$BRAF^{V600E}$ Mutation and Outcome of Patients with Papillary Thyroid Carcinoma: A 15-Year Median Follow-Up Study

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Background: The $BRAF^{V600E}$ mutation is the most frequent genetic alteration in papillary thyroid carcinoma (PTC). The role of $BRAF^{V600E}$ mutation as a poor prognostic factor has been controversially reported in series with short-term follow-ups. In this study we verified the prognostic value of the $BRAF^{V600E}$ mutation in PTC patients with a long-term follow-up.

Methods: We studied 102 PTC patients with a median follow-up of 15 yr. The $BRAF^{V600E}$ mutation was analyzed by PCR-single-strand conformational polymorphism and sequencing. The correlation between the presence/absence of the $BRAF^{V600E}$ mutation, clinicopathological features, and outcome of PTC patients were evaluated.

Results: The $BRAF^{V600E}$ mutation was found in 38 of 102 (37.3%) PTC patients, and was significantly more frequent in patients older than 60 yr ($P = 0.02$), in advanced stages ($P = 0.03$), and in cases with vascular invasion ($P = 0.02$). At univariate analysis the worst outcome for PTC patients was significantly correlated with clinicopathological features (*i.e.* age, tumor size, extrathyroid extension, lymph node and distant metastases, advanced stage, vascular endothelial growth factor expression, and vascular invasion) and the $BRAF^{V600E}$ mutation ($P < 0.002$). However, at multivariate analysis only the $BRAF^{V600E}$ mutation showed an independent correlation with the worst outcome ($P = 0.03$). Moreover, the survival curves of PTC patients showed a lower percentage of survivors in the $BRAF^{V600E}$-mutated group ($P = 0.015$).

Conclusions: In this study the $BRAF^{V600E}$ mutation correlated with the worst outcome for PTC patients, who were not only at a higher risk not to be cured but also for death. In particular, the $BRAF^{V600E}$ mutation was demonstrated to be a poor prognostic factor independent from other clinicopathological features. **(*J Clin Endocrinol Metab* 93: 3943–3949, 2008)**

Papillary thyroid carcinoma (PTC) is the most common endocrine malignancy (1). Several oncogenes and rearrangements with different prevalence and specificities (Ras, p53, RET/PTC, tyrosine kinase and PAX8- peroxisome proliferator activated receptor γ) involved in the pathogenesis of thyroid tumors have been isolated in the last few years (2). An activating mutation of the B isoform of the Raf kinase gene, located on exon 15, has recently been found to result in a valine to glutamic acid substitution at amino acid 600 ($BRAF^{V600E}$ mutation), and has been found to be the most common mutation in PTC (3, 4). The $BRAF^{V600E}$ mutation contributes to the destabilization of the kinase encoded by the gene, which then becomes constitutively activated and promotes tumorigenesis through the MAPK pathway. Its prevalence is highly variable, ranging from 29–83% among different publications (5, 6).

The $BRAF^{V600E}$ mutation shows a high specificity for PTC as well as anaplastic thyroid carcinoma, especially when deriving

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Abbreviations: IHC, Immunohistochemistry; 131-I, iodine-131; PTC, papillary thyroid carcinoma; SSCP, single-strand confirmational polymorphism; TNM, Tumor-Node-Metastases; VEGF, vascular endothelial growth factor.

from the dedifferentiation of PTC. Conversely, it has never been described in other thyroid cancer histotypes such as follicular thyroid carcinoma, medullary thyroid carcinoma, or benign thyroid neoplasms (7). The relationship between the $BRAF^{V600E}$ mutation and the clinical and pathological features of PTC remains controversial. Some authors have demonstrated that $BRAF^{V600E}$ mutations are more frequently present in PTC with an advanced tumor stage at diagnosis, whereas in other studies this association has not or has only been partially confirmed (6–11). Recently, the relationship between the $BRAF^{V600E}$ mutation and a higher aggressiveness of PTC has been attributed to a significantly higher expression of vascular endothelial growth factor (VEGF) in $BRAF^{V600E}$-mutated PTC patients (12).

Independent of the correlation of the $BRAF^{V600E}$ mutation with a more advanced stage at diagnosis, the question whether $BRAF^{V600E}$ mutation represents a poor prognostic factor for the outcome of PTC patients has yet to be elucidated. Only few and controversial evidence has been reported to date in studies with very short-term follow-ups (6, 9, 11). The aim of the present study was to evaluate the prognostic significance of the $BRAF^{V600E}$ mutation in a series of PTC patients who were diagnosed between 1985 and 1992. To determine the long-term prognostic significance, we searched for the $BRAF^{V600E}$ mutation in 102 PTC tissues, and correlated the presence of the mutation with both the outcome and other clinicopathological features of PTC patients. The survival curves of PTC patients were also analyzed according to the presence or absence of the $BRAF^{V600E}$ mutation in tumoral tissues.

Patients and Methods

Identification of the study group

A total of 200 archived paraffin-embedded thyroid tumoral tissues belonging to a series of consecutive patients who underwent total thyroidectomy and radioiodine (131-I) treatment for PTC between 1985 and 1992 was selected for DNA extraction. Among them, 102 PTC cases were chosen to represent the study group on the basis of the quality of DNA isolated. The comparison of the clinicopathological features and the outcome of the 98 cases excluded from this study demonstrated that there was no difference with respect to those of the 102 patients included.

All patients underwent total thyroidectomy with or without neck lymph node dissection at the Department of Surgery and were followed up at the Department of Endocrinology of the University Hospital of Pisa, Italy. As standard procedure, all patients were treated with radioiodine (30–100 mCi 131-I) to ablate the postsurgical thyroid remnant.

This study was approved by the institutional review board. Patients who were still alive provided their consent to the study.

Clinicopathological features of the study group

Of the 102 PTC patients, 20 were male and 82 female. The mean age at diagnosis was 42.8 ± 15.4 yr (median 39.5, range 12–78). An accurate retrospective analysis of the pathological features revealed that the mean size of the tumor was 2.0 ± 1.4 cm (median 1.5). With the exception of a few cases, we obtained information regarding multifocality, lymph node metastases, extrathyroid extension, and distant metastases that were present in 38.5% (37 of 96), 40% (40 of 100), 12.4% (12 of 97), and 6% (six of 100) of the cases, respectively. The classical variant was the most frequently observed (88 of 102: 86.3%), followed by oxyphilic (eight of 102: 7.8%) and follicular variants (five of 102: 4.9%). The tall cell variant was present in only one case. According to the De Groot's classification (13), 59 of 99 (59.6%) patients were in class I, 27 of 99 (27.3%) in class II, eight of 99 (8%) in class III, and five of 99 (5%) in class IV. Following the Tumor-Node-Metastases (TNM) classification (14), 60 of 98 (61.2%) were at stage I, 18 of 98 (18.4%) at stage II, five of 98 (5.1%) at stage III, and 15 of 98 (15.3%) were at stage IV.

Outcome

At the time of this study, the 102 PTC patients had a mean follow-up of 13.0 ± 5.4 yr (median 15, range 1–19). The majority of patients were actively followed at the Department of Endocrinology of the University Hospital of Pisa. Those few who were apparently not followed up were recontacted by telephone, and all of them consented to participate in a new clinical control to assess the presence or absence of recurrence.

In agreement with the recent guidelines for the diagnosis and management of thyroid cancer (15, 16), patients were considered cured (*i.e.* free of disease) when neck ultrasound and diagnostic whole body scans were negative, as well as when serum thyroglobulin and antithyroglobulin antibodies were undetectable in either hypothyroidism or after recombinant TSH stimulation. Patients who died of causes unrelated to PTC were considered cured or not cured according to their clinical status at time of death.

At the end of the study, 83 patients (81.4%) were cured, 13 (12.7%) had persistent disease, and six (5.9%) had died of metastatic PTC.

Pathology

All histological slides were independently reviewed by two pathologists (C.U. and F.B.) of the Department of Oncology at the University Hospital of Pisa. The sections (5-μm thick) obtained from tissues were fixed in 10% neutral buffered formalin, embedded in paraffin blocks, and were stained with hematoxylin and eosin for histological examination. The histological diagnosis was made according to the standard classification (17).

DNA extraction

Serial 5-μm sections were taken from paraffin blocks for DNA extraction from the primary tumor. The presence of tumor tissue was confirmed in the first and last section for each section series. Tumor tissue was manually microdissected from one to two sections, and samples were lysed and digested with proteinase K. DNA extraction was performed using the spin column procedure (QUIamp Mini Kit; QIAGEN, Inc., Valencia, CA) and finally reconstituted in 30 μl A/E buffer.

Detection of $BRAF^{V600E}$ mutations by PCR-single-strand conformational polymorphism (SSCP) and DNA sequencing

PCR-SSCP screening for the $BRAF^{V600E}$ mutation was performed with regard to exon 15 using the following primers: 5′(forward)-tca taa tgc ttg ctc tga tag ga, and 5′(reverse)-ggc caa aaa ttt aat cag tgg a (the resulting amplicon for exon 15 is 215 bp). Previously reported conditions were used for both PCR and SSCP (18). Altered migration patterns in two or three independent PCR-SSCP runs were considered to be indicative of DNA mutations. Purified PCR products were then sequenced on an ALF II automated sequencer (Amersham Biosciences, Freiberg, Germany) using a Thermo Sequenase Cy5 Dye Terminator Cycle Sequencing Kit (Amersham Biosciences). DNA sequences were compared with those of the normal BRAF gene exon 15 in the GenBank database using the Basic Alignment Search Tool software available at the National Center for Biotechnology Information (Bethesda, MD).

DNA extracted from ARO and TPC, two human thyroid cancer-derived cell lines known to be heterozygous and negative for the $BRAF^{V600E}$ mutation, respectively, were used as positive and negative controls.

Immunohistochemistry (IHC) for VEGF

There were 88 PTC samples available to study the VEGF expression by IHC. Tissue sections were incubated with primary antibody against

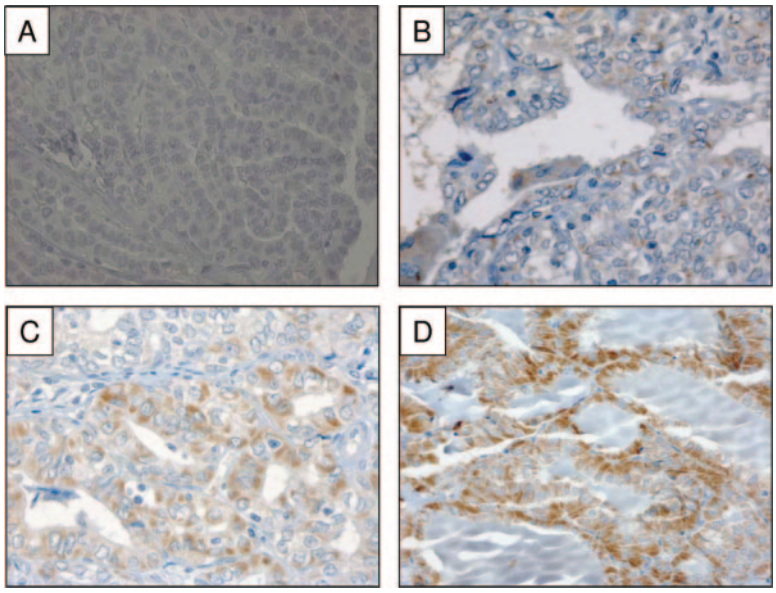

FIG. 1. IHC for VEGF (×40) in PTC. A, Negative control. B–D, Positive cases with different degrees of staining: weak (+), moderate (2+), and strong (2++), respectively.

VEGF (Santa Cruz Biotechnology, Inc, Santa Cruz, CA; 1:20). Negative controls (*i.e.* tissue sections incubated with PBS instead of the anti-VEGF antibody) and positive controls were also stained. Staining was scored according to the method previously proposed (12). Examples of the scoring are shown in Fig. 1. The same PTCs were also analyzed for the presence of vascular invasion.

Statistical analysis

We adopted the χ^2 test and Student's *t* test to analyze the clinical and pathological data of the patients with and without the $BRAF^{V600E}$ mutation. The multiple logistical regression test was used to determine the independent effect of the $BRAF^{V600E}$ mutation and the other clinical and pathological features on the outcome of PTC patients. Survival curves were analyzed using the Kaplan-Meier method, and statistical significance was assessed using the log-rank test. Data analysis was performed using StatView 4.5 software (Abacus Concepts Inc., Berkeley, CA). A *P* value less than 0.05 was regarded as statistically significant.

Results

Relationship between the $BRAF^{V600E}$ mutation and clinical and pathological features

Two groups of patients were distinguished according to the presence or absence of the $BRAF^{V600E}$ mutation in the tumoral tissue: group 1 comprising $BRAF^{V600E}$-mutated cases (38 of 102, 37.3%); and group 2 comprising $BRAF^{V600E}$-nonmutated cases (64 of 102, 62.7%).

TABLE 1. Correlation between $BRAF^{V600E}$ and clinical-pathological features of PTC

Clinical features	Group 1 BRAF+ (n = 38)	Group 2 BRAF− (n = 64)	*P* value
Mean follow-up ± SD (median)	11.6 ± 5.8 yr (14)	13.8 ± 5.0 yr (15)	0.2
Mean age ± SD (median)	45.9 ± 16.5 yr (43)	41.0 ± 14.6 yr (39)	0.1[a]
>60 yr	10/38 (26.3)	6/64 (9.3)	*0.02*[b]
Male sex	7/38 (18.4)	13/64 (20.3)	0.8
Mean tumor size ± SD (cm)	1.9 ± 1.3	2.0 ± 1.5	0.9[a]
>3 cm	5/37 (13.5%)[c]	11/61 (18%)[c]	0.7
Multifocality	14/35 (40.0%)[c]	23/61 (37.7%)[c]	0.8
Lymph node metastases	17/37 (45.9%)[c]	23/63 (36.5%)[c]	0.3
Extrathyroid extension	5/35 (14.3%)[c]	7/62 (11.3%)[c]	0.6
Distant metastases	3/37 (8.1%)[c]	3/63 (4.8%)[c]	0.5
De Groot's classes			0.5
I	19/37 (51.3%)[c]	40/62 (64.6%)[c]	
II	11/37 (29.8%)[c]	16/62 (25.8%)[c]	
III	4/37 (10.8%)[c]	4/62 (6.4%)[c]	
IV	3/37 (8.1%)[c]	2/62 (3.2%)[c]	
III + IV	7/37 (18.9%)[c]	6/62 (9.6%)[c]	0.2
Stage			*0.03*
I	18/37 (48.7%)[c]	42/61 (68.9%)[c]	
II	6/37 (16.2%)[c]	12/61 (19.7%)[c]	
III	4/37 (10.8%)[c]	1/61 (1.6%)[c]	
IV	9/37 (24.3%)[c]	6/61 (9.8%)[c]	
III + IV	13/37 (35.1%)[c]	7/61 (11.5%)[c]	*0.005*
VEGF expression (score 2 and 3)	19/33 (57.5%)[d]	31/55 (56.4%)[d]	0.4
Vascular invasion	15/33 (45.4%)[d]	12/55 (21.8%)[d]	*0.02*

Unless stated, values are number/total number (%). *P* values in *italics*, statistically significant.

[a] Age and tumora size analyzed as continuous variables with a *t* test.

[b] Data-related age cutoff found by analyzing different age cutoff.

[c] In a few cases, some pathological features were unknown.

[d] Of 102 PTC patients, 88 were also analyzed for VEGF expression and vascular invasion.

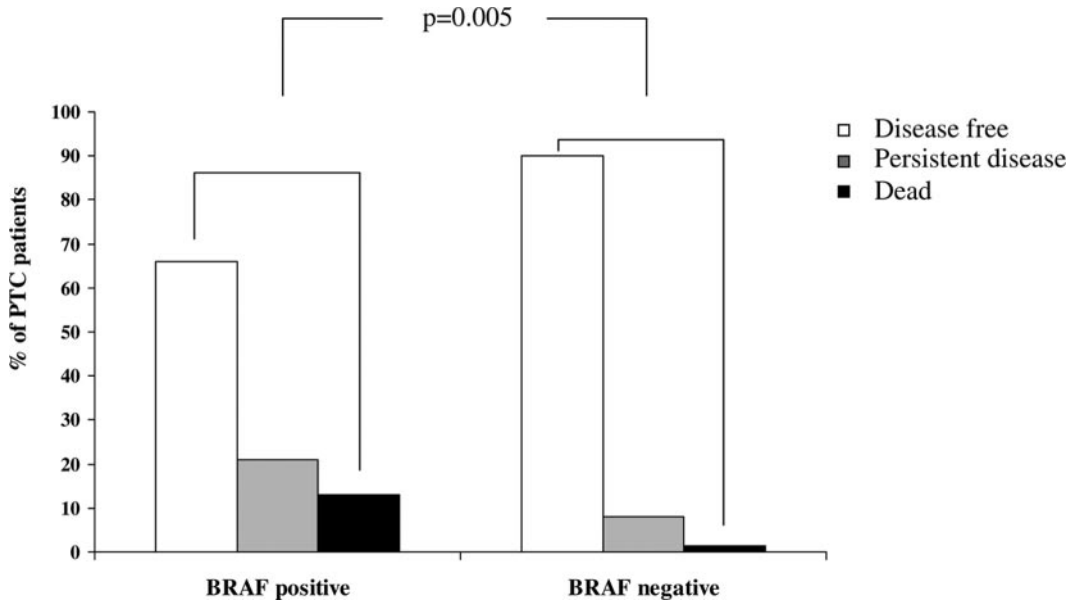

FIG. 2. Outcome of 102 PTC patients with (n = 38) and without (n = 64) the BRAF^{V600E} mutation in the tumoral tissue. A significantly higher prevalence of persistence of disease and/or death was observed in cases with the BRAF^{V600E} mutation with respect to those without.

Epidemiological, clinical, and pathological features analyzed in groups 1 and 2 are reported in Table 1.The univariate analysis showed that age at diagnosis older than 60 yr ($P = 0.02$), stage ($P = 0.03$), and vascular invasion ($P = 0.02$) were significantly correlated with the BRAF^{V600E} mutation, whereas gender ($P = 0.8$), tumor size ($P = 0.9$ and $P = 0.7$, by t test and χ^2 test, respectively), multifocality ($P = 0.8$), lymph node metastases ($P = 0.3$), extrathyroid extension ($P = 0.6$), distant metastases ($P = 0.5$), and VEGF levels of expression ($P = 0.4$) were not (Table 1).

As shown in Fig. 2, in the group of patients with the BRAF^{V600E} mutation, 25 (65.8%) were free of disease, eight (21.1%) had persistent disease, and five (13.1%) had died of thyroid carcinoma. On the other hand, in the group of patients without BRAF^{V600E} mutation, 58 (90.6%) were free of disease, five (7.8%) had persistent disease, and one (1.6%) had died of thyroid carcinoma. The different outcome of patients with and without BRAF^{V600E} mutation was statistically significant, regardless of whether the group of patients with persistent disease was analyzed separately from the group of deceased patients (Fig 2) or when they were pooled together (Table 2) ($P = 0.005$ and $P < 0.002$, respectively).

Correlation of BRAF^{V600E} and other clinicopathological features of PTC with the final outcome

As shown in Table 2, the univariate analysis of clinical and pathological features potentially influencing the outcome of our PTC patients revealed that advanced age at diagnosis (>60 yr), tumor size larger than 3 cm, the presence of lymph node metastases, extrathyroid extension, presence of distant metastases, advanced clinical presentation (both according to TNM and De Groot's classification), VEGF expression (score 2 and 3), and vascular invasion were significantly correlated with a worse prognosis. In addition, the BRAF^{V600E} mutation was significantly correlated with a poorer outcome using a univariate analysis ($P < 0.002$).

Despite the great number of prognostic factors identified by univariate analysis, the multivariate logistical regression test showed that only BRAF^{V600E} mutation independently correlated with a poorer outcome of our PTC patients ($P = 0.03$) (Table 3).

Furthermore, when the survival curves of PTC patients with and without the BRAF^{V600E} mutation were evaluated, a significantly lower percentage of surviving patients was found in the group harboring the BRAF^{V600E} mutation ($P = 0.015$) (Fig. 3).

Discussion

Although PTC shows a generally good prognosis with a very high survival rate, 10–15% of the cases are not cured by the initial treatment (*i.e.* total thyroidectomy and postsurgical thyroid remnant 131-I ablation). These cases usually require more aggressive treatments that often fail in reaching a definitive cure for the patients (19). Several prognostic factors for the early identification of this smaller, but not negligible, percentage of higher risk PTC patients have previously been described (13, 20–23).

After the recent evidence that the BRAF^{V600E} mutation is found in about 40% of PTC patients (5, 6), the correlation of the mutation with more aggressive features of PTC and its prognostic role has been explored by several authors who obtained interesting opposing results. Recently, these controversial results have been discussed in two very comprehensive reviews (24, 25), which have shown that the most consistent correlations are those observed between the mutation and both an older age and an advanced stage at diagnosis. As in our multicentric study (6) as well as in the present PTC Italian series, we found that the BRAF^{V600E} mutation was significantly more frequent in older patients and, in particular, in those older than 60 yr. This finding

TABLE 2. Correlation of $BRAF^{V600E}$ and other clinical-pathological features of PTC with outcome

Clinical features	Free of disease (n = 83)	Persistent disease and dead patients (n = 19)	*P* value
Mean age ± SD	41.5 ± 13.9 yr	48.7 ± 20.2 yr	0.06[a]
>60 yr	9/83 (10.8)	7/19 (36.8)	*0.005*[b]
Male sex	15/83 (18.1)	5/19 (26.3)	0.4
Mean tumor size ± SD (cm)	1.8 ± 1.2	2.6 ± 1.8	*0.009*[a]
>3 cm	8/80 (10%)[c]	8/18 (44%)[c]	*0.0004*
Multifocality	28/80 (35%)[c]	9/16 (56.3%)[c]	0.1
Lymph node metastases	27/82 (32.9%)[c]	13/18 (72.2%)[c]	*0.002*
Extrathyroid extension	5/81 (6.2%)[c]	7/16 (43.8%)[c]	*<0.0001*
Distant metastases	1/82 (1.2%)[c]	5/18 (27.8%)[c]	*<0.0001*
De Groot's classes			*<0.0001*
I	55/81 (67.9%)[c]	4/18 (22.2%)[c]	
II	21/81 (25.9%)[c]	6/18 (33.4%)[c]	
III	4/81 (4.9%)[c]	4/18 (22.2%)[c]	
IV	1/81 (1.3%)[c]	4/18 (22.2%)[c]	
III + IV	5/81 (6.2%)[c]	8/18 (44.4%)[c]	*<0.0001*
Stage			*0.0006*
I	54/80 (67.5%)[c]	6/18 (33.4%)[c]	
II	16/80 (20%)[c]	2/18 (11.1%)[c]	
III	3/80 (3.7%)[c]	2/18 (11.1%)[c]	
IV	7/80 (8.8%)[c]	8/18 (44.4%)[c]	
III + IV	10/80 (12.5%)[c]	10/18 (55.5%)[c]	*<0.0001*
VEGF expression (score 2 and 3)	40/73 (54.8%)[d]	13/15 (86.6%)[d]	*0.02*
Vascular invasion	18/73 (24.6%)[d]	9/15 (60%)[d]	*0.006*
$BRAF^{V600E}$ mutation	25/83 (30.1%)[c]	13/19 (68.4%)[c]	*<0.002*

Unless stated, values are number/total number (%). *P* values in *italics*, statistically significant.

[a] Age and tumor size analyzed as continuous variables with a *t* test.

[b] Data-related age cutoff found by analyzing different age cutoff.

[c] In a few cases, some pathological features were unknown.

[d] Of 102 PTC patients, 88 were also analyzed for VEGF expression and vascular invasion.

is consistent with the data that an advanced age at diagnosis is a poor prognostic factor in several scoring systems (14, 20, 26, 27). However, it is worth noting that we obtained a statistical significance only when the patients' age was analyzed by separating the study group into two categories (younger and older than 60 yr), but not when the age was analyzed as a continuous variable. As a matter of fact, the $BRAF^{V600E}$ mutation is very uncommon in papillary childhood thyroid cancer (28, 29), whereas it is the most prevalent oncogene in adult PTC patients, with several controversial findings showing a significantly increased frequency in older patients (25).

As far as the correlation with an advanced stage at diagnosis is concerned, in this series we found a negative correlation when the De Groot's classification was used and a positive correlation when the TNM staging was used. In this regard, it is worth noting that several authors have attempted to find the best scoring system in predicting the outcome of PTC patients (30–32). Alternatively, the results of these studies have indicated either one of these systems as being the best. We agree with the observation of Leboulleux *et al.* (33), who clearly demonstrated that TNM is good at predicting the death event, but because it does not consider the lymph node metastases, it is poor at predicting the recurrence and persistence of the disease, which are indeed the major clinical difficulties in the outcome of PTC patients (27, 31).

TABLE 3. Multivariate logistical regression analysis of parameters correlated with a poorer outcome for PTC patients

Clinical features	Odds ratio	95% CI	*P* value
Age at diagnosis >60 yr	1.25	0.08–19.28	0.87
Tumor size (continuous variable)	0.51	0.25–1.04	0.06
De Groot's classes	10.97	0.72–166.84	0.08
Stage (TNM)	1.02	0.89–1.16	0.73
VEGF (score 2–3)	1.20	0.95–1.51	0.12
Vascular invasion	0.97	0.10–9.00	0.98
BRAF mutation	14.63	1.28–167.29	*0.03*

Despite the dissimilar results obtained in the different series analyzed, the role of the $BRAF^{V600E}$ mutation as a poor prognostic marker is generally accepted, primarily because it has been found to be more frequently associated with PTC diagnosed at a more advanced stage (24). However, in our opinion this correlation is not necessarily predictive of a poor prognosis, as suggested by the evidence that childhood PTC is usually very aggressive at diagnosis but very well responsive to conventional therapies (34, 35). Interestingly, the prevalence of the BRAF mutation in childhood PTC is low (28, 29), suggesting that an advanced stage not associated with the presence of the BRAF mutation is more prone to a good outcome.

As far as the prognostic role of the $BRAF^{V600E}$ mutation is concerned, there are only two studies (6, 9) that have analyzed

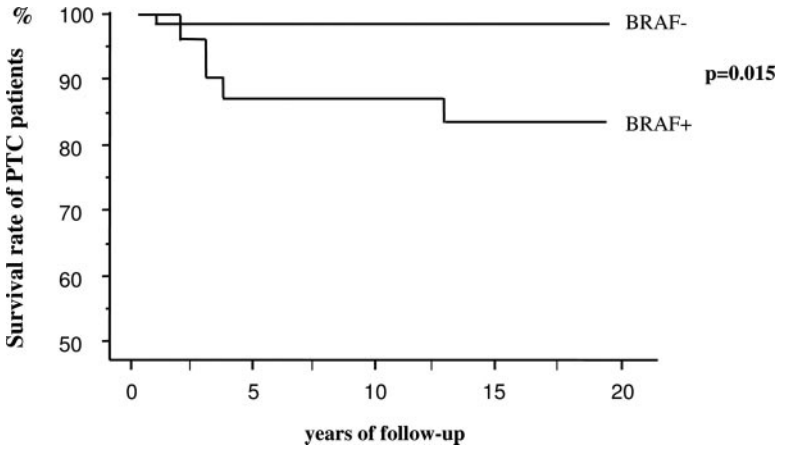

FIG. 3. Survival curves of PTC patients with and without the BRAF^{V600E} mutation. Patients with thyroid tumors harboring the BRAF^{V600E} mutation showed a significantly lower survival rate ($P = 0.015$, by log-rank test).

the relationship between the oncogene activation and the outcome of PTC patients with a relatively long follow-up (6 and 7.3 yr, respectively). In the present series, using a median follow-up of 15 yr (the longest to our knowledge), the BRAF^{V600E} mutation has been shown to act as a poor prognostic factor for the outcome of PTC patients (cured *vs.* not cured or deceased patients). In particular, we have demonstrated that the presence of the BRAF^{V600E} mutation represents *per se* a risk factor for the unsuccessful treatment of PTC patients independent from all other well-known negative prognostic factors and, in particular, from the extension of the tumor at the time of surgical treatment, either when considered according to De Groot's classification or TNM staging. This finding was consistent with previous observations, demonstrating a negative prognostic role of the BRAF mutation for the outcome of PTC patients with a relatively short follow-up (11). Moreover, in this study we have provided the evidence that PTC patients with the BRAF^{V600E} mutation not only have a greater probability to be not cured but also that they have a higher probability to die of the tumoral disease. This is demonstrated by their significantly worse 19-yr survival rate with respect to PTC patients lacking the mutation.

One possible explanation for this strong impact of the BRAF^{V600E} mutation on the outcome may be related to the recent evidence that the mutation correlates with a lower expression of sodium/iodide symporter (NIS) and thyroperoxidase (TPO) mRNA and related proteins (36–38), and that the BRAF^{V600E} mutation is associated with a higher risk of developing PTC recurrence with a poor ability to take up 131-I (11, 39). Because the success of the radioiodine 131-I treatment, which represents the most important and effective therapeutic procedure for PTC, depends mainly on the ability of tumoral cells to incorporate 131-I, it is conceivable that tumors with lower expression of both NIS and TPO are more resistant to this treatment. According to this evidence, we hypothesize that the role of the BRAF^{V600E} mutation as a poor prognostic factor is related primarily to the dedifferentiation process more than to its correlation with more advanced stages of PTC at diagnosis. This hypothesis needs to be confirmed in future studies.

Although we could not confirm a significantly higher expression of VEGF in BRAF^{V600E}-mutated PTC patients (12), we found a significant correlation of the BRAF^{V600E} mutation with an increase in vascular invasion. This finding may represent another possible explanation for the correlation of the BRAF^{V600E} mutation and a worse prognosis. It is worth noting that vascular invasion has been a poor prognostic factor for the outcome of PTC patients both in the present study and in other series already published (40). We can hypothesize that BRAF^{V600E}-mutated PTC patients are more prone to new angiogenesis and vascular invasion that would be responsible for either the tumor growth or extrathyroid extension.

In conclusion, our 15-yr follow-up study clearly demonstrated a significant correlation between the BRAF^{V600E} mutation and a worse outcome of patients with PTC. The BRAF^{V600E} mutation was found to be significantly more frequent in older patients and in cases with an advanced stage according to the TNM staging, but not with the clinical De Groot's classification. However, despite these correlations the multivariate analysis demonstrated that the BRAF^{V600E} mutation is a poor prognostic factor independent from other negative prognostic features such as advanced age, larger tumor size, the greater local extent, presence of local or distant metastases, and vascular invasion. Finally, to our knowledge this is the first study demonstrating that BRAF^{V600E}-positive PTC patients have not only a worse outcome in terms of persistent disease, they also have a lower survival rate.

Acknowledgments

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