BRAF mutations in pT1 tumors invading the thyroid capsule should be considered a marker of worse prognosis

Basolo F, Torregrossa L, Giannini R, Miccoli M, Lupi C, Sensi E, Berti P, Elisei R, Vitti P, Baggiani A, Miccoli P. Correlation between the *BRAF* V600E mutation and tumor invasiveness in papillary thyroid carcinomas smaller than 20 millimeters: analysis of 1060 cases. J Clin Endocrinol Metab 2010 doi:101210/jc.2010/jc.2010-0337.

SUMMARY

BACKGROUND

The incidence of thyroid cancer has been increasing worldwide for the past several decades. Papillary thyroid cancer (PTC) comprises nearly 90% of all thyroid cancers, about half of which are tumors ≤10 mm, and approximately 87% are papillary thyroid cancers ≤20 mm. Although small PTCs usually have a good outcome, there is disagreement concerning the management of tumors measuring 10 to 20 mm. Previously classified as pT2, these tumors are now are classified as pT1 according to the sixth edition of the tumor–node–metastases (TNM) staging system. PTCs <2 cm that are limited to the thyroid gland and have no lymph-node metastases are generally considered to be low-risk tumors that can be treated with thyroidectomy. Still, tumors with extrathyroidal extensions require more aggressive management, as an unfavorable outcome is more likely. *BRAF* V600E activating mutation, with a prevalence of approximately 45%, has become a promising prognostic factor in the risk stratification of PTC. The aim of this retrospective study was to correlate *BRAF* V600E mutations with the clinical and pathological features in a large and homogeneous series of patients with PTC tumors <2 cm.

PATIENTS AND METHODS

The Pathology Descriptions

Thyroid specimens were retrieved from archival formalin-fixed and paraffin-embedded thyroid specimens maintained in the Department of Surgical Pathology, University of Pisa, from 1060 consecutive patients, 254 of whom were men (24%) and 806 women (76%), with PTCs ≤20 mm that were diagnosed from January 2006 through April 2009. All of the patients were treated and observed at the Institute of Endocrinology at the University of Pisa and had total or near-total thyroidectomy and excision of suspicious cervical lymph nodes by surgeons in the Department of Surgery of the same university.

Two pathologists reviewed the diagnosis to ensure that the specimens were in accord with the World Health Organization classification of thyroid malignancy. Tumors were staged according to the sixth edition of the TNM staging system as pT1 for tumors ≤20 mm and pT3 for tumors with minimal extrathyroidal extension of the tumor.

Tumors were divided into four groups: (1) totally encapsulated—tumors completely surrounded by a well-defined fibrous capsule that separated the tumor from the surrounding nonneoplastic thyroid tissue, (2) not encapsulated without thyroid capsular invasion—tumors without a complete capsule around the tumor, extension into the collateral thyroid parenchyma, and absences of contact with the adjacent thyroid capsule, (3) thyroid capsular invasion—tumors with clear tumor infiltration of the thyroid capsule with no extension into the perithyroidal soft tissues, and (4) extrathyroidal extension—tumors that showed signs of infiltration, including even minimal infiltration beyond the confines of the thyroid capsule into the adjacent soft tissues. When there was thyroid capsule invasion, at least 10 consecutive serial tissue sections were evaluated per sample to exclude tumor extension into the perithyroidal soft tissues.

BRAF Mutation Analysis

BRAF detection was performed by polymerase-chain-reaction single-strand conformation polymorphism, and direct DNA sequencing was performed according to standard procedures.

RESULTS

TNM Tumor Sizes and Stages (Figure 1)

A total of 581 of 1060 patients (54%) had histologically diagnosed papillary thyroid microcarcinoma (PTMC), defined as a tumor ≤10 mm. Among this group, 225 (21.3%) had classic PTC, 174 (16.4%) had follicular variant PTC, 64 (6%) had tall-cell variant, and 16 (1.5%) were categorized as "others," including solid, trabecular, and oxyphilic variants (Figure 1).

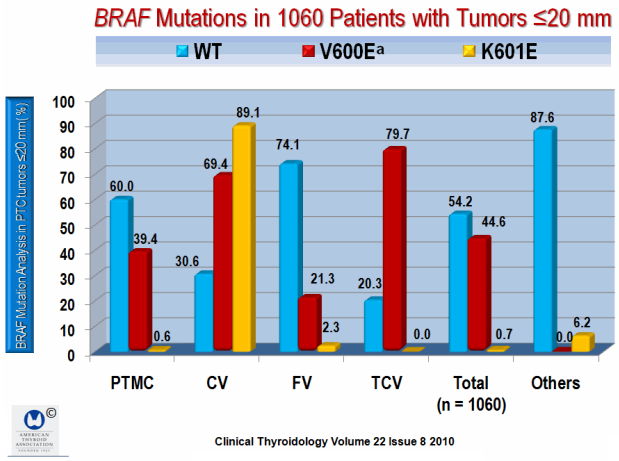


Figure 1. This figure shows the *BRAF* mutational analysis in 581 of 1060 patients with PTCs ≤20 mm. CV = classical variant of PTC; FV = follicular variant of PTC; PTMC = papillary thyroid microcarcinoma; TCV = tall-cell variant of PTC; WT = wild-type; V600Ea = an association between *BRAF* V6000E mutation and PTC variant. Others had rare variants of PTC, including solid, trabecular, and oxyphilic. P<0.0001 for both PTMC and FV vs. TVC. The data for this figure are derived from Table 1 of Basolo et al.

The mean (±SD) tumor size was 10.3±5.3 mm (median, 10.0). Tumor multifocality was found in 404 cases (38.1%). Minimal extrathyroidal neoplasm extension (pT3) was found in 311 PTCs (29.3%), and lymph-node metastases were found in 186 (17.5%). According to the TNM staging system, 917 patients (87%) had stage I tumors, 108 (10.3%) had stage III tumors, and 29 (2.7%) had stage IV tumors *(Figure 2)*.

BRAF *Mutations*

BRAF alterations were found in 486 of 1060 patients (45.8%). *BRAF* V600Ewas identified in 473 patients (44.6%). *BRAF* K601E was found in 8 patients (0.7%), and *BRAF* VK600-1E in 2 (0.2%). In addition, a complex *BRAF* exon 15 deletion–insertion mutation (1798–1811) was found in 3 patients, with a concomitant 2-bp insertion (1798–1799), and 1 patient had a 2-bp insertion (1796–1797) with a concomitant 8-bp insertion (1798–1805).

Correlation between BRAF *V600E and Clinical Pathological Features (Figure 3A)*

Concerning patients with PTC variants, *BRAF* V600E was found in 229 of 581 patients with PTMC (39.4%), in 156 of 225 with classic PTC (69.4%), in 37 of 174 with follicular PTC variants (21.3%), and in 51 of 64 with tall-cell variants (79.7%). *BRAF* K601E was found in 3 patients with PTMCs with follicular patterned-type architecture, in 4 patients with the follicular variant of PTC, and in 1 with the prevalent trabecular pattern–type of architecture designated as "others"; *BRAF* VK600-1E was found in only 2 patients, 1 with follicular variant PTC and 1 with trabecular variant PTC (Figure 3).

A total of 13 patients with *BRAF* K601E, *BRAF* VK600-1E, and *BRAF* deletion mutations were excluded.

Univariate Analysis Odds Ratio (Figure 3B)

In univariate analysis that considered the clinical risk factors of patient age and sex, only age at diagnosis was significantly associated with the presence of a *BRAF* mutation. The percentage of *BRAF* among patients younger than 45 years of age was 49.4%, while the percentage of *BRAF* positivity was 40.8% in patients 45 years of age or older(P = 0.006). *BRAF* mutations were significantly associated with tumor size (P = 0.0001), multifocality (P<0.0001), absence of tumor capsule (P<0.0001), extrathyroidal extension (P<0.0001), and presence of lymph-node metastases (P<0.001). Also, patients with *BRAF* mutations more frequently had stage III or IV as compared with stage I tumors (67.6 vs. 4.5%, P<0.0001) (Figure 3B).

The association of *BRAF* mutation and the clinical–pathological features expressed as an odds ratio (OR) ranged from 0.71 for age at diagnosis to 3.74 for the presence of extrathyroidal extension. Similar results were obtained only for tumors <1 cm, with the exception of tumor multifocality (Figure3B).

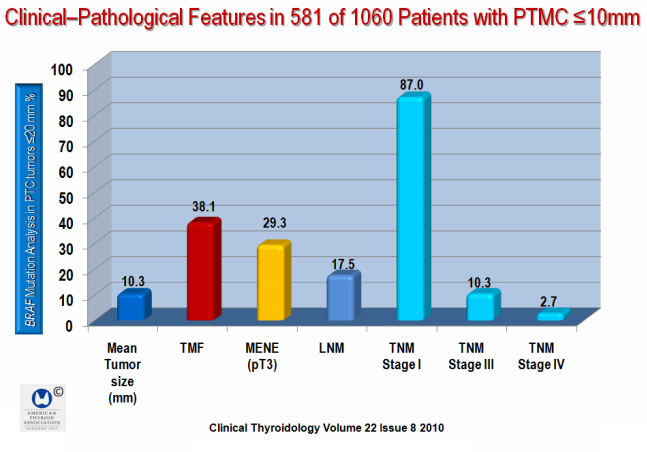
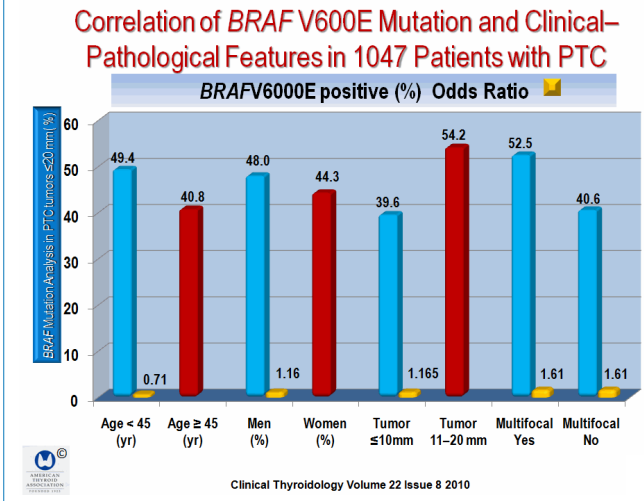
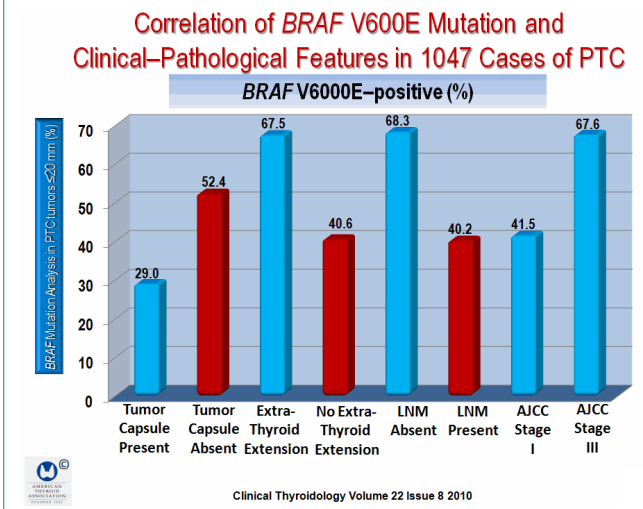


Figure 2. This figure shows the tumor features, including tumor size, tumor multifocality (TMN), minimal extrathyroidal neoplasm extension (MENE), and lymph-node metastases (LNM) and TNM staging in 581 (54.8%) that had histologically diagnosed PTMC.





Figures 3A and 3B. This figure shows the correlation of *BRAF* V600E and the clinical–pathological features in 1047 patients. (3A) AJCC = American Joint Committee on Cancer; LNM = lymph-node metastases. (3B)The data in this figure and in Figure 4 are derived from Table 2 in Basolo et al.

Multivariate Analysis (Figures 4 and 5)

Multivariate analysis identified the following independent variables: age at the time of diagnosis, tumor size, and tumor multifocality. Absence of tumor capsule, extrathyroidal extension, lymph-node metastases, and AJCC stage were all significantly associated with a high frequency of *BRAF* mutations. When only the 723 patients with nonencapsulated tumors were considered, all clinical–pathological features increased their statistical association with *BRAF* mutations (P<0.001) in both the univariate and multivariate analyses.

Association between BRAF V600E and Tumor Invasion (Figures 4 and 5)

PTCs were subdivided into four associations: group A comprised 324 totally encapsulate tumors; group B had 305 nonencapsulated tumors without thyroid capsule invasion; group C had 107 tumors with thyroid capsular invasion; and group D had 311 tumors with extrathyroidal invasion.

The percentages of *BRAF* V600E were as follows: group A, 94 0f 324 tumors (29%); group B, 97 of 305 (31.8%) tumors; group C, 72 of 107 (67.3%); and group D, 210 of 311 tumors (67.5%). The percentage of *BRAF* V600E cases for the group of totally encapsulated tumors and the value of group B did not differ significantly (29 vs. 31.8%, P>0.005). However, the percentage of *BRAF* V600E cases was significantly higher for group C than for group B (67.3 vs. 31.8%, P<0.0001). Also, a statistically significant difference was found between group B and group D (31.8 vs. 67.5%, P<0.0001, but there was no significant difference between PTCs invading the thyroid capsule (group C) and the tumors with extrathyroidal extension (group D; 67.3 vs. 67.5%; P>0.05) The correlation between *BRAF* V600E and the degree of tumor infiltration was present in different histologic variants. There was no significant difference between encapsulated tumors (Figures 4 and 5).

Verification of the correlation of *BRAF* V600E and the degree of tumor infiltration was found in different histological variants of PTC, including *BRAF* mutated PTMC, follicular and classical as well as tall cell PTC variants, taking into account the degree of tumor infiltration

CONCLUSION

There were no significant differences between totally encapsulated tumors and tumors invading only the surrounding parenchymal tissue, and *BRAF* was significantly more common in PTCs invading the thyroid capsule than in tumors that were not invading the thyroid capsule (P<0.0001), and there was no statistically significant difference in *BRAF* alterations between pT1 tumors with thyroid capsule invasion and pT3 tumors.

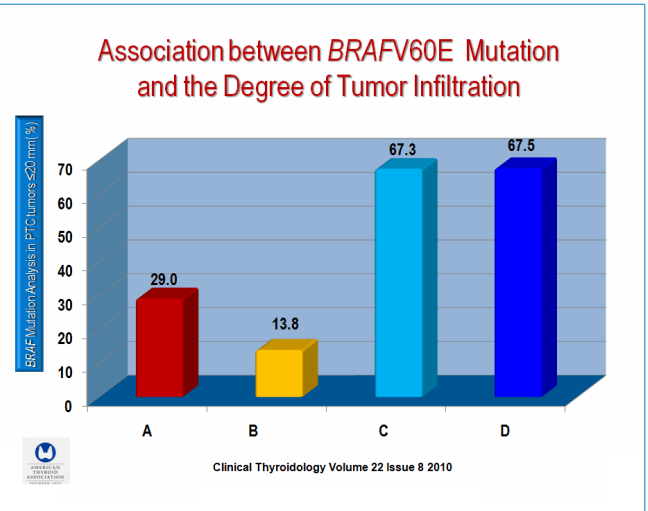


Figure 4. This figure shows the association between *BRAF* V600E mutation and the degree of tumor infiltration. The percentages of *BRAF* V600E cases in each of four tumor groups: group A, totally encapsulated; group B, not encapsulated, without thyroid capsule invasion; group C, thyroid capsule invasion; group D, extrathyroidal extension. P<0.0001 for all comparisons. This figure and is constructed from the data in Figure 2 in the study by Basolo et al.

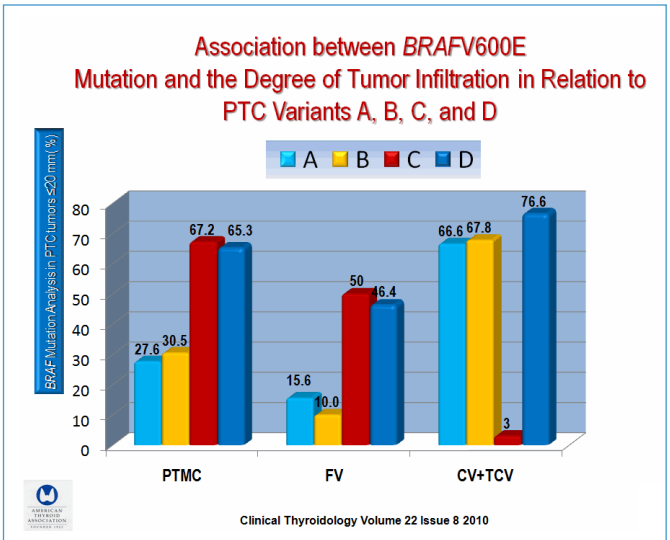


Figure 5. This figure shows the association of *BRAF* V600E in relation to PTC variants A, B, C, and D, as summarized in the Figure 4 legend. CV = classical variant of PTC; FV = follicular variant of PTC; PTMC = papillary thyroid microcarcinoma; TCV = tall-cell variant of PTC This figure is constructed from the data in Figure 3 in the study by Basolo et al.

COMMENTARY

Although the large majority of small papillary thyroid cancers generally have a favorable prognosis, the prognostic features of these small tumors have until now not been fully defined. A PTC ≤20 mm can be staged as pT1 or pT3, but the designation of pT3 means that the tumor is infiltrating beyond the thyroid capsule and perithyroidal soft tissues. Still, a PTC ≤20 mm is considered to be low-risk when the tumor is confined to the thyroid gland (pT1), although PTCs of the same size but with extrathyroidal extension (pT3) have a high potential for tumor persistence or recurrence, thus requiring more aggressive therapy (1;2). The authors of this study make the point that evaluating the degree of tumor infiltration beyond the thyroid capsule by histologic examination is a unique way to label a PTC ≤20 mm as pT1 or pT3, thus making a dividing line between low- and high-risk PTCs.

The authors examined *BRAF* mutation as a means of facilitating the differentiation between pT1 and pT3 tumors. *BRAF* mutation has been investigated by many studies that showed that this mutation is related to poor clinical–pathological parameters (3). However the correlation of patient age and the presence of *BRAF* mutations has been the subject of controversy (4). However, Basolo et al. found a correlation between age at the time of diagnosis and the presence of *BRAF* mutations in almost half the patients (49.4%), with only 41% of patients 45 years of age or younger (P = 0.006), which is in accord with the study by Lee et al. (3).

In the study by Basolo et al., multivariate analysis confirmed the association of age at diagnosis, tumor size, and multifocality, absence of tumor capsule, extrathyroidal extension, lymph-node metastases, and AJCC stage. As a result, the authors focused their study on the association of *BRAF* mutations with the degree of tumor infiltration. The study found that tumors classified as pT1 were significantly less likely to have *BRAF* mutations (40.6%), as compared with tumors classified as pT3, which had a 67.5% rate of *BRAF* mutations.

The tumors in this study were subdivided into four groups (Figure 4) to take into account the degree of tumor infiltration in each of the groups, which yielded the following results. There was no significant difference between totally encapsulated tumors and tumors invading only the surrounding parenchymal tissue (Figure 4). Also, *BRAF* mutations were significantly more common (67.3%) in PTCs invading the thyroid capsule than in tumors that were not invading the thyroid capsule (Figure 5) (P<0.1000). And there was no statistically significant difference in *BRAF* mutations between pT1 tumors with thyroid capsular invasion (67.3%) and pT3 tumors (67.5%).

Basolo et al made several important conclusions. The presence of *BRAF* mutations in pT1 tumors invading the thyroid capsule should be considered a marker of an unfavorable prognosis, and that *BRAF* V600E status of a tumor could be useful, even for pT1 tumors, to improve the risk stratification and management of PTCs, especially for PTMC tumors and small tumors with follicular variants of PTC.

This study and the study by Durante et al. provide a new look on the clinical assessment and treatment of papillary thyroid microcarcinomas that is likely to change the management of these common tumors. Both studies are likely to challenge the current ATA guideline (5) that recommends that thyroid lobectomy alone may be sufficient treatment for small (<1 cm), low-risk, unifocal, intrathyroidal papillary carcinomas in the absence of prior head and neck irradiation or radiologically or clinically involved cervical lymph-node metastases. (Recommendation rating: A)

Both this and the Durante study should be read in their entirety to fully understand the scope of these studies that are likely to set the pace for the management of papillary thyroid microcarcinomas.

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