BRAF Mutation in Papillary Thyroid Cancer and Its Value in Tailoring Initial Treatment

A Systematic Review and Meta-Analysis

Ralph P. Tufano, MD, MBA, Gilberto V. Teixeira, MD, PhD, Justin Bishop, MD, Kathryn A. Carson, ScM, and Mingzhao Xing, MD, PhD

Abstract: Clinicians have long sought to characterize biological markers of neoplasia as objective indicators of tumor presence, pathogenicity, and prognosis. Armed with data that correlate biomarker activity with disease presence and progression, clinicians can develop treatment strategies that address risks of disease recurrence or persistence and progression. The B-type Raf kinase (BRAF V600E) mutation in exon 15 of the BRAF gene has been noted to be a putative prognostic marker of the most prevalent form of thyroid cancer, papillary thyroid cancer (PTC)—a tumor type with high proclivity for recurrence or persistence. There has been a remarkable interest in determining the association of BRAF mutation with PTC recurrence or persistence. Using many new studies that have been published recently, we performed a meta-analysis to investigate correlations of BRAF mutation status with PTC prognosis, focusing on the recurrence or persistence of the disease after initial treatment.

The study was based on published studies included in the PubMed and Embase databases addressing the BRAF mutation and the frequency of recurrence of PTC. We selected studies with data that enabled measurement of the risk ratio for recurrent disease. We also analyzed the factors that are classically known to be associated with recurrence. These factors included lymph node metastasis, extrathyroidal extension, distant metastasis, and American Joint Committee on Cancer (AJCC) stages III/IV.

We used 14 articles that included an analysis of these factors as well as PTC recurrence data, with a total of 2470 patients from 9 different countries. The overall prevalence of the BRAF mutation was 45%. The risk ratios in BRAF mutation-positive patients were 1.93 (95% confidence interval [CI], 1.61–2.32; $Z = 7.01$; $p < 0.00001$) for PTC recurrence, 1.32 (95% CI, 1.20–1.45; $Z = 5.73$; $p < 0.00001$) for lymph node metastasis, 1.71 (95% CI, 1.50–1.94; $Z = 8.09$; $p < 0.00001$) for extrathyroidal extension, 0.95 (95% CI, 0.63–1.44; $Z = 0.23$; $p = 0.82$) for distant metastasis, and 1.70 (95% CI, 1.45–1.99; $Z = 6.46$; $p < 0.00001$) for advanced stage AJCC III/IV.

Thus, in this meta-analysis, the BRAF mutation in PTC was significantly associated with PTC recurrence, lymph node metastasis, extrathyroidal extension, and advanced stage AJCC III/IV. Patients with PTC harboring mutated BRAF are likely to demonstrate factors that are associated with an increased risk for recurrence of the disease, offering new prospects for optimizing and tailoring initial treatment strategies to prevent recurrence.

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Abbreviations: ^{131}I = radioiodine 131, AJCC = American Joint Committee on Cancer, BRAF = B-type Raf kinase, CI = confidence interval, CT = computed tomography, DNA-MASA = DNA-mutant allele-specific amplification, FDG-PET = 2-deoxy-2-(^{18}F)-fluorodeoxyglucose positron emission tomography, MEK-ERK = mitogen-activated protein kinase/extracellular-signal-regulated kinase, MESH = Medical Subject Headings, MRI = magnetic resonance imaging, PCR = protein chain reaction, PCR-SSCP = PCR-single-strand conformation polymorphism, PTC = papillary thyroid cancer, Tg = thyroglobulin, TSH = thyroid stimulating hormone, wt = wildtype.

INTRODUCTION

Papillary thyroid cancer (PTC) is the most common thyroid malignancy, accounting for 88% of thyroid cancer cases.[9] It has an overall favorable prognosis with an average 10-year survival rate of 93%, although up to 10% of patients eventually die as a result of the disease.[24] The prognostic factors favoring metastasis, recurrence, and death from differentiated thyroid carcinomas include patient factors of age under 15 years and above 45 years, male sex, and history of familial thyroid cancer; and tumor factors of a primary tumor >2 cm, multifocal or bilateral presence of disease in the thyroid, extrathyroidal extension, subtypes like tall and columnar cell variants, nuclear atypia and tumor necrosis, vascular invasion, lymph node metastasis, distant metastasis, and low iodine uptake.[75] Recurrence frequently occurs in the neck due to lymph node metastasis or thyroid bed remnant disease (79%), and less commonly in distant sites (21%).[49]

Recent investigations have identified molecular markers that may carry diagnostic, prognostic, and therapeutic value in the management of PTC. This complementary information can supplement characterization of the clinical and pathologic features of the disease and may help provide a tailored approach with the goal of mitigating the risk of recurrence.[2,18,67,79] BRAF mutation has been the subject of intensive investigation, as many investigators have tried to assess if the mutated gene is associated with a worse prognosis for PTC.[71] Since the first reports describing the BRAF mutation in melanoma, glioma, colorectal, ovarian, lung, and liver cancers and sarcoma cells,[8] numerous studies have been published correlating mutated BRAF with thyroid malignancy, and in particular PTC.[48,53,68,82] This mutation is specific for papillary and poorly differentiated and anaplastic thyroid carcinomas of epithelial derivation. It is not seen in follicular, Hürthle cell, and medullary carcinomas. Importantly, BRAF mutation is not present in benign tumors, including follicular and Hürthle cell adenomas.[5,54]

From the Department of Otolaryngology/Head and Neck Surgery (RPT, GVT), Department of Pathology (JB), and Department of Medicine (MX), Johns Hopkins Medical Institutions, Baltimore; and Department of Epidemiology (KAC), Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland.

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Reprints: Ralph P. Tufano, MD, MBA, The Johns Hopkins Hospital, JHOC, 601 North Caroline Street, 6th floor, Baltimore, MD 21287-0910 (e-mail: rtufano1@jhmi.edu).

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BRAF is a B-type Raf kinase, located in chromosome 7, and is the most potent activator of the mitogen-activated protein kinase/extracellular-signal-regulated kinase (MEK-ERK) pathway. The most common hotspot mutation in the BRAF gene is a thiamine transversion to adenine at nucleotide position 1799 (T1799A) in exon 15. This causes a conversion of valine to glutamate of amino acid 600 in the BRAF protein, creating a constitutively active BRAF kinase, which has been proven to be an oncogene in human cancer.[8,15] The incidence of BRAF mutation in PTC varies from 32%[83] to 73.3%[35] in patients with recurrent/persistent disease. The mutation represents a valuable molecular marker that could be examined preoperatively on fine needle aspiration biopsy specimens to estimate the risk of recurrence for thyroid cancer and would delineate a risk stratification to guide a tailored approach to tumors that express BRAF mutation.[79] BRAF mutation has been thought to confer a worse prognosis for PTC due to its association with lymph node metastasis, extrathyroidal extension, capsule invasion, vascular invasion, multifocality, bilateral tumors, older age, tumor size, aggressive subtype, impaired iodine uptake, recurrence, and death.[4,7,12,14,16,19,22,30,40,41,45,52,58,57,61,64,71,74,81] Tumor recurrence is associated with a 5-fold increase in the risk of disease-related death.[47] Tumor recurrence is an unexpected event that may promote reoperation, excessive radioiodine exposure, and an increase in morbidity related to the disease and additional treatments.[13,22,60,65] A prognostic marker that predicts the biological and clinical aggressiveness of PTC for a particular patient could lead to an appropriately customized initial treatment that may help reduce the risk of recurrence.[72]

Defining a Recurrence/Persistence Event for PTC

The American Thyroid Association's definition of cure for patients with PTC is based on the absence of clinical or radiographic imaging evidence of tumor and undetectable serum thyroglobulin (Tg) levels during thyroid stimulating hormone (TSH) suppression and stimulation without interfering antibodies.[6] These criteria were used to define PTC recurrence in the studies included in the present analysis. Because disease persistence and recurrence of thyroid cancer may not be separable in a practical sense, the aggregation of the terms "recurrent" and "persistent" disease seems appropriate to frame this event, particularly in well-differentiated thyroid carcinoma.

The evaluation for and diagnosis of recurrent/persistent disease should include physical examination; imaging studies like ultrasound, computed tomography (CT), and magnetic resonance imaging (MRI); nuclear medicine exams like whole body scan with radioiodine (^{131}I), technetium scan and 2-deoxy-2-(^{18}F)-fluorodeoxyglucose positron emission tomography (FDG-PET); combined techniques (FDG-PET/CT); blood tests (serum Tg and anti-Tg antibodies), and anatomopathologic exams (histopathology and cytopathology).[6,43] Biopsy-proven diagnosis remains the gold standard for evidence in confirming disease recurrence/persistence.

In suspected cases of PTC recurrence/persistence, imaging studies with neck ultrasound and sometimes CT or MRI are essential to determine the anatomic location of the regional remnant disease. Most recurrences affect the neck region.[13,49] In the absence of a confirmatory histopathologic diagnosis and appropriate image of the remnant PTC, it is reasonable to accept a ^{131}I uptake outside of the thyroid bed on posttreatment diagnostic whole body scan as recurrent/persistent disease, especially if it is associated with elevated levels of Tg without anti-Tg-Ab interference. This diagnostic method of recurrence gives a high specificity but only moderate sensitivity.[43] Recently, researchers using FDG-PET and FDG-PET/CT examination for PTC investigation found that although it was less specific than abnormal ^{131}I uptake for making the diagnosis of recurrent/persistent disease, there was excellent sensitivity (88.6%) and specificity (89.3%), mainly in thyroid bed recurrences.[27]

Posttreatment recombinant TSH-stimulated Tg measurements below 0.5 ng/mL offer a reliable method to detect remission of the disease. A serum Tg blood level over 2.0 ng/mL confers only a 55% chance of remission.[37] The elevation of serum Tg level alone, without anti-Tg-Ab interference, is not specific for diagnostic confirmation of recurrent/persistent disease. In these cases, the suspected recurrent/persistent disease could be remnant thyroid tissue that was incompletely ablated by radioactive iodine. The diagnosis of recurrent/persistent disease in PTC cases can be a challenge for the practitioner because of the different clinical, laboratory, and imaging manifestations.

The differences in surgical approach and adjuvant radioactive ^{131}I therapy adopted in different centers, the progressive changes over time of therapeutic strategies in the same centers, the changes in criteria to diagnose a tumor recurrence, and the different durations and types of follow-up are factors that contribute to the difficulties in comparing results reported by different centers, mainly in retrospective studies.[62] Determining an optimal initial treatment strategy for patients with PTC is thus confusing. As an endpoint for the prognosis of patients with PTC, recurrence/persistence of tumor could be related to the biological aggressiveness of the disease. The ability of a biomarker to confer a worse prognosis or a higher likelihood of recurrence/persistence in the remnant thyroid bed, regional lymphatic nodes, or distant sites such as lungs and bones may help to effectively customize initial treatment to improve patient outcomes.[49,65]

Given limitations in current clinical approaches to the prognostication of recurrent PTC prior to initiating treatment, we explored the association between recurrent/persistent disease and BRAF expression, as well as other variables that have been implicated in the recurrence/persistence of PTC. Extrathyroidal extension, advanced AJCC stage, lymph node metastasis, and distant metastasis were all assessed. Despite the variations in the definition of recurrent/persistent disease observed in the literature, we adhered to the authors' definitions published in the studies for this meta-analysis. Most of the authors defined recurrence based on a variable combination of results including radiographic and nuclear medicine imaging studies, serum Tg level, and cytologic or pathologic findings.[23,35,51,58,64,66,70,80,81,83] The aim of this meta-analysis was not to create rules to define what recurrent/persistent PTC disease is, but to alert the reader that depending on the institutional protocols and authors' definitions, sometimes the perceived "recurrent" disease is disease that was not detected before or at initial treatment, although it was present.

METHODS

Meta-Analysis Methods

We performed the meta-analysis by searching the MEDLINE database (National Library of Medicine, Bethesda, MD) and Embase database (Elsevier, Amsterdam, The Netherlands) using the terms "BRAF," "thyroid," "prognosis," "recurrence," "carcinoma," and the Medical Subject Headings (MeSH; National Library of Medicine) terms "thyroid neoplasm," "proto-oncogene proteins B-raf," "prognosis," and "recurrence." Studies were selected based on the presence of sufficient data to permit the analysis of the risk ratio for recurrent/persistent disease in patients treated for PTC who had a BRAF mutation test in the postsurgical specimen or in cytology from the fine needle aspiration biopsy prior to treatment. Studies from the same authors

were selected based on the newest and most informative article to avoid including repetitive data. We analyzed data extracted from these articles for the risk ratio of the variables: recurrence/persistence, extrathyroidal extension, lymph node metastasis, distant metastasis, and clinical/pathologic AJCC stage by BRAF mutation status. We also explored the method used to determine BRAF expression, the study design, year, country, treatment protocol, and criteria for defining recurrent/persistent disease. We calculated the risk ratio of each event by BRAF status in a pooled estimative analysis using the Mantel-Haenszel model designed by the RevMan 5.0 software for Cochrane meta-analysis (http://ims.cochrane.org/revman). The data from the studies were organized in tables attached to the respective plotted forest graph made by the software. The homogeneity assumption was assessed by the chi-square test. The test of overall effect was assessed by the Z-value, and statistical significance was set at $p < 0.05$.

Primary PTC ≤1.5 cm Group Study Methods

In addition to the meta-analysis, we conducted a retrospective chart review of 39 consecutive patients treated for fine needle aspiration-confirmed primary PTC in a dominant nodule with a maximum diameter of ≤1.5 cm (T1 classification according to AJCC staging system), during 2005, at The Johns Hopkins Hospital (Baltimore, MD). All 39 patients underwent total thyroidectomy. In 28 (72%) patients, lymph nodes were present to be assessed by anatomopathologic macroscopic and microscopic evaluation. These were lymph nodes attached to the thyroid gland, or collected by separate sampling during the surgery. Two patients were suspected to have lymph node metastases prior to or at the time of surgery and underwent a lymphadenectomy to clear those nodes. Data collected included age, sex, BRAF status, anatomopathologic features (extrathyroidal extension, surgical margins, focality, lymph node metastasis), postoperative treatment with radioactive iodine (^{131}I), and outcome (recurrence).

The BRAF V600E mutation was identified by performing pyrosequencing on the amplified fragment of a region of the BRAF gene that spans codon 600, using the PyroMark Q24 instrument (Qiagen, Germantown, MD). DNA was extracted from formalin-fixed, paraffin-embedded tissue. The tissue was microdissected using Zymo Research reagents and purified with QIAmp protocol (Qiagen). Protein chain reaction (PCR) primer and sequencing primer sequences were as follows: exon forward 5′_-GAAGACCTCACAGTAAAAATAG-3′; reverse 5′_-biotin~ATAGCCTCAATTCTTACCATCC-3′; and seq primer 5′_-AGGTGATTTTGGTCTAGCTACAG-3′. A 2 μL volume of the genomic DNA was amplified by PCR using standard conditions (95°C × 15 min; 95°C × 20 sec; 53°C × 30 sec; 72°C × 20 sec; 72°C × 5 min). The resulting PCR product was immobilized onto Streptavidin Sepharose-coated beads (GE Healthcare, Piscataway, NJ). The DNA was then washed, denatured, and transferred to a plate containing 0.75 μL of the BRAF sequencing primer in annealing buffer. The primed single-stranded DNA templates were then loaded on a PyroMark Q24, where real-time sequencing of the sequence surrounding codon 600 exon 15 was performed using PyroMark Reagents (Qiagen). The complementary DNA strand was built up by sequential addition of different dNTPs, and the nucleotide sequence was determined from the signal peaks in the pyrogram trace, which are generated by the detection of light from an enzymatic reaction following the successful addition of a dNTP to the growing strand.[63]

Patient age was summarized with median and range, and categorical data using frequencies and percentages. The association of BRAF status with anatompathologic features and recurrence was calculated using the Fisher exact test. Odds ratio calculation was based on the Mantel-Haenszel test. $P < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS v. 19.0 for Windows software (SPSS, Chicago, IL).

RESULTS

Literature Search

The literature search strategy retrieved 472 articles from PubMeD using the MeSH terms "thyroid neoplasm" and "proto-oncogene proteins B-raf." A second match in an advanced search selecting "prognosis" in a broad search revealed 133 articles. Another restrictive term "recurrence" was added to the first search where we found 37 articles. We read these 170 articles and selected 14 articles that met the criteria for this meta-analysis. Two other articles were excluded: 1 because of the overlap of cases with a previous report and the inclusion of other types and variants of PTC,[14] and a second because a different protocol was used to treat patients, which created a bias when compared with other included studies.[28] The Embase search crossing the terms "thyroid," "carcinoma," and "BRAF" revealed 452 articles, all of which overlapped with the MEDLINE search.

Meta-Analysis Results

The selected studies were published between 2004 and 2011, and included 2470 cases of PTC studied for detection of the BRAF V600E mutation in extracted DNA. All studies were retrospective, using stored formalin-fixed paraffin-embedded samples and frozen surgical specimen samples. Two studies used cells derived from fine needle aspiration biopsy samples collected preoperatively or during the surgical procedure.[80,83] The meta-analysis was based on studies with samples from 9 different countries (Germany, Italy, the United States, Ukraine, Spain, Saudi Arabia, Korea, Australia, and Czech Republic), and 1 was a multicenter study.[81] All studies analyzed PTC and its respective variants. The described treatments included total and partial thyroidectomy, sometimes associated with elective neck dissection of the central compartment and the lateral neck region. Some studies included therapeutic neck dissection (for obvious lymph node metastasis) of the central compartment and the lateral neck region. The indication for a postsurgical ablative dose of radioactive iodine and a postsurgical therapeutic radioactive iodine dose was not standardized in these studies, showing variations among institutions, countries, and during the study period. Some studies reported recurrence without details of location, while others described recurrence in the neck region (thyroid bed and lymph node metastasis) or in distant sites.

The most commonly used method for determining the presence of the BRAF V600E mutation was direct sequencing of DNA after PCR amplification alone (7 studies),[1,14,29,35,51,52,58] followed by a calorimetric method (2 studies),[80,81] DNA PCR-single-strand conformation polymorphism (PCR-SSCP) direct sequencing (2 studies),[14,70] DNA PCR and fluorescence melting curve analysis (2 studies),[23,83] and DNA-mutant allele-specific amplification (DNA-MASA) sequencing (1 study).[66]

Among the studies, the overall prevalence of the BRAF mutation was 45% (range, 32%–73%), with a total of 1118 cases expressing the BRAF V600E mutation (Table 1). The data revealed an incidence of tumor recurrence in BRAF-mutated patients of 24.9% and in BRAF wildtype (wt) patients of 12.6% (Table 2). The risk ratio for recurrence in these cases was 1.93 ($\chi^2 = 30.16$, $I^2 = 57\%$, $Z = 7.01$, $p < 0.00001$) (Figure 1). The distribution of the cases among the studies is shown in Table 3. For lymph node metastasis, the incidence in BRAF-mutated

TABLE 1. Patients With Papillary Thyroid Cancer Stratified by BRAF Mutation in 14 Studies

Study	No. of Patients	BRAF+ No. (%)	BRAF- No. (%)
Fugazzola, 2006[14]	47	18 (38)	29 (62)
Xing, 2005[81]*	219	107 (49)	112 (51)
Riesco-Eizaguirre, 2006[64]	67	28 (42)	39 (58)
Sapio, 2006[66]	43	19 (44)	24 (56)
Kim, 2006[35]	203	149 (73)	54 (27)
Kebebew, 2007[30]	209	111 (53)	98 (47)
Abubaker, 2008[1]	296	153 (52)	143 (48)
Elisei, 2008[12]	102	38 (37)	64 (63)
Xing, 2009[80]*	100	40 (40)	60 (60)
Yip, 2009[83]	332	106 (32)	226 (68)
Musholt, 2010[51]	290	122 (42)	168 (58)
O'Neill, 2010[58]	101	60 (59)	41 (41)
Sykorova, 2010[70]	242	81 (34)	161 (66)
Howell, 2011[23]	219	86 (39)	133 (61)
Total	2470	1118 (45)	1352 (55)

Abbreviations: BRAF(+) = BRAF mutated, BRAF(-) = BRAF wildtype.

*The data from Xing (2009) included in this table do not overlap with the data in Xing (2005). These data were updated from Xing (2010).[78]

patients was 54.1%, and in BRAF wt patients it was 36.8% (see Table 2), with a risk ratio for lymph node metastasis of 1.32 (χ^2 = 39.81, I^2 = 70%, Z = 5.73, p < 0.00001) (Figure 2). For extrathyroidal extension, the incidence in BRAF-mutated tumors was 46.2%, and in BRAF wt tumors it was 23.6%, with a risk ratio of 1.71 (χ^2 = 56.26, I^2 = 84%, Z = 8.09, p < 0.00001) (Figure 3). For distant metastasis, the incidence in BRAF-mutated patients was 8.0%, and in BRAF wt patients it was 7.9%, with a risk ratio of 0.95 (χ^2 = 10.79, I^2 = 44%, Z = 0.23, p = 0.82) (Figure 4). For advanced stage AJCC III/IV, the incidence in BRAF-mutated patients was 35.4%, and in BRAF wt patients it was 19.6%, with a risk ratio of 1.70 (χ^2 = 17.13, I^2 = 42%, Z = 6.46, p < 0.00001) (Figure 5).

Primary PTC ≤1.5 cm Group Study Results

The group with primary PTC ≤1.5 cm consisted of 39 patients, 26 (67%) female and 13 (33%) male. Median age was 48 years (range, 17–72 yr). Sixteen (41%) patients were aged <45 years, and 23 (59%) patients were aged ≥45 years. The BRAF-mutated gene was present in 24 (62%) patients. As for the anatomopathologic features, we observed lymph node metastasis in 12 (31%) patients, extrathyroidal extension in 8 (21%) patients, multifocality in 23 (59%) patients, and compromised or <1 mm surgical margins in 21 (54%) patients. Disease recurrence was assessed in 22 (56%) patients who were followed at our institution, and of these 22 patients, 2 (9%) had recurrence. The median follow-up period for the 22 patients was 28 months (range, 10–74 mo).

We were able to assess the records of 25 patients to determine whether adjuvant treatment with radioactive iodine was given: 18 (72%) patients were treated with postoperative radioactive iodine, and 7 (28%) were not. The most important indication for radioactive iodine treatment was the presence of lymph node metastases. All 7 patients with lymph node metastases (in this group of 25 patients) received radioactive iodine after surgical treatment (100%), and in the remaining 18 patients without lymph node metastases, 11 (61%) were treated with

TABLE 2. PTC Characteristics by BRAF Status for Each of the 14 Studies

	Recurrence		Lymph Node Metastasis		Extrathyroid Extension		Distant Metastasis		Advanced Stage AJCC	
Study	# (BRAF+)	# (BRAF-)	# (BRAF+)	# (BRAF-)	# (BRAF+)	# (BRAF-)	# (BRAF+)	# (BRAF-)	# (BRAF+)	# (BRAF-)
Fugazzola, 2006[14]	5 (18)	5 (29)	12 (16)	13 (23)	-	-	-	-	7 (18)	9 (29)
Xing, 2005[81]*	23 (92)	9 (96)	58 (107)	24 (112)	44 (107)	18 (112)	-	-	31 (107)	16 (112)
Riesco-Eizaguirre, 2006[64]	9 (28)	3 (39)	9 (28)	9 (39)	18 (28)	11 (39)	1 (28)	1 (39)	23 (28)	16 (39)
Sapio, 2006[66]	2 (11)	4 (11)	0 (19)	7 (24)	-	-	0 (19)	1 (24)	1 (19)	6 (24)
Kim, 2006[35]	32 (149)	4 (54)	116 (149)	37 (54)	107 (149)	31 (54)	-	-	62 (149)	17 (54)
Kebebew, 2007[30]	38 (111)	18 (98)	-	-	-	-	-	-	-	-
Abubaker, 2008[1]	44 (153)	25 (143)	93 (144)	83 (128)	77 (153)	70 (143)	21 (147)	9 (140)	48 (142)	37 (134)
Elisei, 2008[12]	13 (38)	6 (64)	17 (37)	23 (63)	5 (35)	7 (62)	3 (37)	3 (63)	13 (37)	7 (61)
Xing, 2009[80]*	15 (40)	6 (60)	28 (73)	21 (117)	17 (73)	13 (117)	-	-	15 (73)	13 (117)
Yip, 2009[83]†	18 (106)	8 (100)	42 (83)	18 (69)	56 (106)	15 (100)	-	-	-	-
Musholt, 2010[51]	35 (87)	38 (126)	77 (112)	71 (120)	-	-	8 (116)	23 (137)	-	-
O'Neill, 2010[58]‡	6 (55)	10 (40)	20 (60)	16 (41)	8 (60)	7 (41)	3 (60)	2 (41)	20 (60)	8 (41)
Sykorova, 2010[70]	8 (67)	3 (133)	31 (81)	44 (161)	30 (81)	42 (161)	3 (81)	9 (161)	23 (81)	26 (161)
Howell, 2011[23]	11 (86)	3 (133)	23 (63)	14 (82)	44 (86)	13 (133)	-	-	40 (86)	22 (133)
Total	259 (1041)	142 (1126)	526 (972)	380 (1033)	406 (878)	227 (962)	39 (488)	48 (605)	283 (800)	177 (905)

Abbreviations: See previous table. (#) = Number of available cases used in the calculation.

*The data from Xing (2009) included in this table do not overlap with the data in Xing (2005). These data were updated from Xing (2010).[78]

†Of the 226 cases that were BRAF-, only 100 had clinical/pathologic data available.

‡The data represent clinical recurrence.

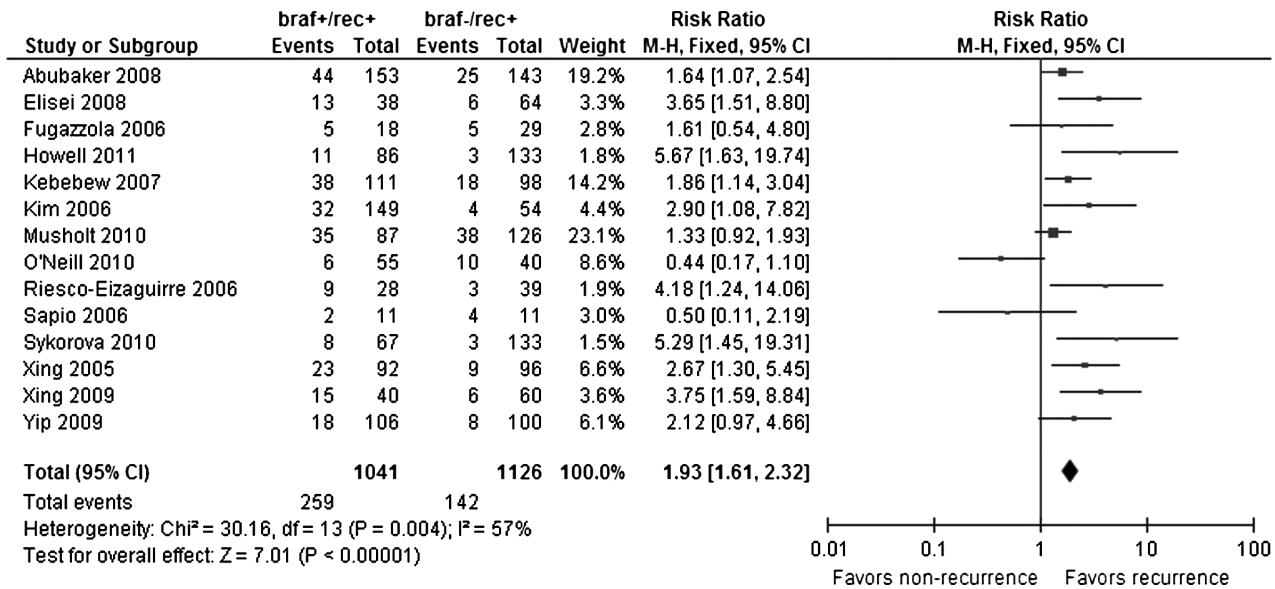

FIGURE 1. Risk ratio for persistence/recurrence associated with BRAF status. Notes: Data from the studies organized in table attached to the respective plotted forest graph made by the software RevMan 5.0, as described in Methods section. The graph demonstrates the effect size and 95% CI. See Table 1 for reference numbers for the studies listed. Abbreviations: M-H = Mantel-Haenszel, braf+ = BRAF mutated, braf− = braf wildtype, rec = persistence/recurrence.

radioactive iodine ($\chi^2 = 3.78$, $p = 0.05$). The 2 recurrence/persistence cases in our study were aged <45 years, had lymph node metastases detected with their first surgical treatment, and received postoperative radioactive iodine. Coincidentally, these 2 recurrence/persistence cases had the BRAF-mutated gene.

The association of BRAF status with the anatomopathologic features, demographic data, and the recurrence of the disease are presented in Table 4. We found no statistically significant associations of BRAF status with the clinical/pathologic characteristics analyzed in this small patient cohort. However, the risk ratios were consistent with those found in other studies.

DISCUSSION

Since the first description as a mutation related to the MEK-ERK pathway in human tumors,[8] the BRAF V600E mutation has been studied as a biological marker for tumor aggressiveness and prognosis.[21,27,73,76] Among the 3 Raf isoforms,

TABLE 3. Distribution of Cases Among 14 Studies Showing the Risk for Recurrence/Persistence According to BRAF Status*

Study	Patient Group	BRAF Mutated (%)	BRAF wt (%)	Clinical Follow-Up (median, mo)	Risk Ratio (95% CI)	P
Fugazzola, 2006[14]	Italian	5/18 (28)	5/29 (17)	NR	1.61 (0.54–4.80)	0.47
Xing, 2005[81]	American	23/92 (25)	9/96 (9)	15	3.37 (1.47–7.74)	0.006
Kim, 2006[35]	Korean	32/149 (21)	4/54 (7)	88	2.90 (1.08–7.82)	0.022
Riesco-Eizaguirre, 2006[64]	Spanish	9/28 (32)	3/39 (8)	36	4.18 (1.24–14.06)	0.021
Sapio, 2006[66]	Italian	2/11 (18)	4/11 (36)	72	0.50 (0.11–2.19)	0.31
Kebebew, 2007[30]	American	38/111 (34)	18/98 (18)	72	1.86 (1.14–3.04)	0.012
Abubaker, 2008[1]	Middle Eastern	44/153 (29)	25/143 (17)	66	1.64 (1.07–2.54)	0.027
Elisei, 2008[12]	Italian	13/38 (34)	6/64 (9)	180	3.65 (1.51–8.80)	0.003
Xing, 2009[80]	American	15/40 (38)	6/60 (10)	24	5.40 (1.87–15.57)	0.002
Yip, 2009[83]	American	18/106 (17)	8/100 (8)	8.9	2.12 (0.97–4.66)	0.05
Sykorova, 2010[70]	Czech	8/67 (12)	3/133 (2)	39	5.88 (1.51–22.94)	0.008
Musholt, 2010[51]	German	35/87 (40)	38/126 (30)	66	1.33 (0.92–1.93)	0.08
O'Neill, 2010[58]	Australian	6/55 (11)	10/40 (25)	106	0.44 (0.17–1.10)	0.02
Howell, 2011[23]	American	11/86 (13)	3/133 (2)	18	5.67 (1.63–19.74)	0.001

Abbreviations: NR = not reported.

*Notes: The p values were calculated using the Fisher exact test. The raw data were provided by Dr. E. Kebebew (for Kebebew, 2007) and Dr. K. S. Al-Kuraya (for Abubaker, 2008), through personal communication. The data in the 2 Xing references do not overlap.

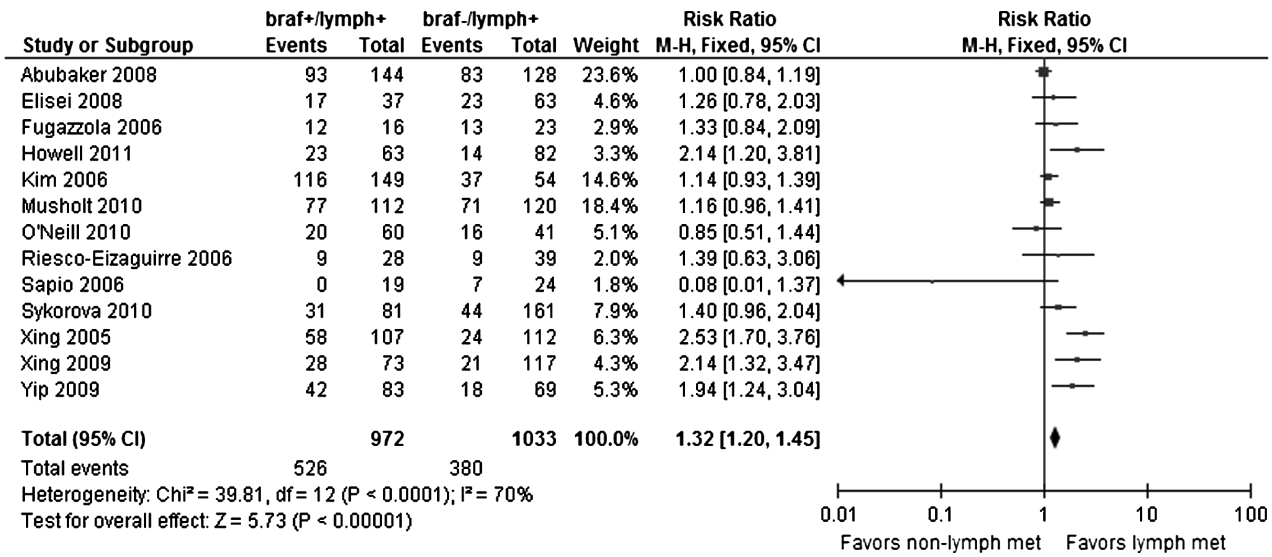

FIGURE 2. Risk ratio for lymph node metastasis associated with BRAF status. (See Figure 1 for notes and abbreviations.) Abbreviations: lymph+ = with lymph node metastasis, non-lymph met = no lymph node metastasis; lymph met = lymph node metastasis.

BRAF is the strongest activator of the downstream MEK. Downstream of MEK are ERK1/2, and phosphorylation of ERK activates substrates located in the nucleus and cytoplasm. This regulation is essential to maintain biological homeostasis, and aberrant activation of this pathway would determine tumor transformation.[71] After observing the high prevalence of the mutated BRAF gene in thyroid tumors, specifically in PTC cases,[5,48,53] some authors began reporting a relationship between thyroid malignancy prognosis and the mutated BRAF gene,[32,77] although this association was not observed in some other studies.[36,44] Currently this mutation is believed to be the most promising molecular marker in guiding therapeutic decision making and prognostication for thyroid cancer.[17,21,26,38,57,65,72]

Well-differentiated thyroid cancer is a disease with an overall 5-year survival rate of 97.3% and an age-adjusted death rate of 0.5 per 100,000 people.[3] Despite this good outcome and low disease-related death, the tumor can recur, causing increased disease-related morbidity. Some clinical factors have been shown to be associated with a worse prognosis. These include advanced age, male sex, tumor size, extrathyroidal extension, race, lymph node metastasis, and distant metastasis.[84] The factor with the most negative impact on disease-related morbidity is the recurrence of disease. Recurrent disease results in impaired quality of life, risk of reoperations, and exposure to high cumulative radioiodine dose, which leads to an elevated risk of complications, morbidity, and death.[60,84]

The BRAF V600E mutation has been widely reported to be associated with PTC prognosis, showing an adverse influence on tumor aggressiveness.[39,76] However, some studies did not find a significant association with prognosis.[7,28,36,44,58,66] In the present updated meta-analysis, we searched for the incidence of PTC recurrence/persistence, selecting papers that permitted extraction of sufficient data to calculate the risk ratio. All the studies included in the current meta-analysis were retrospective

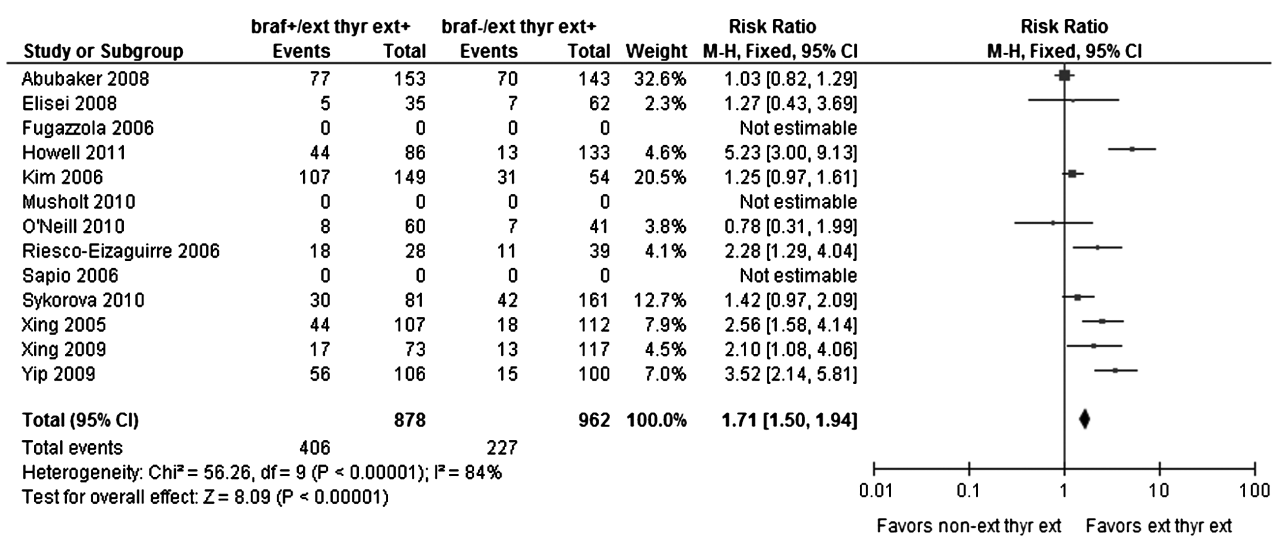

FIGURE 3. Risk ratio for extrathyroidal extension associated with BRAF status. (See Figure 1 for notes and abbreviations.) Abbreviations: ext thyr ext = extrathyroidal extension.

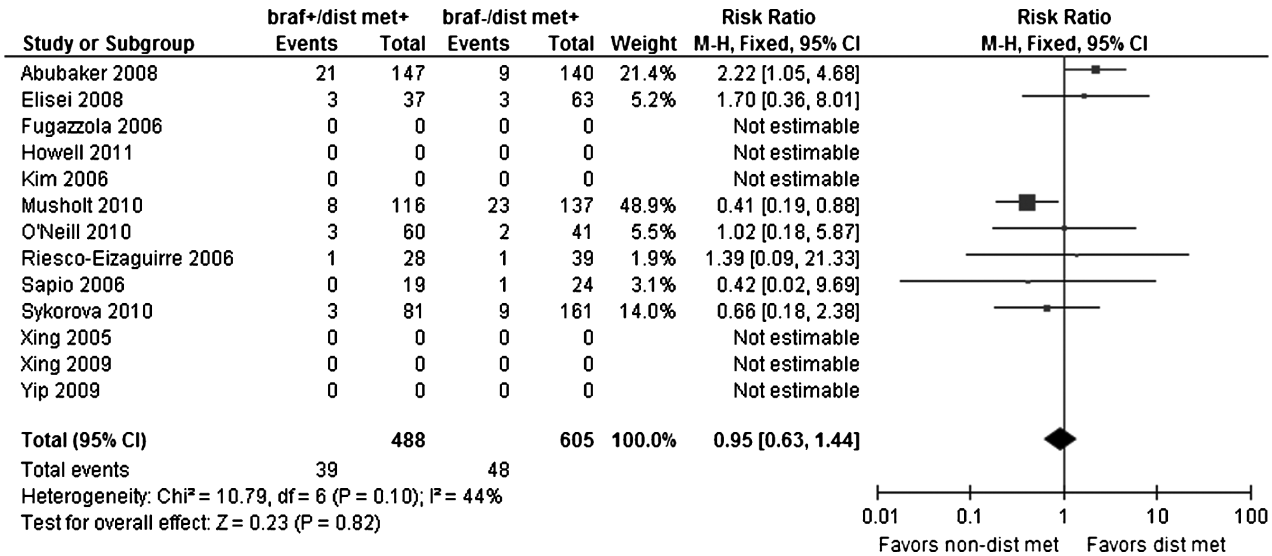

FIGURE 4. Risk ratio for distant metastasis associated with BRAF status. (See Figure 1 for notes and abbreviations.) Abbreviations: dist met = distant metastasis.

and analyzed different populations with heterogeneous study designs. This should be taken into account when interpreting the results of this study.

The most important association with prognosis in thyroid tumors is seen in PTC cases,[5,39,56] where the prevalence of this mutation is 45%, with a range of 29%-83%.[77] For follicular variants of PTC, the prevalence of this mutation is 20%, and for tall cell variant it has been reported as 50%.[28] As BRAF mutation is predominantly in PTC, for the current study we included only publications analyzing PTC. We found a 45% incidence of the BRAF V600E mutation, confirming its worldwide prevalence observed in PTC cases.[77] The benefit of finding a prognostic marker like BRAF V600E is only realized if it is able to be assayed before the definitive treatment is rendered. It would then allow for delineation of a customized approach based on the prediction of the tumor's biological behavior. The BRAF V600E mutation seems to fulfill these criteria.[33,50,55,80] In 2009, Xing et al[80] reported that BRAF mutation tested for on fine needle aspiration biopsy specimens before surgery was predictive for increased prevalence of extrathyroidal extension, thyroid capsular invasion, lymph node metastasis, and recurrence/persistence of the disease, and suggested that this information could be used to tailor the extent of the initial surgery.

The methods described for detecting BRAF V600E mutation in the studies included in the current meta-analysis showed a variation in the techniques. The techniques included direct sequencing of DNA after PCR amplification alone (the most common method),[1,14,28,35,51,52,58] or an associated calorimetric method,[80,81] DNA PCR-SSCP direct sequencing,[14,70] DNA PCR and fluorescence melting curve analysis,[23,83] and DNA-MASA sequencing.[66] Recently, the pyrosequencing analysis for detection of a BRAF V600E mutation was compared with the conventional direct DNA sequencing analysis. The 2008 study by Kim et al[33] showed the pyrosequencing method to be more sensitive, faster, less expensive, and superior, mainly in fine needle aspiration biopsy specimens of thyroid nodules. The

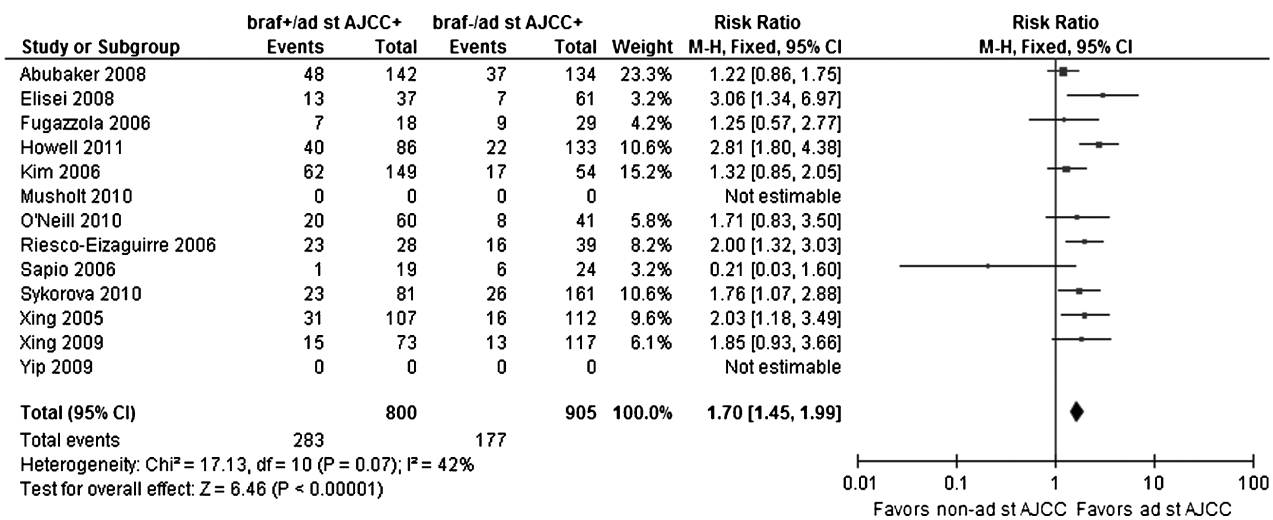

FIGURE 5. Risk ratio for advanced stage AJCC associated with BRAF status. (See Figure 1 for notes and abbreviations.) Abbreviations: ad st AJCC = advanced stage AJCC.

TABLE 4. Association of BRAF-Mutated Gene Status With Anatomopathologic Features and Recurrence in 39 Small (T1) PTC Patients

Feature	BRAF+ (n = 24) No. (%)	BRAF- (n = 15) No. (%)	Fisher Exact Test P Value	Risk Ratio (95% CI)	P
Age			0.19		
≥45 yr	12 (52)	11 (48)		2.75 (0.68–11.11)	0.15
<45 yr	12 (75)	4 (25)		Ref.	
LN metastasis			0.31		
Present	9 (75)	3 (25)		2.35 (0.45–16.50)	0.43
Absent	15 (56)	12 (44)		Ref.	
Extrathyroidal extension			0.45		
Present	6 (75)	2 (25)		2.13 (0.31–24.83)	0.65
Absent	18 (58)	13 (42)		Ref.	
Focality			0.52		
Multifocal	13 (57)	10 (43)		0.60 (0.12–2.69)	0.67
Unifocal	11 (69)	5 (31)		Ref.	
Surgical margins			0.53		
Compromised or ≤1 mm	14 (67)	7 (33)		1.58 (0.36–7.12)	0.70
Free	10 (56)	8 (44)		Ref.	
Recurrence*			0.99		
Present	2 (100)	0		NC	
Absent	13 (65)	7 (35)			

Abbreviations: LN = lymph node, NC = not calculated.

*Studied in a total of 22 patients with available follow-up records.

pyrosequencing analysis was the method used in our BRAF mutation test for the small primary PTC group studied in the present meta-analysis. In addition, a qualitative fluorescent rapid multiplex real-time PCR was designed to witness another BRAF mutation VK600-1E allowing the detection of homozygous and heterozygous status of these deletions.[59] The variations observed in the applied methodologies described in the current meta-analysis could interfere with the sensitivity of the mutation detection.

The aggressiveness of the PTC and an increased risk of recurrence/persistence has been observed with clinicopathologic factors such as age <15 years and >45 years, male sex, history of familial thyroid cancer, and tumor factors such as diameter >2 cm, multifocal, bilateral, extrathyroidal extension, subtypes like tall and columnar cell types, nuclear atypia and tumor necrosis, vascular invasion, lymph node metastasis, distant metastasis, and low iodine uptake.[75] BRAF V600E mutation has been related to almost all of these factors, which are used in predicting a disease recurrence/persistence event.[4,7,12,14,16,19,22,29,40,41,45,52,57,58,61,64,71,74,77,81] In the current meta-analysis we addressed the disease characteristics of extrathyroidal extension of the tumor, advanced AJCC stage, lymph node metastasis, and distant metastasis, to assess the relationship between these occurrences and the BRAF status of the tumor. All of these features are associated with recurrence/persistence of PTC.

BRAF and Lymph Node Metastasis

In the meta-analysis, the prevalence of lymph node metastasis was increased in patients who were BRAF mutation positive, with a risk ratio of 1.32 (see Figure 2). Increased risk was observed in 11 of 13 studies.[1,14,23,28,35,51,64,70,80,81,83] The risk was decreased in only 2 studies.[58,66] Although the risk ratio for lymph node metastasis was only moderately elevated, the majority of recurrent/persistent disease is due to this factor.[49,84] There is a bias in the estimates of prevalence of lymph node metastasis based on different institutional treatment protocols. O'Neill et al[58] (2010) reported selective elective central compartment neck dissections in 55% of BRAF-mutated cases and 59% of BRAF wt cases, finding lymph node metastasis in 33.3% and 39%, respectively. Howell et al[23] (2011) described an ipsilateral superficial prophylactic central compartment lymph node dissection for preoperatively diagnosed PTC on fine needle aspiration and bilateral central compartment neck dissection for preoperative ultrasound suspected or intraoperative detection of lymph node metastasis. In the study by O'Neill et al, the incidence of recurrence was 10.9% for BRAF-mutated patients and 25% for wt BRAF patients, and in the study by Howell et al it was 12.8% for BRAF-mutated patients and 2.3% for wt BRAF patients. This more aggressive approach, including elective cervical lymphadenectomy, described in these 2 studies probably influenced the outcome in the incidence of nodal recurrences. It is noteworthy that in the study by O'Neill et al,[58] BRAF mutation was significantly associated with clinically obvious PTC recurrence that required more aggressive treatments, although an apparent inverse association was observed when biochemical recurrence with mildly elevated Tg was also included. Kim et al[35] (2006) showed an increased prevalence of lymph node metastasis in the Korean population with lymph node metastasis in 77.8% of BRAF-mutated cases and 68.5% in BRAF wt cases, with a 17.7% overall incidence of recurrence. The difference in the prevalence of lymph node metastasis, comparing BRAF-mutated cases to BRAF wt cases, was statistically significant in 5 studies.[23,30,80,81,83] The overall prevalence of lymph node metastasis in the current meta-analysis was 45.2%, with 54.1% for BRAF-mutated cases and 36.8% for BRAF wt cases. This sample of a large number of patients from 9 countries showed an elevated prevalence of known lymph node metastasis regardless of BRAF status. Diagnostic methods, including anatomopathologic studies, blood assays, and radiologic

exams, have led to an increase in the incidence of diagnosed lymph node metastasis in the past few years.[25,28,46,84] Indeed, one of the most important aims of determining the relevance of BRAF expression preoperatively is to predict a worse prognosis, and in mutated cases, recommend a more comprehensive approach addressing the lymphatic drainage.[50]

BRAF and Extrathyroidal Extension

Recurrent/persistent disease significantly contributes to PTC morbidity. A residual invasive tumor has the potential risk to invade cervical structures such as the trachea, requiring more aggressive treatment with an increased chance of treatment morbidity. Extrathyroidal extension is an important factor related to PTC prognosis, contributing to an increased risk of local recurrence/persistence of the disease, despite the size of the tumor (see Figure 3).[46] The thyroid remnant is responsible for 8%–20% of recurrent cases.[28,49] In the current meta-analysis we found a strong association between BRAF-mutated expression and the risk of extrathyroidal extension, with a risk ratio of 1.71. The literature supports that this tendency has been the most commonly reported association between BRAF mutation and other clinical/pathologic features that have been reported to be associated with BRAF status. Xing et al[81] (2005), Riesco-Eizaguirre et al[64] (2006), Xing et al[80] (2009), and Yip et al[83] (2009) all found a statistically significant association between BRAF V600E mutation and extrathyroidal extension. Extrathyroidal extension carries an increased risk for recurrence of PTC and papillary microcarcinoma,[46] and decreased survival.[84] Furthermore, gross extrathyroidal extension influences disease-free survival and cause-specific survival.[28] In most situations, extrathyroidal extension is only a postoperative/anatomopathologic finding. The benefit of determining the BRAF status before surgery is that it could be used to inform the patient and justify the decision to perform a more extended and possibly aggressive surgical approach in BRAF-mutated tumors.[80]

BRAF and Distant Metastasis

Distant metastasis is an uncommon event in PTC cases, being observed in 1%–5% of cases.[49,84] It is generally associated with lymph node metastasis,[49] and carries a worse prognosis compared to other clinicopathologic features. Studies included in the current meta-analysis revealed a prevalence of distant metastasis in 8.0% of BRAF-mutated cases and 7.9% of BRAF wt cases, which was not a statistically significant association (see Figure 4). The total number of reported cases of distant metastases in these studies was 87 of 1093 patients, so there was limited power in detecting an association. BRAF V600E mutation is associated with impaired tumor cell iodine uptake, conferring a poor prognosis based on resistance to the conventional radioactive iodine adjuvant therapy recommended in those cases.[50] The effectiveness of radioactive iodine ablation is dependent on the ability of thyrocytes to take up iodide via a sodium-iodide symporter (NIS) mediated process, which is located on the cell membrane. Xing et al[80] reported the initial finding that BRAF mutation was associated with decreased radioactive iodine avidity in recurrent/persistent PTC, consistent with the 2006 study by Riesco-Eizaguirre et al[64] showing a dramatically decreased NIS expression and impaired NIS targeting to membranes in BRAF-mutated PTC.

BRAF and AJCC Stage of Disease

Because the AJCC staging system is a sum of tumor features including lymph node status and evidence of distant spread of the disease, we hypothesized that there was an association between BRAF status and AJCC stage. Confirming our hypothesis, AJCC stage III/IV was reported to have a strong association with BRAF mutation in the current meta-analysis, conferring a risk ratio of 1.70 (see Figure 5). The AJCC staging system defines stage III/IV as cases with tumor classification over T3 or any T with lymph node metastasis or distant metastasis in patients aged >45 years. Patients aged <45 years qualify for stages I and II only.[6] Some studies in the literature reported an association between age >45 years or 60 years and BRAF status, showing an increased incidence of this mutation in older patients.[2,11,14,30,31,52,54] Twelve of 14 studies evaluated in the current meta-analysis searched for an association between BRAF status and age. Elisei et al[12] (2008) found a relation between BRAF mutation and age over 60 years. Howell et al[23] (2011) observed that recurrent/persistent disease appeared to affect predominately patients who were both elderly and BRAF mutated, and these 2 criteria alone (age and BRAF status) were not enough to confer an increased risk for recurrence in their population. Xing et al[81] (2005), Riesco-Eizaguirre et al[64] (2006), and Elisei et al[12] reported a statistically significant association between BRAF-mutated tumors and advanced stage AJCC III/IV. BRAF mutation is an event that portends a more aggressive course of the tumor, and even in papillary microcarcinoma it conferred an increased incidence of extrathyroidal extension, multifocality, and nodal metastasis.[41,42]

The Role of BRAF V600E Mutation in PTC Recurrence/Persistence

The 5-year survival rate for PTC confined to the thyroid gland is 99.7%; for tumor spread to locoregional lymph nodes, 96.9%; and for distant metastatic disease, 57.8%.[69] Surveillance to detect disease recurrence or persistence is one of the most important goals of follow-up. Few patients will die of the disease, but a significant portion of these patients will experience a disease recurrence/persistence event that may require additional treatment and intensive follow-up that may lead to morbidity. To predict those patients more likely to have recurrent/persistent disease and define where more aggressive initial treatment may be justified to avoid recurrence, many clinicopathologic features have been studied, some of which are not available before initial treatment. The BRAF V600E mutation is an important pretreatment prognostic marker for PTC cases that may allow for a more customized initial treatment plan to reduce the risk of recurrence/persistence.

Several factors are associated with an increased prevalence of BRAF V600E mutation. High iodine intake, sometimes implicated in PTC pathogenesis, is one such factor. Male patients have also been reported to have an increased prevalence of BRAF V600E mutation.[20,39] Several studies have demonstrated the benefit of BRAF V600E mutation status assessed on fine needle aspiration biopsy as a complementary diagnostic tool.[33,51,80,83] The current meta-analysis showed that the BRAF V600E mutation was associated with lymph node metastasis, extrathyroidal extension, and advanced stage AJCC III/IV, disease characteristics that are directly related to the risk of recurrence/persistence of the disease. Beyond these findings, it has been suggested that the BRAF V600E mutation has a connection with patients with older age, larger tumor size, and aggressive subtypes.[2,11,12,14,30,31,35,52,54,57,64] The relationship of BRAF V600E mutation and other cell processes showed an association with an impaired NIS expression and also a decrease in iodide-metabolizing gene expression of TSH-R, Tg, and TPO.[11,57,64] Based on imaging, Barollo et al[4] (2010) reported a decreased uptake of ^{131}I in primary and recurrent/persistent disease in BRAF-mutated cases, with an inverse ability to concentrate ^{18}F-FDG in these cases. In their 2012 meta-analysis,

Kim et al[34] observed an elevated risk for extrathyroidal invasion, lymph node metastasis, and advanced stage in BRAF-mutated carriers. In summary, BRAF V600E mutation is associated with the factors stated above, which are related to an increased risk of recurrence/persistence of the PTC disease. Therefore, testing for the BRAF V600E mutation may be an effective predictor of prognosis in PTC cases. This information may be critical to appropriately tailor treatment to minimize tumor recurrence for an individual patient with PTC.

Primary PTC ≤1.5 cm Group Study

A molecular marker like BRAF that could help to predict prognosis would be most useful in early-stage PTC for customizing initial treatment.[72] The extent of initial thyroid resection (thyroid lobectomy vs. total thyroidectomy), the decision to perform an elective cervical nodal dissection at the time of thyroidectomy, and the decision to give radioactive iodine postoperatively are all controversial in early-stage PTC.[6] In our study of patients with primary PTC, 2 patients had a recurrence/persistence event in the form of lymph node metastasis in the central neck. Both of these patients were aged <45 years. The age is significant because in the American Joint Committee on Cancer (AJCC) staging system, age <45 years is stage 1 even with lymph node metastasis, and suggests a favorable overall prognosis. Unfortunately, the AJCC staging scheme does not necessarily differentiate the risk of a recurrence/persistence event for this patient population with PTC. Both patients with a recurrence/persistence event in our study also carried the BRAF mutation. The current meta-analysis has elucidated a clear association with lymph node metastasis and the BRAF mutation. Knowledge of a BRAF-positive primary PTC by fine needle aspiration before surgery could influence the surgeon to perform a formal elective central neck dissection to reduce this risk of lymph node recurrence/persistence. Our results in this study for lymph node metastases and extrathyroidal extension showed a pattern similar to the results found in the meta-analysis. Though not statistically significant, these 2 features were more prevalent among BRAF-mutated patients (see Table 4).

In our small primary PTC group study, the BRAF-mutated gene expression was easily studied using a pyrosequencing technique on paraffin-embedded tumor samples, which is the more sensitive, fast, and less expensive method to detect the mutation, compared to direct DNA sequencing.[10,33] Knowledge of this information at this point in the treatment (after surgery) may influence the decision to give radioactive iodine and the dose. Carefully designed prospective studies are necessary to confirm the utility of BRAF testing for customizing treatment in patients with PTC.

Conclusion

BRAF mutation evaluation represents a molecular marker test that has moved from "bench to bedside" and can be tested on paraffin-embedded, frozen tissue, and fine-needle aspirated samples of PTC for clinical decision making. Based on the current updated meta-analysis, BRAF testing is confirmed to hold great promise to refine initial treatments of PTC to minimize tumor recurrence.

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