COMMENTARY

Molecular markers guided surgical management of indeterminate thyroid nodules

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Fine needle aspiration cytology (FNAC) is considered the gold standard in preoperative settings to evaluate thyroid nodules because it is cost-effective and accurate. However, it is not able to resolve 5%-40% of cases classified as "Indeterminate" according to the Bethesda classification system 2017. The cases with indeterminate cytology (Bethesda III, IV) harbor an intermediate risk of malignancy that varies between 10% and 40% across different institutions [1]. Diagnostic lobectomy is recommended to definitely rule out malignancy in these cases. Furthermore, cases with malignant diagnostic lobectomy go through two-step surgery that is associated with additional risk and operative costs. The addition of the "rule in malignancy" test can augment FNAC to reduce the need for diagnostic lobectomies and can better define the extent of the initial surgery in a fraction of cases.

Currently, two types of molecular tests are available to evaluate thyroid nodules and are classified as the "rule in malignancy" and "rule out malignancy" tests. Traditionally, the "rule in malignancy" test uses a panel of seven genes (BRAF, N-/H-/K-RAS, RET/PTC, PAX8/PPAR-γ). Mutations in these genes are associated either with definite malignancy (100%) or confer a high risk of malignancy (70%-80%). Every gene in the panel requires exclusive interpretation, and the risk of malignancy depends upon individual genes. However, the test is expensive and requires the use of dedicated equipment like a next-generation sequencer. Furthermore, the test has an overall sensitivity of 65% and a false positive rate of 12%-13% in indeterminate cases [2]. Many institutions later limited the test to single gene BRAF(V600E) mutational analysis due to its high prevalence in Papillary thyroid carcinoma (PTC) (40%-80%) and 100% risk of malignancy. The test can be performed using a routine molecular diagnostic technique like PCR in a cost-effective manner [3,4]. Recent studies suggested the use of Telomerase Reverse Transcriptase (TERT) gene promotor mutations (C228T, C250T) in combination with other genes for thyroid cancer diagnostics due to 100% PPV for malignancy. TERT gene promotor mutations are prevalent in 7%-10% of PTC and 17% of follicular neoplasms. The presence of BRAF (V600E) and TERT promotor mutation (C228T, C250T) in malignant nodules and nonexistence in benign neoplasms make them ideal for use in thyroid diagnostic testing [5-7].

The concomitant use of BRAF and TERT gene mutations can be an effective "rule in malignancy" test to augment FNAC and a cost-effective approach in developing countries like Pakistan. The test can be performed using qPCR, ARMS-PCR, or highly sensitive technique like digital PCR. The effectiveness of this test lies in its high PPV of 100%, though the utility is limited by the lower sensitivity of 38%-50% [8]. The preoperative diagnosis of differentiated thyroid cancer in thyroid nodules with indeterminate cytology using the "rule in malignancy" test can streamline the extent of initial thyroidectomy by avoiding the performance of two-step surgery (initial surgery followed by completion thyroidectomy).

National Comprehensive Cancer Network guidelines also recommend the use of the molecular diagnostic test in cases with indeterminate cytology on FNAC. The test can also be used in cases with nodules having benign cytology but suspicious features on ultrasound or clinical examination. American Thyroid Association suggests the use of molecular tests cautiously in cases suspicious of malignancy (Bethesda V). The extent of initial surgery predominantly depends upon

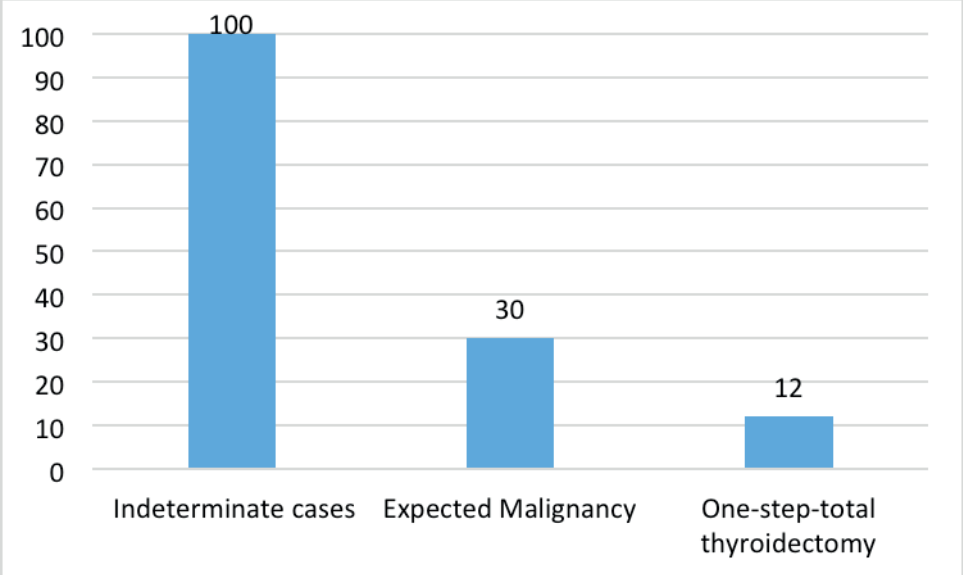

Figure 1. *Impact of molecular testing on change in surgical management.*

the clinical condition present. The risk of malignancy in these cases varies between 45% and 60%. However, in cases with a negative molecular test, the risk of malignancy may reduce by half to 28%, and thus a diagnostic lobectomy is generally reasonable. In such cases, the utility of molecular tests could improve decision-making when combined with clinical and sonographic risk factors for malignancy [1,2,9].

In order to perform BRAF and TERT genes mutational analysis, an additional FNA sample is stored in a 1.5-ml Eppendorf tube containing normal saline. The tubes can be stored at −40°C to −80°C for later use provided results are inconclusive on cytology. Alternatively, repeat FNA is recommended to perform molecular analysis when cytology is indeterminate.

The performance of molecular tests depends upon the prevalence of malignancy in a given Bethesda cytology category. The "rule in malignancy" test will perform better when the prevalence of malignancy is high. The risk of malignancy in cases of indeterminate cytology varies between 10% and 40% across different institutions and countries. The sensitivity of the assay varies between 38% and 50% depending upon the prevalence of tested mutations in a particular population. If the risk of malignancy in indeterminate cases is 30%, and assay sensitivity is 40%, then we can avoid two-stage surgery in 12 out of 30 cases as shown in Figure 1.

To summarize, molecular testing is recommended in all cases (Bethesda III, IV, V) where the plan is to perform diagnostic lobectomy to rule out malignancy. It may change initial management from two-step surgery to one-step total thyroidectomy in 40%-50% of malignant and 10%-15% of overall cases. This will ultimately translate into reduced operative costs, risks, and patient burden.

Conflict of interests
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