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The Role of American Thyroid Association Pediatric Thyroid Cancer Risk Stratification and *BRAF^{V600E}* Mutation in Predicting the Response to Treatment in Papillary Thyroid Cancer Patients ≤18 Years Old

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What is already known on this topic?

American Thyroid Association (ATA) pediatric initial risk stratification was documented to be useful and effective in predicting recurrence and response to treatment in papillary thyroid cancer (PTC). *BRAF^{V600E}* mutation has been found to be associated with an increased risk of lymph node metastasis, recurrence, and poor prognosis in adult patients. There is limited data about the impact of *BRAF^{V600E}* mutation on prognosis in pediatric PTC.

What this study adds?

This study documented that ATA initial pediatric risk stratification effectively predicted the risk of recurrent and persistent disease and final response to treatment in PTC patients ≤18 years old. The presence of *BRAF^{V600E}* mutation was highly predictive for locoregional recurrence but had no significant effect on the final rate of excellent response to treatment.

Abstract

Objective: This study aimed to evaluate the role of risk stratification by the American Thyroid Association (ATA) pediatric thyroid cancer risk levels and *BRAF^{V600E}* mutation to predict the response to treatment in papillary thyroid cancer (PTC) patients ≤18 years old.

Methods: Clinical outcomes during a median period of 6 (2-21.8) years were assessed in 70 patients, according to ATA pediatric risk stratification, *BRAF^{V600E}* mutation status, and dynamic risk stratification (DRS) at final follow-up.

Results: Of 70 patients, 44 (63%), 14 (20%), and 12 (17%) were classified initially as low-, intermediate-, and high-risk, respectively. *BRAF^{V600E}* mutation analysis data was available in 55 (78.6%) patients, of whom 18 (32.7%) had the *BRAF^{V600E}* mutation. According to the final DRS, 61 (87%), two (3%), six (9%), and one (1%) patients were classified as an excellent, incomplete biochemical, incomplete structural, and indeterminate response, respectively. All ATA low-risk patients showed excellent response to treatment, whereas the rate of excellent response was 65.4% in intermediate- and high-risk levels ($p < 0.001$). The rates of excellent response in *BRAF^{V600E}* positive and negative patients were 83% and 92%, respectively ($p = 0.339$). The rate of locoregional recurrence was significantly higher in *BRAF^{V600E}* positive vs negative patients (33.3% vs 2.7% respectively, $p = 0.001$).

Conclusion: ATA pediatric risk stratification is effective in predicting response to treatment in PTC patients ≤18 years old. The presence of *BRAF^{V600E}* mutation was highly predictive for recurrence but had no significant impact on the rate of excellent response to treatment at final follow-up.

Keywords: *BRAF^{V600E}* mutation, dynamic risk stratification, pediatric thyroid cancer, thyroid cancer

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Introduction

Differentiated thyroid cancer (DTC) in pediatric and adolescent populations is uncommon, and constitutes approximately 2-4% of all pediatric malignancies (1,2). However, global trends in the incidence of thyroid cancer in children and adolescents showed rapid increases between 1998-2002 and 2008-2012 in almost all countries (3). Thyroid cancer is the most common endocrine malignancy in the 0-19 year age group (4). Papillary thyroid cancer (PTC) constitutes almost 90% of all thyroid carcinoma in this age group (5). The initial presentation, clinical course, and mortality of DTC in pediatric patients exhibit differences compared to adult patients. The rates of lymph node involvement, distant metastasis, and recurrence are much higher in pediatric and adolescent patients compared to adults, but the mortality rate at 20 years is less than 1% (6,7). The use of the American Joint Committee on Cancer/International Union Against Cancer (AJCC/UICC) TNM staging system is recommended for patients with DTC to predict disease-related mortality (8,9). The role of the AJCC/UICC TNM staging system to predict prognosis in pediatric DTC is limited due to the very low mortality rate (8). Considering the differences between adult and pediatric DTC, the American Thyroid Association (ATA) published Management Guidelines for Children with Thyroid Nodules and Differentiated Thyroid Cancer in 2015 (1). This guideline recommended initial postoperative stratification of pediatric PTC patients into low-, intermediate-, or high-risk levels to predict the patients at risk of persistent or recurrent cervical disease (1). ATA pediatric initial risk stratification was documented to be useful and effective in predicting recurrence in many studies (10,11,12,13,14). The dynamic risk stratification system (DRS), which was suggested to assess the response to therapy in DTC, has been validated in adult patients and has also been evaluated in pediatric DTC patients in some studies (12-20).

BRAF^{V600E} mutation has been found to be associated with an increased risk of lymph node metastasis, recurrence, and poor prognosis in adult patients (21,22). *BRAF*^{V600E} mutation status was incorporated into the ATA 2015 Modified Risk Stratification System for adult DTC patients and continuous risk scale for the assessment of structural recurrence risk to assist clinicians in proper risk stratification when mutation status data were available (8). There is limited data about the impact of *BRAF*^{V600E} mutation on prognosis in pediatric PTC (23,24,25,26). There are some reasons for the limited data. The rate of *BRAF*^{V600E} mutation is low in pediatric PTC compared to adults; it exhibits great differences according to the age of the patient and is very low in young patients (21,27). There is only one study in the literature that evaluated the correlation between *BRAF*^{V600E} mutation status and ATA pediatric initial risk stratification. The authors detected no significant correlation between the *BRAF*^{V600E} mutation status and ATA pediatric initial risk stratification (24). The impact of *BRAF*^{V600E} mutation on response to treatment by DRS has not been previously investigated in pediatric PTC patients.

The aim of this study was to evaluate the role of ATA pediatric thyroid cancer risk stratification and *BRAF*^{V600E} mutation status to predict the response to treatment in pediatric and adolescent PTC patients.

Methods

A total of 119 patients ≤18 years old underwent thyroid surgery in the Division of Endocrine Surgery of Istanbul Faculty of Medicine, Department of General Surgery between 1995 and 2020. Of these 119 patients, 85 (71.4%) were treated for PTC. This retrospective study included 70 (82%) of the 85 patients in whom all clinicopathological and follow-up data were available. *BRAF*^{V600E} mutation analysis was performed in 55 (78.6%) of 70 patients. Preoperative evaluation included thyroid hormone assay, neck ultrasonography (US), fine-needle aspiration biopsy (FNAB) of suspicious nodules, and FNAB or FNAB-thyroglobulin (Tg) washout of suspicious lymph nodes. The extent of initial thyroidectomy was either lobectomy or total thyroidectomy. Modified radical neck dissection (MRND) and therapeutic central neck dissection (TCND) were performed in patients with proven lateral neck metastasis. TCND was also performed in patients without lateral neck metastasis but with pre- or intra-operative evidence of clinically involved central lymph nodes. Routine prophylactic central neck dissection (PCND) was performed after 2010 in our institution in pediatric DTC patients.

Postoperative management of the patients was accomplished with a multidisciplinary approach, including the departments of pediatric endocrinology, nuclear medicine, and endocrine surgery. Stimulated Tg assay (sTg) and neck US was done 4-6 weeks after surgery in all patients. The patients were initially stratified as low-, intermediate-, and high-risk postoperatively, according to the ATA risk stratification system for pediatric and adolescent DTC patients. Postoperative radioactive iodine (RAI) treatment was performed in all ATA intermediate- and high-risk patients. The decision to use RAI treatment in ATA low-risk patients was individualized according to the clinicopathological features and postoperative sTg and anti-Tg (anti-Tg) values. A whole-body scan (WBS) was obtained 1 week after RAI treatment. Thyroid-stimulating hormone

(TSH) suppression treatment was given in all patients aiming to keep TSH levels lower than 0.1 mIU/L.

Neck US, either stimulated or nonstimulated Tg and anti-Tg assay were repeated every 6-12 months according to the clinical course and initial risk stratification. WBS with 2-5 mCi I^{131} was performed 12 months after RAI treatment with concurrent measurement of sTg and anti-Tg in patients who received RAI treatment. The trend of serum anti-Tg levels was evaluated to manage the follow-up strategy in patients with positive Tg autoantibodies (TgAb). In patients with either detectable/rising levels of nonstimulated Tg or rising/persistently high anti-Tg levels, diagnostic WBS and/or contrast-enhanced computerized tomography of neck and chest were performed when neck US was negative.

BRAF^{V600E} Mutation Analysis

BRAF^{V600E} mutation analysis was performed in formalin-fixed, paraffin-embedded thyroid tissue of thyroid tumor specimens. The QIAamp DNA tissue kit (Qiagen, Hilden, Germany) was used for genomic DNA preparation, following the manufacturer's instructions. *BRAF*^{V600E} mutation was determined by pyrosequencing using the Qiagen PyroMark Q24 pyrosequencer (Qiagen, Venlo, Netherlands) according to the manufacturer's instructions, as has been reported previously (28).

Definitions of Recurrence and Response to Treatment

In patients who underwent total thyroidectomy, with or without RAI treatment, a disease-free state was defined as a nonstimulated Tg level < 0.2 ng/mL or sTg < 1 ng/mL (in the absence of TgAb) concurrent with negative imaging at any time during the follow-up. Recurrence was defined as the detection of biochemical or structural evidence of disease following any disease-free period.

The final response to treatment was evaluated at the time of the final follow-up. Patients were classified as an excellent, incomplete biochemical, incomplete structural, or indeterminate response to treatment according to the previously reported response to therapy definitions and based on initial treatment (15,16,17,18, 29).

In patients who underwent total thyroidectomy and RAI treatment, the excellent response was defined as nonstimulated Tg < 0.2 ng/mL or sTg < 1 ng/mL (in the absence of TgAb) and negative imaging. The incomplete biochemical response was defined as nonstimulated Tg > 1 ng/mL or sTg > 10 ng/mL and negative imaging. The indeterminate response was defined as nonstimulated Tg 0.2-1 ng/mL or sTg 1-10 ng/mL, or stable or declining anti-Tg levels and nonspecific imaging findings.

In patients who underwent thyroidectomy without RAI treatment, the excellent response was defined as nonstimulated Tg < 0.2 ng/mL or sTg < 2 ng/mL (in the absence of TgAb) and negative imaging. An incomplete biochemical response was defined as nonstimulated Tg > 5 ng/mL or sTg > 10 ng/mL, or rising Tg or anti-Tg levels over time and negative imaging. An indeterminate response was defined as nonstimulated Tg 0.2-5 ng/mL or sTg 2-10 ng/mL, or stable or declining anti-Tg levels and nonspecific imaging findings.

In patients who underwent lobectomy, an excellent response was defined as nonstimulated Tg < 30 ng/mL (in the absence of TgAb) and negative imaging. An incomplete biochemical response was defined as nonstimulated Tg > 30 ng/mL or rising Tg or anti-Tg levels over time and negative imaging. An indeterminate response was defined as stable or declining anti-Tg levels or nonspecific imaging findings.

An incomplete structural response was defined as evidence of structural and functional disease with any Tg or anti-Tg level, regardless of the extent of initial treatment.

Evaluation of Outcomes

Demographic data, clinicopathological features [history of irradiation, tumor size, subtypes of PTC, multifocality, lymphovascular invasion and extrathyroidal extension (ETE), and autoimmune thyroiditis], *BRAF*^{V600E} mutation status, the extent of initial surgery, data related to RAI treatment, recurrence, and response to treatment were obtained. The clinicopathological features and clinical outcomes were assessed according to ATA risk levels and *BRAF*^{V600E} mutation. The correlations between the clinicopathological features, ATA initial risk level, *BRAF*^{V600E} mutation, and recurrence and final response to treatment were analyzed.

The study was approved by the Ethics Committee of İstanbul University Faculty of Medicine (approval number: 478485, date: 21.09.2021).

Statistical Analysis

Continuous variables with normal distribution are reported as the mean ± standard deviation (SD), non normal distribution as median (range), and categorical variables as numbers and percentages. The Student's t-test or Mann-Whitney U test was used to compare the differences in continuous variables with normal or non-normal distribution, respectively. The chi-square test or Fisher's exact test was used in comparative analyses of categorical variables. A $p < 0.05$ was considered to be statistically significant. Statistical analysis was performed using IBM Statistical Package for the Social Sciences Statistics for Windows, version 20.0 (IBM Corp., Armonk, NY, USA).

Results

Baseline Clinicopathological Characteristics and Treatment in Pediatric Patients with PTC

The median age of the patients was 16 (5-18) years, with a female to male ratio of 55/15 (3.67:1). Eight (11%) patients had a history of head and neck irradiation. The majority (44/70, 63%) of the patients presented with a solitary thyroid nodule. Fifteen (21%) patients had palpable cervical lymph nodes at the time of initial diagnosis. Preoperative neck US revealed metastatic lymph nodes, in both the central and lateral neck in 15 (21%), lateral neck only in five (7%), and central neck only in three (4%) patients. Cytologic examination of FNAB specimens of suspicious thyroid nodules revealed Bethesda 6 cytology in 37 (52%), Bethesda 5 in 11 (16%), Bethesda 4 in 11 (16%), Bethesda 3 in seven (10%), and benign in four (6%) patients.

Total thyroidectomy was performed in 68 (97%) patients, whereas two (3%) patients underwent lobectomy. Lymph node dissection (LND), additional to total thyroidectomy, was done in 42 (60%) patients. The types of LND were TCND with MRND in 21 (50%), PCND in 18 (43%), and TCND only in three (7%) of 42 patients.

The median tumor size was 15 (3-50) mm. Histopathological examination revealed classical, follicular or aggressive variants of PTC in 28 (40%), 29 (41%), and 13 (19%) patients, respectively. Forty-one patients (59%) had multifocality, 17 (24%) ETE, 31 (44%) lymphovascular invasion, and 26 (37%) autoimmune thyroiditis. Lymph node metastasis was found in 28 (40%) patients. RAI treatment was performed in 52 (74%) patients with a median I^{131} dose of 125 (30-300 mCi). Distant metastasis to the lungs was detected in five (7%) patients on WBS after postoperative RAI treatment. The median follow-up was 6 (2-21.8) years.

Twenty locoregional recurrences developed in 12 (17%) patients during the follow-up. No recurrence at distant sites was observed. When the risk of locoregional recurrence according to clinicopathological factors was analyzed, classical variant of PTC ($p = 0.001$), ETE ($p < 0.001$), lymphovascular invasion ($p < 0.001$), and lymph node metastasis ($p = 0.001$) were significant risk factors (Table 1). The total dose of I^{131} administered for RAI treatment in patients who had recurrence was significantly higher than the patients with no recurrence ($p < 0.001$) (Table 1). There was no disease-related mortality.

ATA Initial Pediatric Risk Stratification

According to the ATA initial pediatric risk stratification, 44 (63%), 14 (20%), and 12 (17%) patients were classified as low-, intermediate-, and high-risk, respectively. The comparison of clinicopathological features and clinical outcomes in ATA low-risk patients vs intermediate- and high-risk patients are summarized in Table 2. An ATA low-risk state was significantly associated with smaller tumor size, lower rates of aggressive variant PTC, multifocality, ETE, lymphovascular invasion, lymph node metastasis, LND, RAI treatment and locoregional recurrence, and a higher rate of follicular variant PTC (FVPTC) compared to paptients classified as ATA intermediate- and high-risk (Table 2). None of the ATA low-risk patients had distant metastasis, whereas lung metastasis was observed in 19% of ATA intermediate- and high-risk patients ($p = 0.003$).

All intermediate- and high-risk patients underwent total thyroidectomy, whereas lobectomy was performed in two (4%) of 44 low-risk patients. The median total dose of administered I^{131} was significantly higher in intermediate- and high-risk vs low-risk patients (150 mCi vs 67.5 mCi, respectively; $p < 0.001$) (Table 2).

Final Response to Treatment

Response to treatment according to DRS at the end of follow-up revealed excellent response in 61 (87%), incomplete biochemical response in two (3%), indeterminate response in one (1%), and incomplete structural response (persistent disease) in six (9%) patients. Five of the six patients with persistent disease were ATA high-risk patients and the rate of persistent disease in the high-risk group was 42% (5/12). The rate of excellent response was 100%, 93%, and 33% in ATA low-, intermediate-, and high-risk levels, respectively. The rate of excellent response was significantly lower in ATA intermediate- and high-risk patients when compared to ATA low- risk patients (65.4% vs 100%, $p < 0.001$) (Table 2). When we compared the clinicopathological features in patients with and without excellent response, older age, FVPTC, unifocality, absence of lymphovascular invasion, initial lymph node metastasis or distant metastasis, and ATA low-risk significantly predicted excellent response to treatment (Table 3). The total dose of I^{131} used for RAI treatment was lower in patients with excellent response compared to those without excellent response, but the difference did not achieve statistical significance ($p = 0.055$) (Table 3). The patients who developed locoregional recurrences during the follow-up showed a significantly lower rate of excellent response to treatment at final follow-up compared to those without recurrences (50% vs 95%, $p < 0.001$) (Table 1).

BRAF^{V600E} Mutation Status

BRAF^{V600E} mutation was positive in 18 (33%) of 55 patients with available data. The median age of these 55 patients

was 16 (5-18) years. The median (range) tumor size was 14 (4-50) mm. The correlation between the *BRAF*^{V600E} mutation status and clinicopathological features, ATA initial risk stratification, the extent of surgery and clinical outcomes are summarized in Table 4. Age, gender, history of irradiation and tumor size showed no significant difference between the patients with or without the mutation. Classical variant PTC was significantly associated with the presence of the *BRAF*^{V600E} mutation ($p = 0.01$), whereas the rate of FVPTC was significantly higher in *BRAF*^{V600E} (-) patients compared to *BRAF*^{V600E} (+) patients ($p = 0.01$). The rate of the aggressive variant of PTC showed no significant difference between the *BRAF*^{V600E} (+) and (-) patients. Although the rate of multifocality was higher in *BRAF*^{V600E} (+) patients compared to *BRAF*^{V600E} (-) patients, the difference did not achieve statistical significance (83% vs 57%, respectively; $p = 0.052$). There was no correlation between *BRAF*^{V600E} mutation and lymphovascular invasion, ETE, autoimmune thyroiditis, the extent of thyroidectomy, LND, lymph node metastasis, distant metastasis, and ATA initial risk levels (Table 4). The recurrence rate was significantly higher in *BRAF*^{V600E} (+) patients compared to *BRAF*^{V600E} (-) patients (33% vs 3%, $p = 0.001$). A total of 14 recurrences were observed in six of 18 *BRAF*^{V600E} (+) patients, whereas there was only one recurrence in one of 37 *BRAF*^{V600E} (-) patients ($p < 0.001$). The total dose of I^{131} used for RAI treatment was significantly higher in *BRAF*^{V600E} (+) compared to *BRAF*^{V600E} (-) patients ($p < 0.001$). The rate of excellent response in *BRAF*^{V600E} (+) and (-) patients were 83% and 92%, respectively, and showed no significant difference ($p = 0.3$). The biochemical incomplete, indeterminate and structural incomplete response rates showed no significant difference between the *BRAF*^{V600E} (+) and (-) patients (Table 4).

Discussion

In our study, we found that all ATA low-risk and 93% of intermediate-risk patients had an excellent response to treatment at final follow-up. The presence of *BRAF*^{V600E} mutation was highly predictive for locoregional recurrence but had no significant effect on the final rate of excellent response to treatment.

Thyroid cancer is rare in children, and there is a limited number of studies with a large number of patients. The

Table 1. The clinicopathological features and clinical outcomes in patients with and without locoregional recurrences

	Recurrence (+) (n = 12)	Recurrence (-) (n = 58)	p
Median (range) age, years	14.5 (8-18)	16 (6-18)	0.3
Gender, n (%)			
Female	9 (75)	46 (79)	0.7
Male	3 (25)	12 (21)	
History of irradiation, n (%)	0 (0)	8 (14)	0.1
Tumor size, mm.	20.5 (9-42)	14 (3-50)	0.1
Histologic type, n (%)			
Classical	10 (83)	18 (31)	0.001
Folicular	0 (0)	29 (50)	0.001
Aggresive	2 (17)	11 (19)	0.8
Pathological features, n (%)			
Multifocality	10 (83)	31 (53)	0.056
Extrathyroidial extension	9 (75)	8 (14)	0.0001
Lymphovascular invasion	11 (92)	20 (34)	< 0.001
Autoimmune thyroiditis	4 (33)	22 (38)	0.6
Total thyroidectomy, n (%)	12 (100)	56 (97)	0.5
Total LND, n (%)	10 (83)	32 (55)	0.06
TCND + MRND, n (%)	10 (83)	11 (19)	< 0.001
Lymph node metastasis, n (%)	10 (83)	18 (31)	0.001
Distant metastasis, n (%)	2 (17)	3 (5)	0.1
ATA low risk, n (%)	1 (8)	43 (74)	< 0.001
RAI treatment, n (%)	12 (100)	40 (69)	0.02
Median (range) total I^{131} dose (mCi)	150 (60-300)	100 (30-150)	< 0.001
Excellent response, n (%)	6 (50)	55 (95)	< 0.001

LND: lymph node dissection, TCND: therapeutic central neck dissection, MRND: modified radical neck dissection, RAI: radioactive iodine, ATA: American Thyroid Association

initial presentation, clinical course, and mortality of PTC in children shows major differences compared to adult patients. The rate of multifocal disease ranged between 28% and 57%, ETE between 36% and 59%, central and/or lateral neck metastasis between 60% and 70%, and initial distant metastasis between 4.7% and 14.6% in pediatric DTC (14,24,30,31). Classical variants constitute the majority of PTC in pediatric and adolescent patients (13,14,24,30,31). Although the initial presentation of childhood DTC is more severe compared to adults, the long-term outcome is favorable, with very low mortality rates (3,7). We found a high rate (41%) of FVPTC in our cohort. In a former study from our instution, the rate of FVPTC was reported as 37.2% in adult PTC patients (32). The reported rates of FVPTC in pediatric PTC patients ranged between 10.4% and 36.8%, and was 22.7% in a large database study, which included 1,956 pediatric patients (24,30,31,33,34). The relatively high rate of FVPTC in our pediatric patients might be an incidental finding in a particulary small cohort or might be a reflection of regional and environmental differences in PTC features.

The local or distant recurrence rates are reported to range between 15.9-34% in pediatric and adolescent DTC patients (13,14,30,35). Recurrence was significantly associated with multifocality, large tumors, palpable cervical lymph nodes, lymph node metastasis, ETE or distant metastasis at diagnosis in pediatric and adolescent PTC (30,35). In the study by Welch Dinauer et al (35), the authors showed that focality was the best predictor of recurrence by multivariate analysis. In our study, the increased rate of locoregional recurrence was significantly associated with classical variant PTC, ETE, lymphovascular invasion, lymph node metastasis, and the presence of *BRAF*^{V600E} mutation. However, age, gender, autoimmune thyroiditis,

Table 2. The comparison of the clinicopathological features, extent of surgery and clinical outcomes in ATA pediatric low-and intermediate/high-risk patients

	ATA low risk (n = 44)	ATA intermediate and high risk (n = 26)	p
Median (range) age, years	16 (6-18)	15.5 (5-18)	0.9
Gender, n (%)			
Female	35 (79.5)	20 (77)	0.8
Male	9 (20.5)	6 (23)	
History of irradiation, n (%)	8 (18)	0 (0)	0.02
Median (range) tumor size, mm	10.5 (3-50)	21 (9-45)	0.001
Histologic type, n (%)			
Classical	14 (32)	14 (53.8)	0.07
Folicular	27 (61)	2 (7.7)	< 0.001
Aggresive	3 (7)	10 (38.5)	0.001
Pathological features, n (%)			
Multifocality	21 (48)	20 (80)	0.017
Extrathyroidial extension	0 (0)	17 (65.4)	< 0.001
Lymphovascular invasion	7 (16)	24 (92.3)	< 0.001
Autoimmune thyroiditis	18 (41)	8 (31)	0.4
Total thyroidectomy, n (%)	42 (96)	26 (100)	0.2
Total LND, n (%)	17 (39)	25 (96)	< 0.001
TCND + MRND, n (%)	1 (2.3)	20 (80)	< 0.001
Lymph node metastasis, n (%)	4 (9)	24 (92.3)	< 0.001
Distant metastasis, n (%)	0 (0)	5 (19)	0.003
RAI treatment, n (%)	26 (59)	26 (100)	< 0.001
Median (range) total I^{131} dose (mCi)	67.5 (30-300)	150 (50-300)	< 0.001
Recurrence, n (%)	1 (2.2)	11 (42.3)	< 0.001
Number of recurrences, n (%)	1 (2.3)	19 (73)	0.003
Final DRS, n (%)			
Excellent response	44 (100)	17 (65.4)	< 0.001
Biochemical incomplete/indeterminate	0 (0)	3 (11.5)	0.047
Structural incomplete	0 (0)	6 (23.1)	0.002

LND: lymph node dissection, TCND: therapeutic central neck dissection, MRND: modified radical neck dissection, RAI: radioactive iodine, ATA: American Thyroid Association, DRS: dynamic risk stratification

tumor size, and distant metastasis were not significantly associated with local recurrence in our cohort. Although the rate of multifocality was approximately 1.5-fold higher in patients who developed locoregional recurrence compared to patients with no recurrence, the difference was not statistically significant.

Recently, the ATA recommended that pediatric PTC patients should be initially stratified into ATA pediatric low-, intermediate-, or high-risk levels, based on clinical presentation, tumor size, and evidence of regional invasion and metastasis to identify the patients at risk of persistent or recurrent cervical disease (1). The ATA's initial pediatric risk stratification system has been validated by several studies, and the findings of our study were similar to the results of these other studies (11,12,13,14,20). We observed significantly higher rates of locoregional recurrence and persistent disease in ATA intermediate- and high-risk patients compared to ATA low-risk patients.

The ATA 2015 Modified Risk Stratification System for adult DTC patients does not suggest a routine analysis of *BRAF*^{V600E} status for initial risk stratification (8). However, *BRAF*^{V600E} mutation has been included in the continuous risk scale for the assessment of the risk of structural disease in adults to help clinicians to perform proper risk stratification in cases where mutation information is available (8). The rate of mutation positivity exhibited great difference according to the age of the patient in a pediatric population and was very low in younger children (26,27). In the study by Nikita et al (27), 89% of *BRAF*^{V600E} mutations were detected in patients older than 15 years and 11% in those younger than 15 years old. In our study, we observed that the median age of *BRAF*^{V600E} (+) patients was older compared to *BRAF*^{V600E} (-) patients, but the difference was not significant. The studies that evaluated the correlation between *BRAF*^{V600E} mutation and histopathological features, the extent of disease, and prognosis in pediatric PTC patients showed that *BRAF*^{V600E} mutation was not significantly associated with adverse histopathological features, lymph node metastasis, or distant metastasis and did not predict an aggressive clinical course, as it does in adult PTC (24,25,31,33). *BRAF*^{V600E} mutation was found to be very frequent in classical variant PTC compared to non-classical variants in several studies (25,31,33). Similarly, we observed that the rate of classical variant PTC was approximately 2-fold higher in *BRAF*^{V600E} (+) patients compared to *BRAF*^{V600E} (-) patients, in our

Table 3. The clinicopathological features and treatment in patients with and without excellent response to treatment

	Excellent response (+) (n = 61)	Excellent response (-) (n = 9)	p
Median (range) age, years	16 (6-18)	13 (5-18)	0.045
Gender, n (%)			
Female	49 (80)	6 (67)	0.3
Male	12 (20)	3 (33)	
History of irradiation, n (%)	8	0	0.2
Median (range) tumor size, mm	14 (3-50)	20 (9-45)	0.2
Histologic type, n (%)			
Classical	22 (36)	6 (67)	0.08
Folicular	29 (48)	0 (0)	0.007
Aggresive	10 (16)	3 (33)	0.2
Pathological features, n (%)			
Multifocality	32 (52)	9 (100)	0.007
Extrathyroidial extension	10 (48)	7 (78)	< 0.001
Lymphovascular invasion	23 (38)	8 (89)	0.004
Autoimmune thyroiditis	23 (239	3 (339	0.8
Total thyroidectomy, n (%)	59 (98)	9 (100)	0.5
Total LND, n (%)	33 (54)	9 (100)	0.008
TCND + MRND, n (%)	12 (16)	9 (100)	< 0.001
Lymph node metastasis, n (%)	19 (319	9 (100)	< 0.001
Distant metastasis, n (%)	0 (0)	5 (56)	< 0.001
Recurrence, n (%)	6 (9.8)	6 (67)	< 0.001
ATA low risk, n (%)	44 (72)	0 (0)	< 0.001
RAI treatment, n (%)	43 (70)	9 (100)	0.06
Median (range) total I^{131} dose (mCi)	100 (30-300)	150 (50-300)	0.055

LND: lymph node dissection, TCND: therapeutic central neck dissection, MRND: modified radical neck dissection, RAI: radioactive iodine

study. Geng et al (24) showed that the *BRAF*^{V600E} mutation was significantly associated with both a low AJCC and low AMES tumor stage. The authors reported the rates of *BRAF*^{V600E} mutation as 63.6%, 40%, and 22.2% in ATA low-, intermediate-, and high-risk patients, respectively, with no statistical difference according to the risk level (24). In our study, we also found no significant correlation between the *BRAF*^{V600E} mutation and ATA pediatric initial risk levels. Nor did we observe any significant correlation between the *BRAF*^{V600E} mutation and adverse histopathological features and initial presentation of PTC. Although, *BRAF*^{V600E} mutation was not associated with unfavorable clinicopathological risk factors initially, we observed that it was a significant predictive factor for recurrence in our patients. In our study, the rates of locoregional recurrence in *BRAF*^{V600E} (+) vs (-) patients were 33% vs 3%, respectively.

The DRS has been proposed for re-staging patients according to response to treatment by re-evaluating the clinical, biochemical, imaging, and cytopathologic findings at any time during follow-up (8,15,16,17,18). The DRS has been validated in patients treated with total thyroidectomy and RAI treatment, and a modified DRS system could also be applied to DTC patients who underwent lobectomy or total thyroidectomy without RAI ablation (15,18,29).

Sohn et al (20) showed that the prevalence of structural persistent disease increased as ATA initial risk classification increased in pediatric DTC. Other studies have reported that low-risk patients had the highest probability of an excellent response to initial treatment while high-risk patients had the highest probability of incomplete structural response and the lowest probability of an excellent response (13,14). Our findings were compatible with these studies. We found the

Table 4. The comparison of clinicopathological features, extent of surgery, ATA initial risk stratification, and response to treatment according to *BRAF*^{V600E} mutation status

	BRAF^{V600E} (+) (n = 18)	*BRAF*^{V600E} (-) (n = 37)	p
Median (range) age, years	16 (14-18)	15 (5-18)	0.064
Gender, n (%)			
Female	15 (83)	29 (78.4)	0.6
Male	3 (17)	8 (21.6)	
History of irradiation, n (%)	0 (0)	6 (16)	0.07
Median (range) tumor size, mm	15 (6-50)	14 (4-50)	0.4
Histologic type, n (%)			
Classical	11 (61)	10 (27)	0.01
Folicular	4 (22)	21 (57)	0.01
Aggresive	3 (7)	6 (16)	0.9
Pathological features, n (%)			
Multifocality	15 (83)	21 (57)	0.052
Extrathyroidial extension	6 (33)	8 (22)	0.3
Lymphovascular invasion	10 (55)	12 (32)	0.1
Autoimmune thyroiditis	8 (44)	14 (38)	0.6
Total thyroidectomy, n (%)	18 (100)	36 (97)	0.48
Total LND, n (%)	12 (67)	23 (62)	0.75
TCND + MRND, n (%)	6 (33)	10 (27)	0.6
Lymph node metastasis, n (%)	9 (50)	13 (35)	0.2
Distant metastasis, n (%)	0 (0)	3 (8)	0.2
ATA low risk, n (%)	10 (55)	27 (73)	0.1
RAI† treatment, n (%)	16 (89)	25 (68)	0.09
Median (range) total dose of I^{131} (mCi)	150 (100-300)	75 (30-250)	0.001
Patients with recurrence, n (%)	6 (33)	1 (3)	0.001
Number of recurrences, n (%)	14 (78)	1 (3)	< 0.001
DRS Response to treatment, n (%)			
Excellent	15 (83)	34 (92)	0.3
Biochemical incomplete/indeterminate	2 (11)	1 (2.7)	0.2
Structural incompelete	1 (6)	2 (5.3)	0.9

LND: lymph node dissection, TCND: therapeutic central neck dissection, MRND: modified radical neck dissection, RAI†: radioactive iodine, ATA: American Thyroid Association, DRS: dynamic risk stratification performed at the end of the folllow up

rate of excellent response to be 100 %, 93 % and 33 % in ATA low-, intermediate- and high-risk patients, respectively. In our study, excellent response to treatment was significantly associated with ATA low-risk, older age, FVPTC, unifocal tumors with no invasion and metastasis, and no recurrences during the follow-up.

The impact of *BRAF*^{V600E} mutation status on response to treatment evaluated by DRS has not been previously investigated in pediatric PTC patients. When we analyzed the response to treatment in *BRAF*^{V600E} (+) and (-) patients, no significant difference was found between the two groups in respect of excellent response to treatment at final follow-up. Although the rate of biochemical incomplete/indeterminate response was higher in *BRAF*^{V600E} (+) patients compared to *BRAF*^{V600E} (-) patients, the difference was not statistically significant (11 % vs 2.7 %, p = 0.2). Our findings suggest that *BRAF*^{V600E} mutation might be associated with a higher rate of locoregional recurrence but probably do not increase the long-term risk of incomplete structural response to treatment.

Some studies have reported that younger age was found to be associated with initial high risk and recurrent/persistent disease in pediatric PTC, while others found no correlation with age and prognosis (12,36,37,38,39). We observed that older age was significantly associated with excellent response to treatment. Gender was not associated with either the initial risk stratification or response to treatment in our study and this finding was compatible with the studies in the literature (36,37,38,39).

Study Limitations

This study has some limitations. This is a retrospective study with a relatively small sample size. Pediatric DTC is a rare disease, and studies reporting outcomes of more than 100 children are few. There is the possibility of selection bias as all of the patients in this study were treated in a single tertiary referral center. *BRAF*^{V600E} analysis was performed in 78.5% of the cohort. The small sample size might be insufficient to determine the correlation between *BRAF*^{V600E} mutation and recurrence or response to treatment.

Conclusion

We showed that ATA initial pediatric risk stratification effectively predicted the risk of recurrent and persistent disease and final response to treatment in PTC patients ≤18 years old. All ATA low-risk and 93 % of intermediate-risk patients had an excellent response to treatment at final follow-up. The presence of *BRAF*^{V600E} mutation was highly predictive for locoregional recurrence but had no significant effect on the final rate of excellent response to treatment. During initial risk evaluation of pediatric PTC patients, investigation of *BRAF*^{V600E} mutation status in addition to ATA initial stratification might provide a better estimate of the probability of recurrence in those patients in whom *BRAF*^{V600E} mutation status can be determined. Further studies with a large number of patients are needed to determine the role of the *BRAF*^{V600E} mutation on recurrence and response to treatment in pediatric PTC.

Ethics

Ethics Committee Approval: The study was approved by the Ethics Committee of İstanbul University Faculty of Medicine (approval number: 478485, date: 21.09.2021).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Yasemin Giles Şenyürek, Yalın İşcan, İsmail Cem Sormaz, Şükran Poyrazoğlu, Fatih Tunca, Concept: Yasemin Giles Şenyürek, Yalın İşcan, Fatih Tunca, Design: Yasemin Giles Şenyürek, Fatih Tunca, Data Collection or Processing: Yasemin Giles Şenyürek, Yalın İşcan, İsmail Cem Sormaz, Analysis or Interpretation: Yasemin Giles Şenyürek, Fatih Tunca, Literature Search: Yasemin Giles Şenyürek, Şükran Poyrazoğlu, Writing: Yasemin Giles Şenyürek, Fatih Tunca.

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