Title

Patient Preference in Physician Decision-Making for Patients With Low- to Intermediate-Risk Differentiated Thyroid Cancer

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Prognostic Value of Preoperative Molecular Testing and Implications for Initial Surgical Management in Thyroid Nodules Harboring Suspected (Bethesda V) or Known (Bethesda VI) Papillary Thyroid Cancer

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IMPORTANCE Molecular testing is commonly used in the diagnosis of thyroid nodules with indeterminate cytology. The role of molecular testing in prognosticating oncologic outcomes in thyroid nodules with suspicious or malignant cytology is unclear.

OBJECTIVE To determine whether molecular profiling of Bethesda V (suspicious for thyroid cancer) and VI (thyroid cancer) nodules is associated with improved prognostication and whether it may inform initial treatment.

DESIGN, SETTING, AND PARTICIPANTS This retrospective cohort study included consecutive patients with Bethesda V or VI nodules who underwent surgery, with histopathology showing differentiated thyroid cancer, between May 1, 2016, and July 31, 2019 in the University of California, Los Angeles health system. Data were analyzed between April 2, 2021, and January 18, 2023.

EXPOSURES Masked ThyroSeq, version 3 molecular analysis after completion of initial treatment and acquisition of follow-up data.

MAIN OUTCOMES AND MEASURES Structural disease persistence or recurrence, distant metastasis, and recurrence-free survival were assessed using ThyroSeq Cancer Risk Classifier (CRC) molecular risk groups (low, *RAS*-like; intermediate, *BRAF*-like; high, combination of *BRAF*/*RAS* plus *TERT* or other high-risk alterations) using Cox proportional hazards regression models.

RESULTS In 105 patients with papillary thyroid cancer (median [IQR] follow-up, 3.8 [3.0-4.7] years), ThyroSeq identified genomic alterations in 100 (95%) samples (6 [6%] low risk, 88 [88%] intermediate risk, and 6 [6%] high risk; median [IQR] age, 44 [34-56] years; 68 [68%] female and 32 [32%] male). No patients with low-risk or negative results experienced recurrence. Of the 88 patients with intermediate risk, 6 (7%) experienced local recurrence, with 1 of them also developing distant metastasis. The 6 patients with high risk (all with *BRAF* V600E plus *TERT* mutation) underwent total thyroidectomy followed by radioactive iodine (RAI) ablation. Four patients with high risk (67%) experienced local recurrence, with 3 of them also developing distant metastasis. Thus, patients with high-risk alterations were more likely to experience persistence or recurrence and distant metastasis than patients with intermediate risk. In a multivariable analysis incorporating patient age, sex, cancer size, ThyroSeq molecular risk group, extrathyroidal extension, lymph node positivity, American Thyroid Association risk, and RAI ablation, only cancer size (hazard ratio, 1.36; 95% CI, 1.02-1.80) and ThyroSeq CRC molecular risk group (high vs intermediate and low: hazard ratio, 6.22; 95% CI, 1.04-37.36) were associated with structural recurrence.

CONCLUSIONS AND RELEVANCE Among the 6% of patients with high-risk ThyroSeq CRC alterations in this cohort study, the majority experienced recurrence or distant metastasis despite initial treatment with total thyroidectomy and RAI ablation. In contrast, patients with low- and intermediate-risk alterations had a low recurrence rate. Preoperative knowledge of molecular alteration status at diagnosis may allow for deescalation of initial surgery and refining of the intensity of postoperative surveillance in patients presenting with Bethesda V and VI thyroid nodules.

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Supplemental content

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Molecular testing is now commonly used in the diagnosis of thyroid nodules with Bethesda III (atypia of undetermined significant or follicular lesion of undetermined significance) and Bethesda IV (follicular neoplasm or suspicious for follicular neoplasm) cytopathology.[1-6] By detecting point mutations, changes in gene expression, copy number alterations (CNAs), and fusions, molecular analysis has been increasingly applied to more accurately diagnose papillary, follicular, and Hurthle cell carcinomas while also identifying a small proportion of aggressive cancers amenable to new targeted therapies.[3,7] In contrast, reports on the performance of molecular testing in Bethesda V (suspicious for thyroid cancer) and Bethesda VI (thyroid cancer) nodules are limited. The high risk of thyroid cancer associated with Bethesda V and VI nodules, which exceeds 50%,[8] justifies a clinical decision to proceed with surgery based on cytopathology alone, without the need for further diagnostic information.

However, preoperative molecular testing may also help to prognosticate oncologic outcomes by identifying indolent vs aggressive tumors, which may inform initial surgical management, the decision to pursue radioactive iodine (RAI) ablation, and the intensity of biochemical and radiographic surveillance in the early postoperative period. Recent reports have shown that thyroid cancers with high-risk molecular profiles may display aggressive pathologic features, recur locally, and give rise to distant metastases.[9,10]

In this study, stored samples from Bethesda V and VI nodules obtained at the time of initial fine needle aspiration (FNA) underwent masked molecular analysis, with results then correlated retrospectively with clinical follow-up data to investigate the role of molecular testing for cancer prognosis. The aim of the study was to examine the association between genetic alterations and rates of structural disease recurrence and distant metastasis in patients suspected or known preoperatively to have differentiated thyroid cancer (DTC).

Key Points

Question Is preoperative comprehensive molecular profiling associated with improved prognostication of Bethesda V and VI thyroid nodules with differentiated thyroid cancer, and how does it inform initial surgical treatment?

Findings In this cohort study of 105 consecutive patients with papillary thyroid cancer, those with high-risk molecular profiles at diagnosis were more likely to experience cancer persistence or recurrence and distance metastases and had worse 36-month recurrence-free survival than those with intermediate- and low-risk molecular profiles.

Meaning These findings suggest that preoperative knowledge of molecular profiling data could inform initial treatment in patients with suspected or known papillary thyroid cancer.

Methods

This retrospective cohort study was performed on consecutive patients with thyroid nodules who underwent FNA throughout the University of California, Los Angeles health system from May 1, 2016, to July 31, 2019. Our institution routinely performs an extra pass to collect a sample for molecular testing during all thyroid FNAs.[1,11] These specimens are reflexively sent for molecular testing in the event of a Bethesda III or IV result, leaving the remaining samples preserved at the time of initial FNA and available for future use.

Patients with Bethesda V or VI cytology results who underwent surgery with confirmation of DTC (papillary thyroid cancer [PTC], follicular thyroid cancer, or Hurthle cell thyroid cancer) on surgical pathology were included. Patients were excluded for the following conditions: anaplastic or poorly differentiated thyroid cancer; medullary thyroid cancer; Bethesda I, II, III, or IV cytology; nonoperative treatment; and lack of postoperative follow-up data. One patient with an initial diagnosis of PTC on surgical pathology was found to have a *PAX8*/*GLIS3* fusion characteristic of hyalinizing trabecular tumor,[12] which was later confirmed on consensus pathology review of histology; this patient was notified of their new diagnosis and excluded from our analysis. The University of California, Los Angeles institutional review board approved this study with a waiver of informed consent because the study was retrospective and deemed minimal risk; furthermore, the biopsy process and subsequent tests were all part of the standard of care. This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline.

Patient demographic and clinical variables were reviewed, including largest nodule diameter and Bethesda diagnostic category. Race and ethnicity data were not collected, as these factors are not used in common risk prediction systems for DTC. Operation details were reviewed for procedure type (thyroid lobectomy vs total thyroidectomy with or without central or lateral neck lymph node dissection) and indication for total thyroidectomy when performed. In patients who underwent thyroid lobectomy, completion thyroidectomy was also documented. Surgical pathology was assessed for tumor size, histology, multifocality, margin status, presence of extrathyroidal extension (ETE) or angioinvasion, and lymph node positivity. As molecular testing was not obtained preoperatively, pathologists examining surgical specimens were necessarily blinded to molecular testing results. Histopathologic data were used to classify patients into risk of structural disease recurrence categories based on the 2015 American Thyroid Association (ATA) thyroid cancer guidelines.[13]

ThyroSeq Molecular Testing

Samples from Bethesda V and VI nodules were retrospectively analyzed using ThyroSeq, version 3 (Sonic Healthcare USA) molecular testing at the University of Pittsburgh Medical Center Molecular and Genomic Pathology Laboratory as previously described.[7] Samples had been collected at the time of initial FNA and were stored in the cytopathology department at −80 °C. Samples sent for ThyroSeq testing were coded, and analysis was performed while masked to oncologic outcomes. Clinical management was performed without knowledge of molecular testing results, as the latter only became

available years after surgery. Molecular testing results were then correlated with clinical follow-up data available to date.

ThyroSeq was developed using next-generation sequencing to identify point mutations and gene fusions commonly found in thyroid cancer. ThyroSeq, version 3 detects an expanded spectrum of genetic alterations, including 12 135 single-nucleotide variants and insertions and deletions in 112 genes, 120 gene fusions, 19 gene expression alterations, and 10 CNAs. Evaluation of genetic alterations has led to the establishment of molecular risk groups (MRGs), which are based on the probability of disease aggressiveness.[9] ThyroSeq Cancer Risk Classifier results were risk stratified as follows: negative, no detectable genetic alteration; low-risk MRG, *RAS* and *RAS*-like alterations including *PPARG* and *THADA* fusions, and focal chromosomal type CNA present as the only event; intermediate-risk MRG, *BRAF* V600E, other *BRAF*-like alterations including receptor tyrosine kinase fusions, and CNA of genome haploidization type; and high-risk MRG, presence of an early alteration and a late-hit alteration including *TERT*, *TP53*, *AKT1*, and *PIK3CA* (ie, combination *BRAF*/*RAS* plus *TERT* or other high-risk alterations).

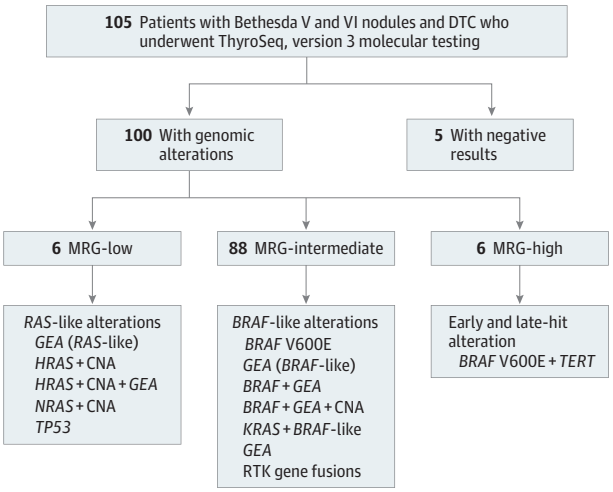
Outcomes

Serum thyroid-stimulating hormone, thyroglobulin, and thyroglobulin antibody levels obtained during follow-up were documented and generally measured every 6 months. Most patients received annual surveillance neck ultrasonography, while repeat whole-body scanning was performed for rising serum thyroglobulin levels at the discretion of the treating physician. Biochemical and structural disease persistence or recurrence and distant metastasis were assessed according to the 2015 ATA guidelines.[13] Structural disease recurrence and distant metastasis were assessed by examining pathologic sodium iodide I 131 uptake on whole-body scanning, when applicable; positive surveillance neck ultrasonography or computed tomography scan findings; or cytologic confirmation by FNA biopsy. Biochemical recurrence was only considered in patients without evidence of structural recurrence. All-cause mortality was also assessed.

Statistical Analysis

Standardized differences were calculated for demographic and clinical variables in pairwise comparisons among patients in each MRG (high vs intermediate/low and high vs intermediate). Univariable Cox proportional hazards regression models were developed to evaluate factors associated with structural recurrence. Kaplan-Meier analysis and multivariable Cox proportional hazards regression models were used to evaluate the association of MRGs with recurrence-free survival (RFS). A forward stepwise model selection was performed with the Cox model to estimate structural recurrence.[14] The initial model included age, sex, and tumor size. Preoperative, histopathologic, and postoperative factors, including MRG, ETE, lymph node positivity, ATA risk of recurrence, and RAI ablation, were selected as the candidate variables added to the model based on Akaike information criterion. Because of the low rate of distant metastasis, no regression analysis was performed for this outcome. Recurrence-free survival was calculated from the time of surgery to structural recurrence. Data analyses were performed between April 2, 2021, and January 18, 2023, using R, version 3.6.1 statistical software (R Foundation for Statistical Computing).

Figure 1. Study Flowchart of Patients With Bethesda V and VI Nodules and Papillary Thyroid Cancer Who Underwent Retrospective ThyroSeq, Version 3 Molecular Testing



ThyroSeq results were risk stratified into molecular risk groups (MRGs) as discussed in the Methods. CNA indicates copy number alteration; DTC, differentiated thyroid cancer; and RTK, receptor tyrosine kinase.

Results

Among 105 eligible adult patients with DTC who underwent resection, ThyroSeq, version 3 identified genomic alterations in 100 samples (95%) as follows: 6 (6%) MRG-low, 88 (88%) MRG-intermediate, and 6 (6%) MRG-high (all *BRAF* V600E plus *TERT*) (**Figure 1**). Among the 100 patients with positive ThyroSeq results, the median patient age was 44 years (IQR, 34-56 years), 68 (68%) were female, 32 (32%) were male, 85 (85%) had Bethesda VI cytology, and median follow-up was 3.8 years (IQR, 3.0-4.7 years) (**Table 1**). The median tumor size was 1.6 cm (IQR, 1.1-2.2 cm), with all patients having PTC. Based on the ATA risk stratification, 41 patients (41%) were at low risk, 51 (51%) were at intermediate risk, and 8 (8%) were at high risk for recurrence. Thyroid lobectomy was performed in 19 patients (19%), of whom 4 (21%) underwent completion thyroidectomy, and total thyroidectomy was performed in the remaining 81 (81%). Thirty-nine patients (48%) underwent concurrent lymph node dissection (either central, lateral, or both). Indications for total thyroidectomy included intraoperative discovery of known or suspicious abnormal lymph nodes or ETE in 40 patients (49%); preoperative known or suspicious abnormal lymph nodes or ETE in 13 patients (16%); nodule size greater than 4 cm in 6 patients (7%); and the presence of contralateral nodules, concurrent hypothyroidism or levothyroxine use, or patient preference in 27 patients (33%). Radioactive iodine ablation was performed in 58 patients (58%).

Table 1. Demographic Characteristics, Surgical Details, and Histology of Patients With Bethesda V and VI Nodules and Papillary Thyroid Cancer Based on ThyroSeq Molecular Risk Groups (MRGs)

Characteristic	No. (%)				Standardized difference (high vs low and intermediate)[a]	Standardized difference (high vs intermediate[a]
	Total (n = 100)	MRG-low (n = 6)	MRG-intermediate (n = 88)	MRG-high (n = 6)		
Age, y, median (IQR)	44 (34-56)	50 (42-65)	42 (33-54)	70 (65-77)	1.65	1.69
Sex						
Female	68 (68)	5 (83)	61 (69)	2 (33)	0.79	0.77
Male	32 (32)	1 (17)	27 (31)	4 (67)		
Bethesda cytology						
V (suspicious for thyroid cancer)	15 (15)	4 (67)	11(12)	0	0.62	0.54
VI (thyroid cancer)	85 (85)	2 (33)	77 (88)	6 (100)		
Lobectomy	19 (19)	3 (50)	16 (18)	0	0.71	0.67
Total thyroidectomy	42 (42)	1 (17)	38 (43)	3 (50)	0.17	0.14
Total thyroidectomy and LND	39 (39)	2 (33)	34 (39)	3 (50)	0.24	0.23
Variant						
Classical	74 (74)	2 (33)	69 (78)	3 (50)	0.01	0.02
Follicular	6 (6)	1 (17)	5 (6)	0	0.40	0.38
Classical/follicular	5 (5)	3 (50)	2 (2)	0	0.26	0.22
Tall cell	10 (10)	0	7 (8)	3 (50)	0.46	0.45
Other[b]	5 (5)	0	5 (6)	0	0.34	0.35
Tumor size, cm, median (IQR)	1.6 (1.1-2.2)	1.4 (0.8-1.8)	1.5 (1.1-2.1)	2.6 (2.0-5.2)	1.03	1.03
Capsular invasion	9 (9)	1 (17)	8 (9)	0	0.46	0.45
Extrathyroidal extension	20 (20)	0	14 (16)	6 (100)		
Microscopic	12 (60)	0	9 (64)	3 (50)	3.36	3.23
Gross	8 (40)	0	5 (36)	3 (50)		
Positive margins	18 (18)	1 (17)	12 (14)	5 (83)	1.94	1.95
Angioinvasion	2 (2)	0	2 (2)	0	0.34	0.35
Positive lymph nodes	41 (41)	1 (17)	36 (41)	4 (67)	0.57	0.54
ATA risk category						
Low	41 (41)	4 (67)	37 (42)	0	1.65	1.61
Intermediate	51 (51)	2 (33)	46 (52)	3 (50)		
High	8 (8)	0	5 (6)	3 (50)		

Abbreviations: ATA, American Thyroid Association; LND, lymph node dissection (either central, lateral, or both).

[a] Standardized difference is the difference in means or proportions divided by the SE. An absolute value of 0.20 is considered a small effect size, 0.50 a medium effect size, and 0.80 a large effect size.

[b] Other is oncocytic or Hurthle cell, hobnail, and diffuse sclerosing.

In 5 patients (5%), ThyroSeq, version 3 results were negative for cancer-associated molecular alterations. The median age of these patients was 57 years (IQR, 56-59 years), 4 were female (80%), 1 was male (20%), 3 (60%) had Bethesda VI cytology, and median follow-up was 5.0 years (IQR, 2.0-5.3 years).

Molecular Risk Groups

An increasing proportion of tumors with established pathologic features associated with more aggressive disease behavior was observed with each step increase in MRG, including median primary tumor size, ETE, and positive surgical margins (Table 1). Additionally, preoperative MRG determined by ThyroSeq aligned closely with the ATA risk of recurrence, a well-validated prognostic system derived largely from surgical pathology findings.[13]

Among the 6 patients with MRG-low profiles, 3 underwent thyroid lobectomy and 3 underwent total thyroidectomy followed by RAI ablation. A majority of MRG-low tumors had *RAS*-like alterations (4 [67%]) and were follicular variants of PTC on histology (4 [67%]). The median tumor size was 1.4 cm (IQR, 0.8-1.8 cm); 0 patients had ETE, 1 (14%) had positive margins, 1 (14%) had positive lymph nodes, and 0 were classified as ATA high risk. No patients with MRG-low profiles experienced local disease persistence or recurrence or developed distant metastasis (**Table 2**).

In the 88 patients with MRG-intermediate profiles, 16 (18%) underwent thyroid lobectomy, while the remaining 72 (82%) underwent total thyroidectomy, with 49 (68%) receiving RAI ablation. Completion thyroidectomy was performed in 4 MRG-intermediate patients who underwent lobectomy (25%): 1 for a contralateral nodule with malignant cytology, 1 for tall cell component (20%-30%; no angioinvasion), 1 for RAI administration due to tumor invasion of the recurrent laryngeal nerve with tumor left on the nerve, and 1 for recurrent hyperparathyroidism. Among the 35 patients (49%) who underwent total thyroidectomy for nononcologic reasons (did not have

Table 2. Oncologic Outcomes of Patients With Bethesda V and VI Nodules and Papillary Thyroid Cancer Based on ThyroSeq Molecular Risk Groups (MRGs)

Outcome	No. (% of category)				Standardized difference (high vs intermediate and low)[a]	Standardized difference (high vs intermediate)[a]
	Total (n = 100)	MRG-low (n = 6)	MRG-intermediate (n = 88)	MRG-high (n = 6)		
Completion thyroidectomy	4 (21)	0	4 (25)	0	0.30	0.31
Malignant tumor in contralateral lobe	1 (25)	0	1 (25)	0		
Radioiodine ablation	58 (59)	3 (50)	49 (68)[b]	6 (100)	1.17	1.15
Whole-body scan results						
Negative	13 (22)	1 (33)	11 (22)	1 (17)	0.46	0.45
Remnant thyroid or neck	44 (75)	2 (67)	38 (76)	4 (67)		
Distant	2 (3)	0	1 (2)	1 (17)		
Locoregional recurrence	10 (10)	0	6 (7)	4 (67)		
Lateral neck	8 (80)	0	4 (67)	4 (100)	1.00	1.00
Central neck	2 (20)	0	2 (33)	0		
Distant metastases	4 (4)	0	1 (1)	3 (50)		
Pulmonary	3 (75)	0	1 (100)	2 (67)	1.00	1.00
Osseous	1 (25)	0	0	1 (33)		
Follow-up duration, y, median (IQR)	3.8 (3.0-4.7)	4.1 (3.5-5.3)	3.8 (3.5-4.5)	4.6 (4.0-5.3)	0.23	0.18

[a] Standardized difference is difference in means or proportions divided by the SE. An absolute value of 0.20 is considered a small effect size, 0.50 a medium effect size, and 0.80 a large effect size.

[b] Out of 72 patients who had a total thyroidectomy who received radioiodine ablation.

abnormal lymph nodes or gross ETE), completion thyroidectomy would have been indicated in 14 (40%) based on histopathology of just the tumor side.

A *BRAF* V600E mutation was present in 77 patients (88%), and the most common histology was classic PTC. The median tumor size was 1.5 cm (IQR, 1.1-2.1 cm); 14 patients (16%) had ETE (5 [36%] gross), 12 (14%) had positive margins, 36 (41%) had positive lymph nodes, and 5 (6%) were classified as ATA high risk. Among the MRG-intermediate patients, 6 (7%) experienced local disease persistence or recurrence (Table 2). One of these patients (1%), who had a combination *BRAF* V600E and *GEA* alteration and initially presented with a 9.4-cm classic PTC, developed distant metastasis to the lungs. All 10 patients with targetable fusions involving tyrosine kinase receptors were in the MRG-intermediate group (7 with *RET*, 2 with *ALK*, and 1 with *NTRK3*).

The 6 patients with MRG-high profiles underwent total thyroidectomy followed by RAI ablation. All patients had combination *BRAF* V600E plus *TERT* alterations. The MRG-high group included tall cell variant PTC (3 [50%]) and classic PTC (3 [50%]). The median tumor size was 2.6 cm (IQR, 2.0-5.2 cm); all had ETE (3 [50%] gross), 5 (83%) had positive margins, 4 (67%) had positive lymph nodes, and 3 (50%) were classified as ATA high risk. Four high-risk patients (67%) experienced local disease persistence or recurrence (between 1.5 and 4.0 years postoperatively), with 3 of the 6 (50%) also developing distant metastasis (2 pulmonary and 1 bone) (Table 2).

All 5 patients with no molecular alterations identified by ThyroSeq had PTC. The median tumor size was 1.2 cm (IQR, 0.6-1.5 cm), 2 patients (40%) had microscopic ETE, 0 had positive margins, 1 (20%) had positive lymph nodes, and 0 were classified as ATA high risk.

Disease Recurrence

Structural disease recurrence was observed in 10 patients (10%), all of whom had *BRAF* V600E mutations. Isolated biochemical recurrence occurred in 1 MRG-intermediate patient (1.0%). Recurrent structural disease was diagnosed at a median of 2.0 years (IQR, 1.0-2.7 years) postoperatively. Patients with vs without recurrence were more likely to be male (7 [70%] vs 26 [27%]; hazard ratio [HR], 4.71; 95% CI, 1.22-18.24), to have larger tumors (median, 2.4 [IQR, 2.2-5.3] vs 1.5 [IQR, 1.0-2.0] cm; HR, 1.54; 95% CI, 1.23-1.93), to have ETE (6 [60%] vs 16 [17%], HR, 5.54; 95% CI, 1.56-19.65), and to be classified as ATA high risk (4 [40%] vs 4 [4%]; HR, 3.48; 95% CI, 1.33-9.16). In MRG-intermediate patients, structural recurrence was more common in males (4 [67%] vs 23 [28%]; HR, 4.24; 95% CI, 0.77-23.18) and in those with larger tumors (median, 2.3 [IQR, 1.3-2.4] vs 1.5 [IQR, 1.1-2.1] cm; HR, 1.38; 95% CI, 1.02-1.85). No biochemical or structural recurrences occurred in the 5 patients with ThyroSeq-negative results.

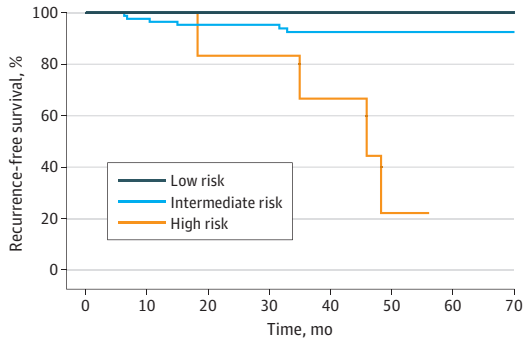
Patients in the MRG-high group were more likely to experience structural recurrence and distant metastasis than those in the MRG-intermediate group (4 [67%] vs 6 [7%]; HR, 9.31; 95% CI, 2.62-32.91 and 3 [50%] vs 1 [1%]; HR, 42.7; 95% CI, 4.41-411.9, respectively) (Table 2). In the base model multivariable analysis incorporating patient age, sex, and cancer size, cancer size (HR, 1.41; 95% CI, 1.09-1.78) was associated with structural recurrence. After forward selection of variables, including ThyroSeq MRG, ETE, lymph node positivity, ATA risk of recurrence, and RAI ablation, only cancer size (HR, 1.36; 95% CI, 1.02-1.80) and MRG (high vs intermediate and low: HR, 6.22; 95% CI, 1.04-37.36) were associated with structural recurrence (**Table 3**). Regression analysis was not performed against the MRG-low group, as there was no recurrence or distant metastasis in this group.

Table 3. Multivariable Analysis Identifying Factors Associated With Structural Recurrence

Variable	HR (95% CI)
Age, y	0.99 (0.94-1.03)
Sex, male vs female	2.21 (0.47-10.46)
Tumor size, cm	1.36 (1.02-1.80)
MRG-high vs MRG-intermediate and MRG-low	6.22 (1.04-37.36)

Abbreviations: HR, hazard ratio; MRG, molecular risk group.

Figure 2. Recurrence-Free Survival Among Patients With Bethesda V and VI Nodules and Papillary Thyroid Cancer Based on ThyroSeq, Version 3 Molecular Risk Groups



No. at risk								
Low risk	6	6	6	6	5	4	2	2
Intermediate risk	88	86	84	74	56	35	18	4
High risk	6	6	6	5	2	1	0	0

Recurrence-Free Survival

Figure 2 details RFS associated with each MRG. Estimated 36-month RFS was highest in the MRG-low group (100%), decreased slightly in MRG-intermediate group (93%), and was lowest in MRG-high group (50%). One patient (1%) with an intermediate-risk profile died during the study of a cause unrelated to their thyroid cancer.

Discussion

In this cohort study of 105 consecutive patients with Bethesda V and VI cytology and PTC on surgical pathology, the prognostic value of genetic alterations was evaluated with ThyroSeq, version 3 molecular analysis after completion of initial management and the acquisition of follow-up data over 3.8 years. We observed that 6% of patients harbored MRG-high profiles (all *BRAF* V600E plus *TERT*), with the majority experiencing structural disease recurrence or distant metastasis despite initial treatment with total thyroidectomy and RAI ablation. In contrast, the rate of disease recurrence and distant metastasis in patients with MRG-intermediate and MRG-low cancers was low. Moreover, MRG-high patients experienced worse RFS. These results suggest that preoperative knowledge of molecular profiling data may inform initial surgical treatment in patients with suspected or known PTC.

Molecular risk groups in DTC, first described by Yip et al[9] in 2021, are based on known associations of molecular alterations with the probability of developing distant metastases. Tumors displaying aggressive clinical behavior were shown to harbor additional late-hit driver alterations, including *TERT*, *TP53*, *PIK3CA*, and *AKT1*, in addition to a primary *BRAF* or *RAS* mutation. In this case-control study by Yip et al, two-thirds of the 62 patients with DTC and distant metastasis had high-risk molecular profiles, which were strongly associated with distant metastasis compared with the intermediate- and low-risk groups. In our study, 75% of patients with distant metastasis were classified as MRG-high, a group we similarly observed to have the most aggressive biology and a 36-month RFS of only 50%.

A recent cohort study by Liu et al[10] examining short-term outcomes in patients with thyroid nodules of all Bethesda categories diagnosed as thyroid cancer on surgical pathology and available molecular testing at the time of initial treatment revealed that recurrence was more likely in MRG-high cancers and that distant metastasis occurred only in this group. In another recent study of patients with Bethesda V nodules followed over 4.3 years, structural or biochemical disease recurrence occurred in 13%, more often occurring in MRG-high (55%) compared with MRG-intermediate (10%) and MRG-low (0%) cancers.[15] Our findings are concordant in that we found an increased risk of locoregional recurrence and distant metastasis between MRG-high (67% and 50%, respectively) and MRG-intermediate (7% and 1%, respectively) and MRG-low (0%) cancers. Importantly, the only instance of distant metastasis we observed in the MRG-intermediate group was found in a patient presenting with advanced disease, with a 9.4-cm PTC and lymph node metastases. The study by Liu et al[10] was dominated by Bethesda III and IV nodules and included not only DTCs but also poorly differentiated and anaplastic thyroid carcinomas. The 21% rate of MRG-high profiles among Bethesda VI nodules, compared with 7% in our study, may be partly due to inclusion of more aggressive histology and suggests that molecular profiling was performed selectively.

Our study focused on the potential for preoperative molecular testing to add value to the initial decision-making process in patients with known or highly suspected DTC (Bethesda V and VI nodules) with regard to the extent of surgery and decision to perform RAI ablation. Bethesda III and IV nodules with positive molecular testing are often either low-risk cancers, benign, or noninvasive follicular thyroid neoplasms with papillary-like nuclear features, which may be due to their molecular profile (often isolated *RAS*).[3,16,17] The most appropriate initial surgery is usually thyroid lobectomy. On the other hand, Bethesda V and VI nodules with poorly differentiated, medullary, or anaplastic features are almost always managed with initial total thyroidectomy. Our cohort study examines the middle tier bracketed by these poles, where molecular testing could allow a pivot from thyroid lobectomy to total thyroidectomy or vice versa. Indeed, according to the current ATA guidelines, thyroid lobectomy alone may be sufficient initial treatment for low-risk DTCs 1 to 4 cm in size.[13] Most of the patients in our study, with suspected (Bethesda V) or known (Bethesda VI) DTC, fall into this treatment group. However, approximately 80% of MRG-intermediate and MRG-low patients underwent total thyroidectomy in our study, of whom

two-thirds received RAI ablation. This finding is consistent with a recent national study showing that 77% of low-risk thyroid cancers are treated with total thyroidectomy,[18] as well as with other studies.[19,20] Thus, an opportunity to deescalate surgery and decrease the burden of permanent surgical complications[21] may be indicated for many patients with thyroid cancer when interpreted within the limitations of the lower power of our findings.

Our study findings suggest that (1) among patients with known or highly suspected PTC, tumors with MRG-high profiles are uncommon; (2) the majority of these tumors are MRG-intermediate by virtue of harboring an isolated *BRAF* V600E mutation; and (3) MRG-intermediate tumors have an acceptably low recurrence rate and may be candidates for deescalation of initial management. The latter window of opportunity is likely best realized by the significant proportion of MRG-intermediate patients (approximately 50% in our study) who undergo total thyroidectomy with or without RAI ablation for "soft" or nononcologic indications, such as contralateral nodules, hypothyroidism, and patient preference, as opposed to "hard" or oncologic reasons, such lymphadenopathy or ETE. However, we also acknowledge that some intermediate-risk patients who receive a lobectomy up front may require completion thyroidectomy (40% in our study when the 2015 ATA guidelines are strictly applied and concordant with prior literature[22]). The structural recurrence rate among the 77 intermediate-risk patients with isolated *BRAF* V600E mutations was 8%, and among all intermediate-risk patients, structural recurrence was more common in male patients and those with larger tumors (2.3 vs 1.5 cm). The question of whether these favorable outcomes are a product of aggressive initial treatment or inherently indolent biology and confirming clinical risk factors for recurrence in MRG-intermediate carcinomas can only be answered by a prospective study.

The prognostic importance of the *BRAF* V600E mutation in thyroid cancer has been studied extensively.[23-26] Though initial reports suggested that *BRAF* V600E portends a poor prognosis,[24,25] this claim runs contrary to the 40% of PTCs harboring this mutation, with most patients enjoying excellent outcomes. More recent literature has focused on poor prognosis arising from interactions between *BRAF* V600E and clinical factors, such as patient age and sex, or a combination of high-risk alterations, such as *BRAF* V600E and a *TERT* promoter mutation.[27-29] Our data support the concept that PTCs harboring isolated *BRAF* V600E mutations may not be inherently aggressive, with further investigation warranted to elucidate whether such tumors may be managed with thyroid lobectomy alone in appropriately selected patients.

We initially hypothesized that preoperative knowledge of molecular profiling results in patients with Bethesda V and VI nodules could allow for escalation of initial management in some patients. However, all patients with MRG-high nodules underwent total thyroidectomy and RAI ablation based on clinical or ultrasonographic features alone, leaving no room for further escalation. The high rate of recurrence and distant metastasis despite aggressive initial treatment suggests that increasing the intensity and duration of postoperative surveillance based on high-risk molecular profiles could be considered. Unexpectedly, our findings have led us to posit that the potential value added by preoperative molecular testing in patients with suspected or known DTC may lie in the opportunity to deescalate initial management in MRG-intermediate tumors, most notably in cases where either total thyroidectomy or lobectomy would be appropriate based on clinical or ultrasonographic characteristics and patient acceptance with both treatment options. However, for molecular testing to be cost-effective as a routine practice for all Bethesda V and VI nodules, the cost would need to decrease and/or widespread insurance coverage would need to be available.

Limitations

We acknowledge several limitations to our study. First, it was a retrospective and nonrandomized study, although the consecutive cohort of patients mitigates selection bias. Second, the length of follow-up was relatively short, and therefore, long-term associations between MRGs and recurrence characteristics remain to be established. Third, most patients in the MRG-intermediate group underwent total thyroidectomy, and many underwent RAI ablation, which may have contributed to their low risk of disease recurrence. Fourth, our results should be interpreted within the limitations of the overall small sample size and event rate and the wide CIs of our findings.

Conclusions

In this cohort study, 6% of patients had ThyroSeq MRG-high carcinomas, and the majority of these individuals experienced disease recurrence or distant metastasis despite initial treatment with total thyroidectomy and RAI ablation. The rate of disease persistence or recurrence in patients with MRG-low and MRG-intermediate cancers was low. Preoperative knowledge of molecular alteration status at the time of initial FNA may allow further individualization of treatment among patients with known or suspected PTC, including extent of initial surgery and intensity of surveillance.

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