyroidectomy. But only 10 to 30% of these nodules will ultimately yield a malignant pathology [4]. This situation places significant financial strain on healthcare systems, payers, and patients alike and for unnecessary surgery [5]. For many years, there has been a significant need for accurate tools that can help identify which of these nodules carry a malignant pathology from those with indolent behavior without the need for invasive lobectomy or thyroidectomy procedures [6]. In this context molecular testing is of great importance as expanding knowledge of the genomic landscape for thyroid carcinoma has demonstrated that molecular alterations are linked to the development of tumorigenesis, histotypes with more aggressive tumor behavior, and tumor recurrence[7,8]. Several publications have suggested that the results of molecular analysis performed on FNAC guide patient management as an additional tool for diagnosis, therapy, and prognosis[9,10].

In recent years, different molecular cytology platforms have become available to help guide clinical management in these patients.

Background:

Before emergence of molecular testing ATA guideline for indeterminate nodules on FNAC was to repeat the FNA, indicate a thyroid lobectomy or continue surveillance with a follow-up ultrasound [11]. In approximately 60–70% of such instances, repeating the FNA will reclassify the nodule to a different Bethesda category, and in approximately 50%, it will result in a benign diagnosis, which has a greater NPV than molecular tests. Further strategies available to help guide management in such indeterminate cases included seeking a second cytopathology opinion and correlating the cytology findings with the sonographic characteristics of the nodule [13].

With the advent of various molecular cytology platforms becoming more readily available, the 2015 ATA management guidelines indicate that “for nodules with AUS/FLUS cytology, after consideration of worrisome clinical and sonographic features, investigations such as repeat FNA or molecular testing may be used to supplement malignancy risk assessment in lieu of proceeding directly with a strategy of either surveillance or diagnostic surgery. Informed patient preference and feasibility should be considered in clinical decision-making (weak recommendation, moderate-quality evidence). If repeat FNA cytology and/or molecular testing are not performed or are inconclusive, either surveillance or diagnostic surgical excision may be performed for an AUS/FLUS thyroid nodule, depending on clinical risk factors, sonographic pattern, and patient preference (strong recommendation, low-quality evidence)” [5].

In these guidelines, clinical judgment plays a significant role, and healthcare professionals need to consider various factors before making decisions. Some information we know regarding ultrasound or molecular profile for all thyroid nodules might not necessarily apply to the subgroup of nodules with indeterminate cytology. There are certain nuances that apply specifically to this group

that need careful consideration[8]. For instance, more than 70% of ATA high suspicion nodules on sonography end up being malignant, with most of those yielding a Bethesda category VI result on FNA; however, when we look at the nodules that have yielded a Bethesda category III result or indeterminate cytology, the predictive value of ultrasound decreases significantly. Similarly, we know that the *BRAF* V600E mutation is highly prevalent in papillary thyroid cancers, with its reported frequency varying from 30 up to 85% among studies[9]. However, when looking at the cancers with Bethesda category III and IV cytology, we find a lower prevalence of BRAF mutations and a higher presence of other mutations such as RAS mutations[10].

When considering the use of molecular testing to risk stratify a thyroid nodule with indeterminate cytology, we should aim for a test with a high negative predictive value (NPV) and high positive predictive value (PPV) to help better triage patients for thyroid surgery. Ideally, performance would be similar to a Bethesda II (benign) and Bethesda VI (malignant) cytology, where there are only 3% false positive and false negative rates [11].

Types of Molecular Tests for Indeterminate Thyroid Nodule:

There are three commercially available molecular tests in use for diagnosis of indeterminate thyroid nodule cytology:

1. Afirma GSC, which is an RNA-based genomic sequencing classifier (GSC) using gene expression to infer the malignant potential of a nodule .
2. ThyGeNext + ThyraMIR, which is a multiplatform test that involves an assessment of genetic alterations of DNA, mRNA, and gene expression regulators known as micro RNA; and
3. Thyroseq GC, a multigene genomic classifier that started as a purely DNA test . In Afirma testing lesions with >95% risk of malignancy if RET mutations found (medullary thyroid cancer), BRAF, and RET:PTC fusion for papillary thyroid cancer.The *Afirma GSC* classifier identifies about one-third of indeterminate cytology samples as suspicious, a result that carries an overall 50% risk of malignancy (ROM). Of those, 44% have an identifiable alteration (variant/fusion) that can be detected with the most recently developed Afirma Expression Atlas (XA), comprised of 593 genes, 905 variants, and 235 fusion pairs, significantly increasing their ROM and providing more granular data that can guide clinical management. 1

With literature review about two-thirds of the Afirma *GSC* tests yield a negative result. The sensitivity and NPV vary between 90 and 100%, while some studies have suggested a lower NPV [14]**.** The PPV is around 60%, which is significantly improved compared to the 40% seen in the older version of the Afirma GEC test [15].

The procedure of Thyroseq testing in its most recent version involves several steps. First, there is an assessment of DNA and RNA adequacy for testing; this is followed by a cellular composition determination, Then, next-generation sequencing (NGS) analysis is conducted for four classes of genetic alterations in 112 genes: (i) mutations (>12,000 variants), (ii) gene fusions (>150 types), (iii) copy number alterations, and (iv) gene expression alterations. The results are processed by a proprietary genomic classifier, and finally, the test result interpretation is based on a knowledge database of >3000 cases with known surgical outcomes, allowing for an assessment of cancer probability and risk of cancer recurrence [16].

Ultimately, reports of the Thyroseq test provide a negative or positive result, and the positive results provide a probability of malignancy or NIFTP (if applicable) as well as very detailed genomic information and clinical management guidance recommendations. A negative result carries a ROM of 3 to 4%, and simple observation is recommended .Nodules with RAS-like mutations or gene expression alterations (GEA) that carry a ROM of 30 to 80% are classified as positive RAS-like and recommended to undergo thyroid lobectomy. Nodules with oncocytic morphology that harbor copy number alterations (CNA) with a ROM of 40 to 80% are classified as positive Hürthle cell type with an intermediate ROM and are recommended to undergo either lobectomy or total thyroidectomy. For nodules classified as positive intermediate-risk, based on the presence of BRAF-like mutations or GEA, total thyroidectomy or lobectomy is recommended based on a ROM ranging from 95 to 100%. Lastly, for nodules classified as positive high-risk, based on mutations associated with a 98 to 100% ROM, total thyroidectomy with or without cervical lymph node dissection is recommended[1].

In *Thy*GeNext/ThyraMIR test, ThyGeNexttests for 10 gene mutations and 38 gene fusions, responsible for the most commonly occurring malignancies and ThyraMIR, tests the expression of 10 miRNA genes.[17].

ThyGeNext/ThyraMIR has a three-tier reporting system that is either negative, moderate, or positive. A study analyzing this test's performance showed that in the negative result group, only 4 out of 81 samples turned out to be malignant vs. 35 out of 47 in the positive result group. The moderate group has a risk of malignancy that is similar to the pretest risk of malignancy, and in the validation cohort, a moderate result occurred in 28% of patients. The test had a benign call rate of 46%, and positive results were found in 26%. [18].

Therapeutic Implications of molecular testing

Molecular testing in thyroid nodule FNA has been primarily used for diagnostic purposes. Because of its high negative predictive value, many surgeries can be avoided in patients with indeterminate cytology and negative molecular testing. Recent advances in our understanding of the association between genetic alterations, cancer phenotype, and risk of aggressive behavior have allowed us to use the information provided by molecular tests to further guide management in different aspects:

1. Surgical Planning: whether total thyroidectomy or lobectomy is appropriate, based on the risk of malignancy and the presence of specific genetic alterations.
2. Prognosis: Molecular testing can also provide prognostic information that helps predict the likelihood of recurrence or aggressive behavior of thyroid cancer.
3. Monitoring Response to Therapy: Molecular testing can be used to monitor response to therapy in patients with advanced thyroid cancer.
4. Hereditary Risk Assessment: Molecular tests are able to identify germline mutations associated with hereditary thyroid cancer syndromes.
5. Treatment Selection: In cases of advanced or recurrent thyroid cancer, molecular testing may inform treatment decisions, such as the selection of targeted therapies or participation in clinical trials based on the presence of specific molecular alterations.

Conclusion:

Molecular tests are valuable tools in the management of thyroid nodules with

indeterminate cytology. Testing algorithms have evolved and improved rapidly in the last few years to improve specificity and PPV while maintaining high sensitivity and NPV. Currently, there are more robust studies to support the clinical validity and utility of Afirma GSC and Thyroseq GC when compared to ThyGeNEXT + ThyraMIR. . It appears that molecular test performance is not meaningfully altered by sonographic risk and that test performance is relatively similar in nodules of various sonographic risk categories. Long-term follow-up studies of outcomes in patients undergoing molecular testing are needed, especially for nonoperated cases with negative test results.

References:

1. Hannoush ZC, Cordero RR,Jara M,Kargi AY. Current State of Molecular Cytology in Thyroid Nodules: Platforms and Their Diagnostic and Theranostic Utility. J. Clin. Med.2024 ,13(6),1759;

2. Cramer H. Fine-needle aspiration cytology of the thyroid: an appraisal. *Cancer.* 2000; 90: 325-329.

3. Gharib H, Papini E, Paschke R. Thyroid nodules: a review of current guidelines, practices and prospects. *Eur J Endocrinol*. 2008; **159**: 493-505.

4 Bongiovanni, M.; Spitale, A.; Faquin, W.C.; Mazzucchelli, L.; Baloch, Z.W. The bethesda system for reporting thyroid cytopathology: A meta-analysis. *Acta Cytol.* **2012**, *56*, 333–339.

5. Uppal, N.; Collins, R.; James, B. Thyroid nodules: Global, economic, and personal burdens. *Front. Endocrinol.* **2023**, *14*, 1113977.

6. van Kinschot, C.M.J.; Soekhai, V.R.; de Bekker-Grob, E.W.; Visser, W.E.; Peeters, R.P.; van Noord, C.; van Ginhoven, T.M. Preferences of patients, clinicians, and healthy controls for the management of a bethesda III thyroid nodule. *Head. Neck* **2023**, *45*, 1772–1781.

7. Xing M. Braf mutationin papillary thyroid cancer:pathogenic role,molecular bases, and clinical implications. *Endocr Rev.* 2007; **28**: 742-762.

8. Puxeddu E, Durante C, Avenia N, et al. Clinical implications of BRAF mutation in thyroid carcinoma. *Trends Endocrinol Metab.* 2008; **19**: 138-145.

9. Rodrigues HG, de Pontes AA, Adan LF. Use of molecular markers in samples obtained from preoperative aspiration of thyroid. *Endocr J*. 2012; **59**: 417-424.

10. Alexander EK, Kennedy GC, Baloch ZW, et al. Preoperative diagnosis of benign thyroid nodules with indeterminate cytology. *N Engl J Med.* 2012; **367**: 705-715.

11. Ravetto C, Colombo L, Dottorini ME. Usefulness of fine-needle aspiration in the diagnosis of thyroid carcinomas.A retrospective study in 37,895 patients. *Cancer Cytopathol* 2000; **90**: 357-363.

12. Poller DN, Ibrahim AK, Cummings MH, et al. Fine-needle aspiration of the thyroid. Importance of an indeterminate diagnostic category. *Cancer Cytopathol.* 2000; **90**: 239-244.

13. Krane JF, Vanderlaan PA, Faquin WC, et al. The atypia of undetermined significance/follicular lesion of undetermined significance:malignant ratio. *Cancer Cytopathol.* 2012; **120**: 111-116.

14. Olson MT, Clark DP, Erozan YS, et al. Spectrum of risk of malignancy in subcategories of "atypia of undetermined significance". *Acta Cytol.* 2011; **55**: 518-525.

15. Horne MJ, Chhieng DC, Theoharis C, et al. Thyroid follicular lesion of undetermined significance: evaluation of the risk of malignancy using the two-tier sub-classification. *Diagn Cytopathol.* 2012; **40**: 410-415.

16. Moses W, Weng J, Sansano I, et al. Molecular testing for somatic mutations improves the accuracy of thyroid fine needle aspiration biopsy. *World J Surg.* 2010; **34**: 2589-2594.

17. Radkay L, Chiosea SI, Seethala RR, et al. Thyroid nodules with KRAS mutations are different from nodules with NRAS and HRAS mutations with regard to cytopathologic and histopathologic outcome characteristics. *Cancer Cytopathol.* 2014; **122**: 873-882.

18. Nikiforov YE, Ohori P, Hodack SP, 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 Metabol*. 2011; **96**: 3390-3397.