Thyroseq II Predicted Thyroid Cancer Rates in a Community Setting

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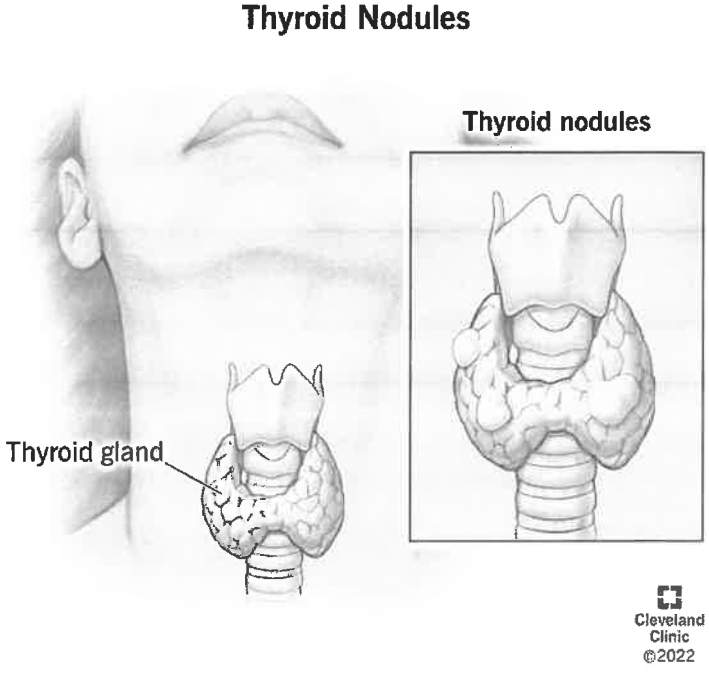
Abstract

Thyroid nodules are found in about 10% of the population. Factors such as family history, radiation exposure and functional thyroid testing are considered in initial evaluation. The nodules are biopsied based on accepted T-RAD radiological criteria. When nodules are biopsied, cytology is reported based on the Bethesda Classification which is described by 6 categories. Category III is atypia of certain significance/follicular lesion uncertain significance. Category IV is suspicious for follicular neoplasm. In the past, surgical removal was the treatment of choice, however, more recently, multiple genetics predictive tests were developed to help surgeons with operative planning. When genetic testing is positive, surgical removal is recommended based on predicted risk of malignancy. Thyroseq is a genetic test developed at the University of Pittsburgh. It has a positive predictive value of 66% and negative predictive value of 97%. This project focused on comparing the predictive value of Thyroseq in Orange County, New York patients. The hypothesis is that the clinically observed rate of malignancy in RAS + patients is lower than that predicted by Thyroseq II. It was found that the differences between the preoperative and postoperative probabilities of malignancy/NIFTP for each mutation type were statistically significant. This also potentially implies that the prevalence of disease in Orange County, New York may be different than that at University of Pittsburgh. Therefore, a more limited surgery may be offered as a treatment option.

Introduction

The Thyroid gland is located in the lower anterior neck. It produces thyroid hormones that regulate metabolism, energy levels, and other vital functions (Brent 2012). Thyroid cancer (TC) accounts for 3.1% of all new cancer diagnoses worldwide, therefore ranking in 9th position with regard to incidence (Nylén et al. 2020). Thyroid malignancy is less common in males with lower survival rates and a more aggressive presentation (Rabhari et al. 2010). Thyroid nodules (Figure 1) have prevalence of about 10%, and about 10% of those are malignant (Bomeli et al. 2010). Nodules can present with a palpable neck mass, difficulty swallowing, and neck pain.

Figure 1: Illustration of a thyroid nodule



Suspicious by radiologic criteria thyroid nodules require a biopsy. Fine Needle Aspiration Biopsies (FNA) reduce the number of thyroid surgeries on patients with benign lesions and identify patients with thyroid cancer for surgery (Wesoła et al. 2017). When nodules are biopsied, cytology is reported based on the Bethesda classification, which comprises 6 categories. Category III and Category IV are considered to be indeterminate and require further genetic testing. Category III is atypia of certain significance/follicular lesion uncertain significance and has a 15% risk for thyroid malignancy (Elomami et al. 2021). Category IV is follicular neoplasm/suspicious for follicular neoplasm and has a 15-30% risk of thyroid cancer. A lack of consistency in thyroid FNA reporting led to divergences in the calculation of the sensitivity and specificity of the method (Wesoła et al. 2017). Variation in the cancer rate in thyroid samples with Follicular Neoplasm(FN)/Suspicious for Follicular Neoplasm(SFN) cytology at different institutions may significantly affect the PPV (Positive Predictive Value) and NPV (Negative Predictive Value) of any diagnostic test (Nikiforov et al. 2014).

In a specific mutation, the RAS mutation, there are 3 proto-oncogenes–HRAS, KRAS, and NRAS–which are found in most human cancers. (Xing 2016). RAS mutations have been understood to have low diagnostic sensitivities and specificities, which can make predictions for these mutations uncertain.

There are oncocytic follicular cells of the thyroid known as Hurthle Cells. They are large, polygonal cells with marked eosinophilic, granular cytoplasm reflective of overly abundant mitochondria (Ahamdi et al. 2016). These cells can be found in both malignant and benign

nodules, and are typically not hereditary. Hurthle Cell carcinoma accounts for approximately 5% of all differentiated thyroid carcinomas. It is very difficult to distinguish between a Hurthle Cell adenoma (non-cancerous tumor) and carcinoma based on cytomorphologic features alone, and are typically noted AUS/FLUS (BIII) or FN/SFN (BIV).

Purpose

In the past, surgical removal was the treatment of choice (Welker et al. 2003), however, in the past decade, multiple genetic tests were developed to try to predict risk of malignancy (Carling et al. 2013). One of those tests is Thyroseq, developed at the University of Pittsburgh. This test has been used by the research mentor to help counsel patients regarding treatment options. This project will focus on comparing the predictive value of preoperative Thyroseq II to postoperative pathology in Orange County, New York patients. The hypothesis is that the clinically observed rate of malignancy in RAS + patients is lower than that predicted by Thyroseq II. This research has direct clinical implications that may potentially decrease the number of unnecessary surgeries.

Methodology

This project was approved by ENT and Allergy associates and Garnet Regional Medical Center IRB. After appropriate deidentification, the preoperative and postoperative pathologies of

550 nodules were reviewed. Dr. Koyfman performed 100% of the surgeries. Data was organized in Google Sheets. Bethesda III and IV categories were identified, genetic aberration documented, and final pathology was reviewed. In the cases where cytology was not provided in the database, Dr. Koyfman's personal notes and any correspondence from other physicians were reviewed.

The data from the study was obtained from the ENT and Allergy Associates database, which contained records of 550 patients who underwent thyroid surgery from 2013-2023. Cases with preoperative and postoperative probability, recorded mutation type (NRAS, KRAS, HRAS), and complete data for analysis were included. Excluded cases were either missing preoperative or postoperative probability data, missing or unspecified mutation type, incomplete data. Any outliers were assessed for accuracy and corrected if necessary. Preoperative and postoperative probabilities of malignancy were expressed as a percentages. Mutation types were categorized into NRAS, KRAS, HRAS, and other mutations. Cases with no mutation were categorized as no mutation. The dataset provided was first filtered to include only the relevant Bethesda scores (BIII and BIV with and without Hurthle Cell change) and mutations (NRAS, KRAS, HRAS), considering potential data input errors such as misspellings. Final pathologies were classified into 'benign', 'malignant', or 'NIFTP' (Non-invasive follicular thyroid neoplasm with papillary-like nuclear features), with 'malignant' encompassing all non-benign or non-NIFTP diagnoses. The preoperative probability was extracted from Thyroseq 2 reports and averaged. Individual NRAS, HRAS, KRAS mutation subsets were combined into 3 categories: HRAS, NRAS and KRAS.

Statistical analysis was performed including means, standard deviations, and frequency distributions. These were calculated for preoperative and postoperative probabilities, as well as mutation types. Independent t-tests were used in comparing preoperative and postoperative probabilities for each mutation type separately. Assumptions for t-tests were assessed and met. The p-values from the t-tests were used to understand statistically significant differences between preoperative and postoperative probabilities for each mutation type.

Results

With respect to the entire dataset postoperatively: 61.82% of the nodules were benign, 34.74% were malignant, and 3.6% were NIFTP. Considering BIII, BIV, BIII Hurthle, and BIV Hurthle, there were a total of 172 cases. Out of those cases, 60.47% were benign, 31.4% malignant, and 5.23% were NIFTP. No preoperative Hurthle BIII nor Hurthle BIV were malignant in the final pathology.

The following data was structured to compare the average preoperative probability provided by Thyroseq for a given Bethesda category or mutation and the postoperative probability based on the results of the data. Table 1 reflects a comparison between preoperative and postoperative probability based on mutation type and p-value.

Table 1

RAS Mutation Type	Preoperative Probability of Malignancy/NIFTP	Postoperative Probability of Malignancy/NIFTP	P-value <0.05
NRAS	65%	14.6%	1.17×10−14
KRAS	57.86%	37.5%	0.0045
HRAS	72%	14.29%	5.49×10−19

The postoperative risk of malignancy/NIFTP for all mutations fell within the 95% confidence interval for KRAS and HRAS mutation. The NRAS fell short of the confidence interval. The P-values for NRAS, KRAS, and HRAS were <0.05 respectively. Since these p-values are <0.05, the differences between the preoperative and postoperative probabilities of malignancy/NIFTP for each mutation type were statistically significant. 11% of NRAS was associated with NIFTP diagnosis. 36.7% of KRAS mutations were associated with NIFTP. 11.8% of HRAS was associated with NIFTP diagnosis.

Table 2 reflects prevalence of mutation with benign and malignant/NIFTP diagnosis.

Table 2

Mutation	Benign	Malignant/ NIFTP	
NRAS	63.89%	25%	11.1%
KRAS	45.45%	27.27%	36.36%
HRAS	70.59%	17.75%	11.76%

Discussion

These results clearly indicate that Thyroseq II overestimates malignant NIFP predictive value in our community setting. This also potentially implies that the prevalence of disease in Orange County, New York may be different than that at University of Pittsburgh. Among the mutations studied, NRAS mutations were the most prevalent (n=36) with 63.89% benign, 25% malignant and 11.1% percent benign. KRAS mutation subset (n=11) 45.0% of benign cases and 27.27% malignant and 36.36% NIFTP cases. The HRAS mutations (n=17) had 70.59% benign, 17.75% malignant and 11.76% NIFTP. NRAS mutation and HRAS mutation appear to be more benign than malignant. There was a nearly even distribution of benign vs malignant/NIFTP pathology in KRAS disease. However, the majority of KRAS have a larger percentage of NIFTP compared to malignant pathology. The preoperative and postoperative probabilities for each mutation type was performed. The results indicated that there is a statistically significant difference in probabilities of malignancy between the preoperative (Thyroseq II) and

postoperative (surgical) pathology for any of the mutation types (NRAS, KRAS, HRAS). This suggests that the predictive value of Thyroseq II is overestimated in the community setting.

There were also a variety of other mutations associated with malignancy, with BRAF V600E being most notable.

Conclusion

The hypothesis that the rate of malignancy in RAS + patients in Orange County, New York is lower than that predicted by Thyroseq II may be true. This can result in a higher rate of partial thyroid surgery vs total thyroidectomy, potentially sparing a patient surgical complications and medications use. Overall, it is important to notice that when divided into malignant vs NIFTP, the true risk of malignancy across all 3 mutations is <30%. The NRAS and HRAS mutations in our sample size were present in a significant number of benign cases which may help counsel patients with those mutations toward a diagnostic lobectomy vs upfront total thyroidectomy. Although the NRAS mutation fell below the confidence interval there appears to be a possible trend toward a lower risk of cancer than that reported by Thyroseq II for the NRAS mutation category. Since the treatment of choice for NIFTP is a lobectomy, it is very evident that a lobectomy could be a treatment of choice for close to 80% of patients with BIII and BIV pathology. A lobectomy could be a preferred treatment for a KRAS mutation as well (combining the 45.45% benign rate with 36.36% NIFTP rate)

There were some notable limitations in this study. There is a possibility for a bias in the dataset based on the fact that all of these surgeries were completed by only one surgeon. An average preoperative probability was used in mutation analysis. Perhaps, if larger numbers of cases are analyzed, individual mutation subsets can be further risk stratified. The NRAS mutation confidence interval fell below the expected range which may require more data points. There is a significant number of cases that are referred out of the county. Additionally, a larger sample size could be used to reduce the possibilities of outliers affecting the results. Finally, the dataset used in this study might not have captured all relevant factors that influence disease outcomes and surgical removals.

The study proves that potentially Thyroseq may be overestimating malignancy and NIFTP in RAS + conditions. The potential implications may be that a more limited surgery may be offered to the patients since up to 80% of the cases could be treated with limited thyroid surgery (lobectomy). Further research is needed with larger datasets.

Future Research

In the future, a similar study should be conducted across multiple zip codes to validate Thyroseq II across a variety of populations.

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