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BRAF analysis before surgery for papillary thyroid carcinoma: correlation with clinicopathological features and prognosis in a single-institution prospective experience

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Abstract

Background: Risk stratification in patients with papillary thyroid carcinoma (PTC) currently relies on postoperative parameters. Testing for *BRAF* mutations preoperatively may serve as a novel tool for identifying PTC patients at risk of persistence/recurrence after surgery.
Methods: The study involved 185 consecutive patients with a histological diagnosis of PTC and *BRAF* analysis performed on thyroid fine-needle aspiration biopsy (FNAB). We assessed *BRAF* status in FNAB specimens obtained before thyroidectomy for PTC, and examined its association with the clinicopathological characteristics identified postoperatively, and with outcome after a mean 55±15 months of follow-up.
Results: One hundred and fifteen of 185 (62%) PTCs carried a *BRAF* mutation. Univariate analysis showed that *BRAF* status correlated with the histological variant of PTC, cancer size, and stage at diagnosis, but not with gender, age, multifocality, or lymph node involvement. *BRAF*-mutated cases had a higher prevalence of persistent/recurrent disease by the end of the follow-up (11% vs. 8%), but this difference was not statistically significant. The Kaplan-Meier curve shows that among the patients with persistent/recurrent disease, *BRAF*-mutated patients needed a second treatment earlier than patients with *BRAF* wild-type, although the difference did not completely reach the statistical significance.
Conclusions: Our study confirmed that preoperatively-identified *BRAF* mutation are associated with certain pathological features of PTC that correlate with prognosis. We speculate that it has a role in identifying PTCs that would generally be considered low-risk but that may reveal an aggressive behavior during their follow-up.

Keywords: *BRAF*; FNAB; prognosis; thyroid cancer.

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Introduction

Differentiated thyroid cancer (DTC) is the most common endocrine malignancy. Its worldwide incidence has been rising gradually in recent decades (due mainly to a considerable improvement in early detection procedures), while the mortality rate has remained relatively stable [1–5]. Well-differentiated papillary thyroid cancer (PTC) is the most common thyroid cancer subtype [6] and usually carries a favorable prognosis, with a survival rate of around 90% at 10 years. Eighty per cent of low-risk patients are successfully treated with primary surgery followed by radio-iodine (^{131}I) ablation [7]. Although PTC is generally indolent and curable, 5%–15% of patients experience local recurrences and/or distant metastases. One in three recurrences lose the

ability to trap ^{131}I, making the most important tool available for treating the tumor ineffectual. The prognosis becomes particularly unfavorable in such patients, their higher morbidity and mortality being due to the lack of effective therapies.

A primary goal of PTC management is consequently to stratify patients accurately in prognostic terms before and after surgery with a view to identifying the small proportion of cases with potentially aggressive disease who warrant tailored treatment and follow-up programs. Several well-standardized indicators (most of them only available postoperatively) are conventionally considered as high-risk factors, including older age at the time of diagnosis, large tumor size, aggressive histological variants, extra thyroidal invasion, lymph node metastasis, and distant metastasis. Each of these clinicopathological features has been found associated with a higher rate of recurrence, progression, and disease-related death [3–12].

Molecular cancer profiling, preferably before surgery, has promise as a novel tool for improving patient risk stratification and prognostics. Molecular status could be useful for tailoring clinical care too. *BRAF* mutations have proved to be the most common genetic event in the onset of PTC, responsible for around 45% of adult cases [13], and several studies have shown that *BRAF* analysis could be a useful, innovative diagnostic and prognostic [14] tool for: i) improving the diagnostic accuracy of fine-needle aspiration biopsy (FNAB) for primary PTC [15–20], and minimal metastatic disease to cervical lymph nodes [21, 22]; and ii) predicting patient outcome, because primary PTCs harboring *BRAF* mutations seem to be more aggressive and more prone to recur and to lose the ability to trap iodine [14, 23, 24].

The aims of the present study were: 1) to correlate preoperative *BRAF* status with classical prognostic indicators in a large consecutive series of patients with PTC managed at a single institution; and 2) to assess prospectively the impact of *BRAF* status per se on a patient's prognosis.

Materials and methods

Patients and follow-up

At our institution, *BRAF* mutation analysis in fine-needle aspiration biopsies (FNAB) is a standard procedure in patients with single thyroid nodules, and/or nodules showing suspect features on ultrasound. Among around 2000 consecutive thyroid FNAB on which *BRAF* status had been explored, we considered the first 185 cases (39 males and 146 females; mean age 48 years, median 49, range 22–81) that proved to be malignant on the final histology report, and for which an adequate follow-up was available at our Institution. Histological diagnoses and staging were done according to the TNM classification [25], and on the grounds of the outcome of the first whole body scan after ^{131}I remnant ablation. All patients involved in this study gave their informed written consent and the Institute's Ethical Regulations on Research on Human Tissues were followed.

After total thyroidectomy, ^{131}I remnant ablation (3.3–7.7 GBq) was performed after withdrawing levothyroxine in 44% of patients (82/186), and after administering recombinant human thyroid-stimulating hormone (rhTSH) in 56% (104/186).

All patients were assessed 4 and 12 months after remnant ablation, and those with a negative post-therapy whole-body scan outside the thyroid bed, negative neck ultrasound (US), no thyroglobulin autoantibodies (TgAb), and a suppressed Tg <2 ng/mL, underwent rhTSH-stimulated Tg assessment according to standard procedures (12 months after their ablation therapy). Beyond the first year, patients were routinely followed up at 6–12-monthly intervals. Additional FNAB cytology, Tg measurements, and other diagnostic studies such as CT and 18F-FDG PET were performed at the physician's discretion, depending on each patient's clinical features, or if persistent disease was suspected. Further surgical and/or ^{131}I treatments were planned if further disease was confirmed. Patients' treatment outcomes were categorized as reported elsewhere [26]. The mean patient follow-up was 55±15 months (min 16, max 93).

DNA extraction and *BRAF* status detection

DNA from FNAB material was isolated using the QIAamp DNA Micro kit (Qiagen, Italy) according to the manufacturer's protocol. The *BRAF* status of exon 15 was assessed both by direct sequencing and by mutant allele-specific PCR amplification (MASA) for the T to A substitution at nucleotide 1799 (V600E), based on descriptions in the literature [27]. We performed our statistical analysis on the direct sequencing results; in the event of discordant results (sequencing versus MASA), we confirmed the findings by assessing *BRAF* status in surgical specimens.

Statistical analysis

Categorical data were summarized using frequencies and percentages. Distributions of the continuous variables were assessed and data were summarized accordingly. Group comparisons of categorical variables were performed using the χ^2-test, or Fisher's exact test. Considering age as a continuous variable, and exploring its relationship with *BRAF*, we also used a ROC curve analysis to define the best age cut-off for predicting *BRAF* status and we sequentially performed a χ^2-test using this cut-off to assess the prevalence of *BRAF* mutations. Multivariate analysis, performed by means of logistic regression, has been used to confirm the independent role of different histopathological variables associated with *BRAF* status. The Kaplan-Meier survival curve has been used to verify the association between *BRAF* status and the time of planned second treatment in patients with persistent/recurrent disease.

Table 1: Correlation between persistent disease or PTC-related death and clinicopathological features of PTC.

		Total	Persistent/recurrent disease or PTC-related death[a]	Disease remission	p-Value
Gender	M	38/184 (21%)	8/38 (21%)	30/38 (79%)	0.037
	F	146/184 (79%)	12/146 (8%)	134/146 (92%)	
Age, years	<45	77/184 (42%)	6/77 (8%)	71/77 (92%)	0.338
	>45	107/184 (58%)	14/107 (13%)	93/107 (87%)	
	<60	143/184 (78%)	11/143 (8%)	132/143 (92%)	0.019
	>60	41/184 (22%)	9/41 (22%)	32/41 (78%)	
Tumor size, cm	Mean	1.64±0.99	2.03±1.22	1.60±0.96	0.04
Extrathyroidal extension	Yes	105/182 (58%)	19/105 (18%)	86/105 (82%)	0.001
	No	77/182 (42%)	1/77 (1%)	76/77 (99%)	
Multifocality	Yes	95/183 (52%)	11/95 (12%)	84/95 (88%)	0.816
	No	88/183 (48%)	9/88 (10%)	79/88 (90%)	
Lymph node metastases	Yes	80/183 (44%)	18/80 (23%)	62/80 (78%)	0.0002
	No	103/183 (56%)	2/103 (2%)	101/103 (98%)	
Histological variants	CV	131/184 (71%)	14/131 (11%)	117/131 (89%)	0.09
	TCV	20/184 (11%)	5/20 (25%)	15/20 (75%)	
	FV	9/184 (5%)	0/9 (0%)	9/9 (100%)	
	OV	24 /184 (13%)	1/24 (4%)	23/24 (96%)	
TNM stage	I	105/184 (57%)	6/105 (6%)	99/105 (94%)	<0.0001
	II	5/184 (3%)	0/5 (0%)	5/5 (100%)	
	III	51/184 (28%)	3/51 (6%)	48/51 (94%)	
	IV	23/184 (13%)	11/23 (48%)	12/23 (52%)	

CV, classical variant; TCV, tall cell variant; FV, follicular variant; OV, oxyphilic variant. [a]One patient who died due to another neoplasm was not considered.

Results

Clinicopathological findings

The histological variants of the 185 PTCs were as follows: classical in 71% of cases (131/184), tall cell in 11% (20/184), follicular in 5% (9/184), and oxyphilic in 13% (24/184). Multifocality, extrathyroidal extension, and lymph node metastases were identified in 52% (95/183), 58% (105/182), and 44% (80/183) of the cases, respectively. Only five patients had distant metastases at initial diagnosis. According to the TNM classification, 57% (105/184) patients were in stage I, 3% (5/184) were in stage II, 28% (51/184) were in stage III, and 12% (23/184) were in stage IV. At the end of the follow-up (mean 55±15 months, range 16–93), 17 patients (9%) had persistent or recurrent disease, 3 (2%) had died of their disease, and one had died due to another neoplasm. Age over 60 years, extrathyroidal extension, lymph node involvement and advanced stage were all significantly correlated with the risk of persistent/recurrent disease or disease-related death (Table 1).

BRAF analysis

Patients were divided into two groups depending on the presence or absence of *BRAF* mutations: group 1 consisted of cases with *BRAF* mutations (115 of 185, 62%), and group 2 of those without *BRAF* mutations (70 of 185, 38%). One patient revealed a K601E mutation, and another had both a BRAFV600E and a p.V600-S605>D mutation (data not shown).

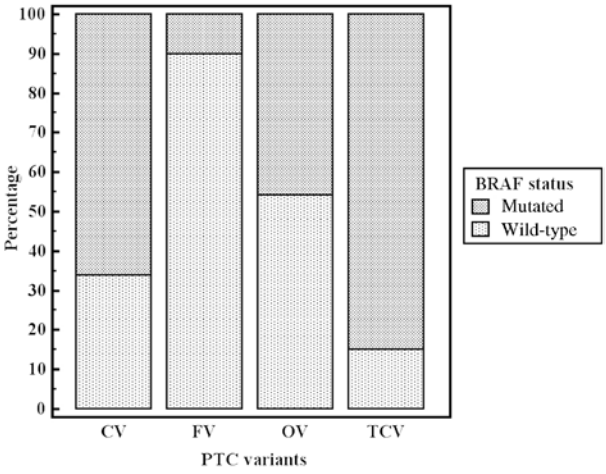

Figure 1: BRAF status according to PTC variants.
CV, classical variant of PTC; TCV, tall-cell variant of PTC; FV, follicular variant of PTC; OV, oxyphilic variant of PTC.

Univariate analysis showed that *BRAF* status correlated with several histological variants of PTC (p=0.0002, Figure 1), cancer size (p=0.002), and advanced stage at diagnosis (p=0.01), but not with gender, multifocality,

Table 2: Correlation between *BRAF*, clinicopathological features and final outcome in patients with PTC.

		Total	*BRAF* mutated	*BRAF* wild type	p-Value
Gender	M	39/185 (21%)	24/39 (62%)	15/39 (38%)	1.00
	F	146/185 (79%)	91/146 (62%)	55/146 (38%)	
Age, years	Mean	49±13	59±14	47±12	0.293
Tumor size, cm	Mean	1.64±0.99	1.52±0.85	1.85±1.18	0.002
Extrathyroidal extension	Yes	105/183 (57%)	72/105 (69%)	33/105 (31%)	0.06
	No	78/183 (43%)	43/78 (55%)	35/78 (45%)	
Multifocality	Yes	95/184 (52%)	64/95 (67%)	31/95 (33%)	0.10
	No	89/184 (48%)	51/89 (57%)	38/89 (43%)	
Lymph node metastases	Yes	80/184 (44%)	50/80 (63%)	30/80 (37%)	1.00
	No	104/184 (56%)	65/104 (62%)	39/104 (38%)	
Histological variants	CV	131/185 (71%)	86/131 (66%)	45/131 (34%)	0.0002
	TCV	20/185 (11%)	17/20 (85%)	3/20 (15%)	
	FV	10/185 (5%)	1/10 (10%)	9/10 (90%)	
	OV	24 /185 (13%)	11/24 (46%)	13/24 (54%)	
TNM stage	I	106/185 (57%)	66/106 (62%)	40/106 (38%)	0.01
	II	5/185 (3%)	0/5 (0%)	5/5 (100%)	
	III	51/185 (28%)	36/51 (71%)	15/51 (29%)	
	IV	23/185 (12%)	13/23 (57%)	10/23 (43%)	
Persistent or recurrent disease	Yes	17/181[a](9%)	13/17 (76%)	4/17 (24%)	0.296
	No	164/181 (91%)	101/164 (62%)	63/164 (38%)	

CV, classical variant; TCV, tall cell variant; FV, follicular variant; OV, oxyphilic variant. [a]One patient who died due to another neoplasm was not considered.

or lymph node involvement; and extrathyroidal extension showed only a trend towards a significant correlation (p=0.06) (Table 2). As for age, on ROC curve analysis, 54 years of age appeared to be the best cut-off for predicting *BRAF* status, mutations being more frequent in patients over 54 years old (72% of patients over 54 had *BRAF* mutations, as opposed to 58% of younger patients), though this difference did not reach statistical significance in the χ^2-test (p=0.05).

When distant metastases were considered, five patients revealed lung metastases on whole body scan after ablation therapy, and two of them had *BRAF* mutations. One of these five patients was not cured by the end of the follow-up and this patient had a *BRAF* mutation. Considering the patients with distant metastasis, both of them have only one neoplastic focus.

On multivariate analysis, only cancer size (OR 0.6, 95% CI 0.5–0.9), tall cell variant (OR 6, 95% CI 1.2–26) and follicular variant (OR 0.06, 95% CI 0.008–0.5) were confirmed as independent factors associated with *BRAF*-mutated status.

When outcome was analyzed, 68% (13/19) of patients with persistent/recurrent disease after surgery had *BRAF* mutations. At the end of the follow-up, PTC persisted or recurred in 11% of the *BRAF*-mutated patients versus 8% of those with *BRAF* wild-type (wt); this difference was not statistically significant (χ^2-test). Eighteen of the 19 patients with persistent/recurrent disease required another treatment (surgery or ^{131}I) during their follow up. Twelve of them (67%) had *BRAF* mutations, while six of them (33%) had *BRAF* wild-type. The Kaplan-Meier curve shows that among the patients with persistent/recurrent disease, *BRAF*-mutated patients needed a second treatment earlier (median 16, 23 months; 95% CI 11.66–20.80) than patients with *BRAF* wild-type (median 29 months; 95% CI 14.76–44.98) (Figure 2); though this difference did not reach statistical significance in the log rank test (p=0.05).

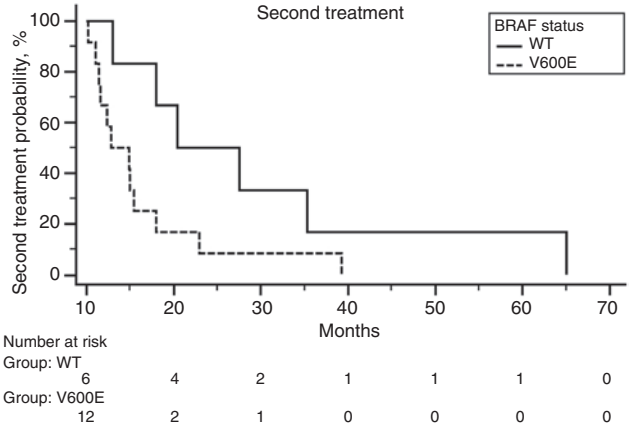

Figure 2: Kaplan-Meier curve for second treatment probability in patients with persistent/recurrent disease.
Correlation between BRAF status and the timing of the second treatment.

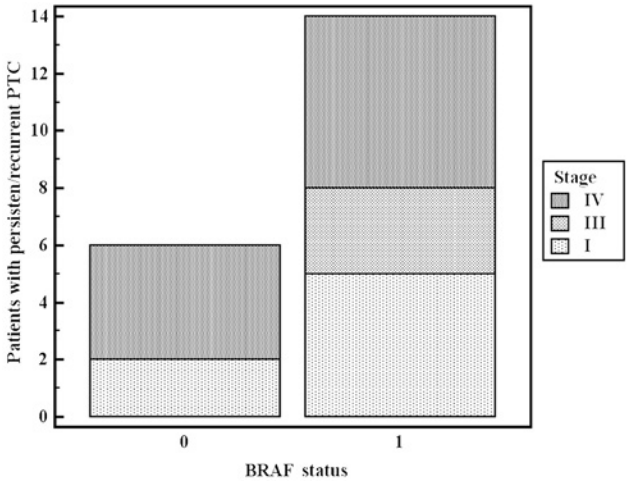

Figure 3: Stage of disease in patients with recurrent/persistent PTC by BRAF status.
BRAF status "0": cases with BRAF wild type. BRAF status "1": cases with BRAF mutations.

It is noteworthy that five of the 13 *BRAF*-mutated patients who had persistent/recurrent PTC were in stage I, three were in stage III, and five were in stage IV, whereas all but one of the *BRAF*-wt patients with persistent/recurrent disease were in stage IV (Figure 3). Only one of the three patients who died of progressive disease carried a *BRAF* mutation.

Discussion

Though relatively infrequent, differentiated thyroid cancer (DTC) is the most common endocrine malignancy. It is the human cancer with the fastest-rising incidence among women, and the second-fastest incidence among men [1–5], so new therapeutic approaches may be needed, particularly for the most aggressive subtypes.

Although many methods are available for treating DTC, little progress has made in improving overall survival for this malignancy. The typical treatment, based on surgical excision, oral levothyroxine suppression, and ^{131}I ablation, where necessary, is successful in nearly 90% of patients with PTC, while survival rates for patients failing to respond to this treatment or presenting with aggressive disease are rather low and there are few other therapeutic options. This makes it important to characterize cases of PTC in prognostic terms, when making decisions not only regarding their initial treatment (e.g. surgical and/or medical, extent of surgery, need for radio-iodine ablation), but also the aggressiveness of their follow-up.

The risk of persistent disease is conventionally judged on the basis of factors that unfortunately cannot be assessed preoperatively. Given the evidence that the BRAFV600E mutation is found in about 40% of PTC patients [28, 29], the relationship between *BRAF* and the potential aggressiveness of PTC has become a focus of interest, but there is a shortage of prospective studies. Three recent reviews [14, 30, 31] reported an association between *BRAF* and extra-thyroidal extension, higher clinical stage, and older age. These risk factors are conventionally associated with higher rates of thyroid cancer recurrence and related mortality [7, 32, 33].

In our samples, we found a proportion of *BRAF* mutations of around 62%, indicating a higher prevalence than the median value in the literature, but recent articles reported similar percentages in European series of PTC: *BRAF* mutations were found in 64% of PTCs in the report from Zatelli et al. [34], and in 62% of cases in the French series described by Porra et al. [29, 35]. Adopting strict criteria for selecting the nodules to assess may influence these proportions. The highly variable reported rates of *BRAF* mutations in PTCs could also relate to the diverse histological variants, epidemiological factors, and age groups analyzed [36]. Our series included a large number of tall cell variants, and this could be another reason for such a high frequency of *BRAF*-mutated cases. Interestingly, the frequency of the BRAFV600E mutation in PTC has risen gradually over the past two decades, from 28%–49% before to 58% after 2006 according to Romei et al. [37], for example, and from 43%–51% before to 88% after 2001 according to Mathur et al. [38].

In our sample, *BRAF* mutations were more common among patients over 54 years old at the time of their diagnosis. This age-related difference showed borderline statistical significance when age was analyzed in the sample as a whole by ROC analysis using a cutoff of 54 years of age ($p=0.05$), but not when the sample was separated into two age groups using a cutoff of 45 years old (the criterion adopted by the TNM), or 60 years old (the cutoff for defining a patient as 'old'). BRAFV600E is the most prevalent mutation in adult PTC patients, but very uncommon in children with PTC [39, 40], and several, albeit controversial studies have pointed to a higher frequency in older patients [30].

In our series, PTCs in *BRAF*-mutated patients were significantly smaller than those in *BRAF*-wt patients. The issue of tumor size is also controversial in the literature: some Authors found an association between *BRAF* mutations and larger tumor size [41], while Xing et al. showed a correlation with smaller tumor size [42]. *BRAF* mutations are frequently detected in papillary microcarcinomas

(in 25%–67.4% of cases evaluated in European and Asiatic series), likewise in papillary carcinomas larger than 1 cm [43]. Papillary microcarcinomas are believed to be an early form of PTC [44–46], and thyroid-targeted BRAFV600E transgenic mice have been shown to develop thyroid tumors with the features of PTC [47]. These findings support the conviction that *BRAF* mutation acts as an initiator in PTC.

The high frequency of *BRAF* mutations in the classical and tall cell variants of PTC (also reported elsewhere [14, 48]) suggests that they might be specific drivers of these PTC phenotypes. In our series, we found a clearly significant association between *BRAF* mutations and the classical (66%), and especially the tall cell (85%) variants of PTC, and a much weaker association with follicular PTC (10%), in which such mutations are quite rare.

As reported in our Results, we found no significant correlation between the BRAFV600E mutation and gender, multifocality or extrathyroidal extension. Further investigations are probably been needed to explain these findings.

We have investigated the mutational status of *BRAF* at presurgical level in FNAB as a standard procedure at our Institution in patients with single nodule, and/or nodules showing suspect features on ultrasound. Indeed, we did not investigate *BRAF* mutation in PTC's foci found only at histological level. In Literature there are evidences that some multifocal PTCs could have an independent clonal origin of distinct tumor foci, with the concurrent presence of both BRAFV600E and *BRAF* wild-type [49, 50]. Indeed, such topic is open to argument extensively, with other studies demonstrating the clonal origin of all cancer's cells [51]. Anyway, this could be a limit of the presurgical analysis of *BRAF* status and it could justify the absence of correlation between *BRAF* mutations and multifocality and extra thyroidal extension.

Consistently with previous reports, our prospective analysis confirmed the association between *BRAF* mutations and clinicopathological factors carrying a poor prognosis. The correlation with advanced stage at diagnosis was particularly obvious, and we found a different distribution of *BRAF* mutations coinciding with different stages of disease in our series. On the other hand, we found no correlation with lymph node metastasis. Reports in the literature seem to be inconsistent regarding this issue, possibly due to differences in the extent of neck dissection performed [14]. It is also worth mentioning a possible bias because *BRAF* analysis was conducted in FNAB specimens early on in our study, in patients who were usually clinically N0. To better elucidate the relationship between *BRAF* status and PTC metastatic potential, further studies analyzing the *BRAF* status both in all foci of PTC at primary level and in corresponding lymph node metastasis are been needed.

Surprisingly, distant metastasis – the most significant risk factor for PTC-related mortality – seems to be unassociated with *BRAF* mutation status. Only two large studies have identified a significant association [52, 53], while a recent study on 47 patients with aggressive PTC who developed distant metastases failed to demonstrate any significant association between the BRAFV600E mutation and systemic disease: only 30% of the patients with distant metastases had *BRAF* mutations [54]. In our study, two of five patients with distant metastases at diagnosis carried *BRAF* mutations. Because distant metastases are uncommon in patients with PTC, few studies have collected a sufficient number of cases to enable their relationship with *BRAF* mutations to be analyzed. In addition, since we considered *BRAF* status preoperatively, it may be that few of our patients had distant metastases because our FNAB cytology enabled patients to be identified and treated earlier, before they could develop disease that is more aggressive.

In a recent retrospective study that pooled data on approximately 2900 patients from 16 centers around the world, Xing et al. [55] found the BRAFV600E mutation an independent predictor of PTC recurrence, which was twice as common for *BRAF*-mutated PTC than for *BRAF*-wt disease (20.9% vs. 11.6%). Even in stage I PTC, 12.1% of *BRAF*-mutated patients experienced persistent/recurrent disease as opposed to 7.3% of *BRAF*-wt cases, after adjusting for age, gender, center and classical pathological factors indicative of a poor prognosis.

Our prospective study involved a follow-up of around 5 years for most patients: a clinical window within which around 76% of PTC cancer recurrences are expected to occur, as recently demonstrated [56]. Although the difference was not statistically significant in our sample, we found that 11% of *BRAF*-mutated patients had persistent or recurrent PTC versus only 8% of those with *BRAF*-wt cancer. PTC is not usually a lethal carcinoma and in our series only three patients died of progressive disease, so to verify the prognostic significance of *BRAF* mutations we used as endpoint not the survival information but the necessity of a second treatment during the follow-up. Interestingly we noted that among the patients with persistent/recurrent disease, *BRAF*-mutated patients needed a second treatment earlier than patients with *BRAF* wild-type, although the difference did not completely reach the statistical significance. This data allows us to speculate that in *BRAF*-mutated patients with persistent disease we have to do a stricter follow-up than in other patients

because the progression of the disease seems to be more rapid. We also found it noteworthy that 38% of our *BRAF*-mutated patients with persistent/recurrent disease had stage I PTC at the time of their diagnosis, whereas all but one of the *BRAF*-wt patients with persistent/recurrent disease were already in stage IV: 7% of our *BRAF*-mutated stage I PTCs persisted or recurred as opposed to 2% of the *BRAF*-wt cancers. Given that stages I and II are conventionally considered indicators of a better prognosis, we speculate that preoperative *BRAF* analysis could identify patients whose disease is more likely to reveal an aggressive behavior during their follow-up, even though they would have been classified as low-risk PTC on the basis of classical factors such as tumor stage. It is hard to say for sure how aggressively small, intrathyroidal low-risk PTCs should be treated. Although the mortality rate for small PTCs is low, a proportion of the affected patients show lymph node metastasis or persistent/recurrent disease after surgery [57]. That is why it is important to distinguish small, apparently low-risk intrathyroidal PTCs with a worse prognosis from those with an indolent course. Some variables, such as histological variant and extrathyroidal extension have prognostic value, but they can only be considered postoperatively, whereas *BRAF* mutations can be detected before surgery. Elisei et al. demonstrated a correlation between the BRAFV600E mutation and persistent disease at 5-year follow-up in patients with apparently low-risk intrathyroidal PTC; and these authors also showed that patients revealing *BRAF* mutations in their primary tumor usually have more radioiodine treatments [58]. Our results are consistent with this association, although it did not reach statistical significance in our sample due to our low percentage of recurrent/persistent cases.

We cannot conclude that *BRAF* mutations predict a poor prognosis in PTC. On multivariate analysis, we found *BRAF* mutations associated with histological variants that carry a poor prognosis and with smaller tumor size, which is generally a positive prognostic factor. Our data nonetheless suggest, consistently with the literature, that detecting *BRAF* prior to surgery could help us to identify cases of PTC that would generally be considered low-risk, but that might reveal an aggressive behavior during their follow-up. Further prospective studies will be needed to clarify this point.

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