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Morphological features predictive for *BRAF*^{V600E} mutation in papillary thyroid microcarcinomas

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Abstract

Background: The B-Raf proto-oncogene serine/threonine kinase (BRAF) V600E (*BRAF*^{V600E}) mutation represents a very specific marker for papillary thyroid carcinoma (PTC), including microcarcinomas (PTMCs). However, assessment of the *BRAF*^{V600E} mutational status is expensive and not available in all pathology laboratories. *Aim*: We aimed to evaluate if we can identify those morphological features that could predict the presence of the *BRAF*^{V600E} mutation in a series of PTMCs. *Materials and Methods*: Nineteen PTMCs with analysis of 25 tumor foci were included. The following histological features were evaluated: size of the tumor, multifocality, extrathyroidal extension, tumor's border, characteristic PTC nuclear features, tumor-associated stromal reaction and histological variant. All PTMCs foci were subject to real-time polymerase chain reaction (RT-PCR) amplification targeting the *BRAF* gene. *BRAF*^{V600E} mutation was assessed by high resolution melting (HRM) analysis and confirmed by Sanger sequencing. Morphological features associated with *BRAF*^{V600E} positive and *BRAF*^{V600E} negative PTMCs were compared using the two-tailed Fisher's exact test, with α set at ≤0.05. *Results*: Out of the 25 PTMC foci, 16 (64%) were *BRAF*^{V600E} negative, whereas nine (36%) were *BRAF*^{V600E} positive. Our data showed that subcapsular localization (p=0.013), conventional histological type (p=0.05) and tumor-associated stromal reaction (moderate/extensive fibrosis) (p=0.032) were significantly associated with the mutation. *Conclusions*: We have demonstrated the value of several morphological features in predicting a *BRAF*^{V600E} mutation profile in PTMCs. All these parameters should be documented in the histopathological report, as they seem to be associated with this mutation and could serve as a risk stratification tool in the selection of patients in need for adjuvant post-surgery therapy.

Keywords: thyroid, papillary microcarcinoma, *BRAF* gene, morphology.

Introduction

The *World Health Organization* (WHO) [1] defines papillary thyroid microcarcinoma (PTMC) as a papillary thyroid carcinoma (PTC) incidentally found measuring ≤1 cm. Many studies over the world have reported a significant increasing incidence of PTMC in the last decades [2–5]. Despite this growing incidence of PTMC, the disease-specific mortality rate remains extremely low, reported to be 0.5% [6]. Due to all these, it has now become increasingly challenging for clinicians to manage patients with PTMC, as they must carefully weigh the benefits of treatment to potential harms [7]. While the great majority of patients with PTMC will have an excellent overall survival, some patients could develop recurrence and/or metastasis in the follow-up period that might require re-operation or radioactive iodine ablation. Identifying that small subset of PTMC patients with potential unfavorable outcome is important in order to ensure a correct management and treatment for those patients.

Great emphasizes has lately been granted to the molecular alteration that characterize PTC. B-Raf proto-oncogene serine/threonine kinase (*BRAF*) gene mutations, in particular, have received considerable attention. *BRAF* mutations occur on the "p" arm of the seventh chromosome, where the gene is located. The most common mutation identified in thyroid cancer is a thymine to adenine transversion at nucleotide 1799 (T1799A), causing a substitution of valine by glutamic acid at codon 600 on the BRAF protein (V600E) [8]. The mutation activates the mitogen-activated protein kinase (MAPK) signaling pathway [9], which plays a major role in the regulation of cell growth, division and proliferation [10]. *BRAF*^{V600E} mutation has been reported in 40–70% of PTCs [11–13], making it the most frequent genetic abnormality in PTC.

A large, recent meta-analysis has demonstrated a positive association between the *BRAF*^{V600E} mutation profile and the risk of recurrence in PTMC [14]. The authors highlighted the potential utility of *BRAF*^{V600E} genotyping in the post-operative planning of the patients with PTMC and concluded that *BRAF*^{V600E} mutation may serve as a helpful tool in the risk-stratification of these patients.

However, the assessment of the *BRAF*^{V600E} mutational status in all PTMCs is expensive and not available in all pathology laboratories. On the other hand, Virk *et al.* have demonstrated that *BRAF*^{V600E} mutated PTMCs are morphologically distinct [15] and proposed a set of

morphological criteria able to predict their *BRAF*^{V600E} mutation status with great accuracy and substantial inter-observer reproducibility [16]. Thus, the aim of our study was to evaluate if we can identify some morphological features that could predict the presence of the *BRAF*^{V600E} mutation in our series of PTMCs.

Materials and Methods

Study group and inclusion criteria

All consecutive PTMCs registered at the Department of Pathology at the Emergency County Hospital, Tîrgu Mureş, Romania, from January 2011 to January 2014 were re-evaluated. Criteria for inclusion were: (*i*) a histopathological diagnosis consistent with PTMC; (*ii*) tumor size ≥5 mm, as the morphological features may not be adequately developed in smaller tumors, and thus, may be unreliable in predicting the mutational status [16]; (*iii*) adequacy of tumor tissue for deoxyribonucleic acid (DNA) extraction for molecular analysis.

The Ethics Committee from the University of Medicine and Pharmacy of Tîrgu Mureş agreed to the present study and informed consent was obtained from all patients included in the study.

Pathological and clinical data

For all the cases, Hematoxylin–Eosin (HE) stained slides were independently reviewed by three pathologists (ACNB, EAS, AB). All the controversial cases were evaluated in panel on a multi-headed microscope and a consensus regarding the discordant features was reached.

PTMCs were referred to PTCs of 1 cm or less, incidentally found [1] (Figure 1A). The diagnosis of PTC was based only on the nuclear features, as defined in the *WHO Classification of Tumors of Endocrine Organs* [1]. We evaluated the following pathological features: the size of the tumor, the extrathyroidal extension, the multifocality, the tumor position in relation to the thyroid capsula (peripheral/subcapsular *versus* intrathyroidal), the surgical positive resection margin, the tumor interface with non-neoplastic thyroid tissue (well delimited, not encapsulated/encapsulated *versus* infiltrative) (Figure 1, A and B), the histological variant (conventional, follicular, tall cell, oncocytic) (Figure 1, C and D), the nuclear features (Figure 2), the tumor cells characteristics (the presence of "plump pink" cells) (Figure 2D), the tumor-associated stromal reaction (absence of fibrosis, fibrosis grade 1+, fibrosis grade 2+, see below) (Figure 3), the presence of psammoma bodies, stromal calcification, intratumoral lymphocytic infiltrate, back-to-back arrangement, intratumoral multinucleated giant cells and lymph node involvement (Table 1).

In accordance with the *2016 American Joint Committee on Cancer (AJCC) Cancer Staging Manual* [17], extrathyroidal extension was referred to as tumor invasion into the strap muscles (sternohyoid, sternothyroid or omohyoid muscles). Multifocality was defined as the presence of two or more isolated/non-contiguous tumor foci in one or both thyroid lobes.

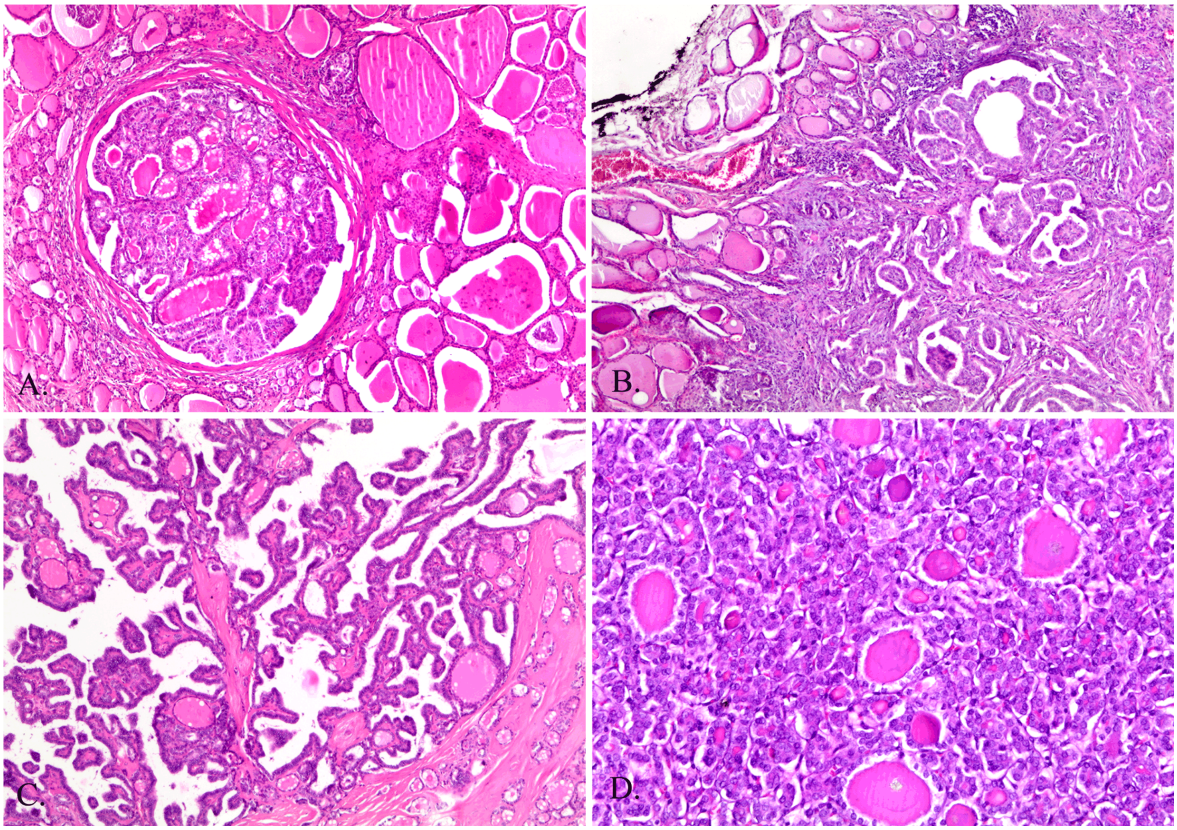

Figure 1 – ***Papillary thyroid microcarcinoma referred to as papillary thyroid carcinomas of 1 cm or less, incidentally found (A); assessment of the tumor's border: an encapsulated (A)*** **versus** ***an infiltrative tumor (B); conventional histology, characterized by papillary architecture that was pure or admixed with a variable proportion of follicles (C); follicular variant, with an exclusively follicular pattern and virtually no papillary structures (D). HE staining: (A and B) ×40; (C and D) ×100.***

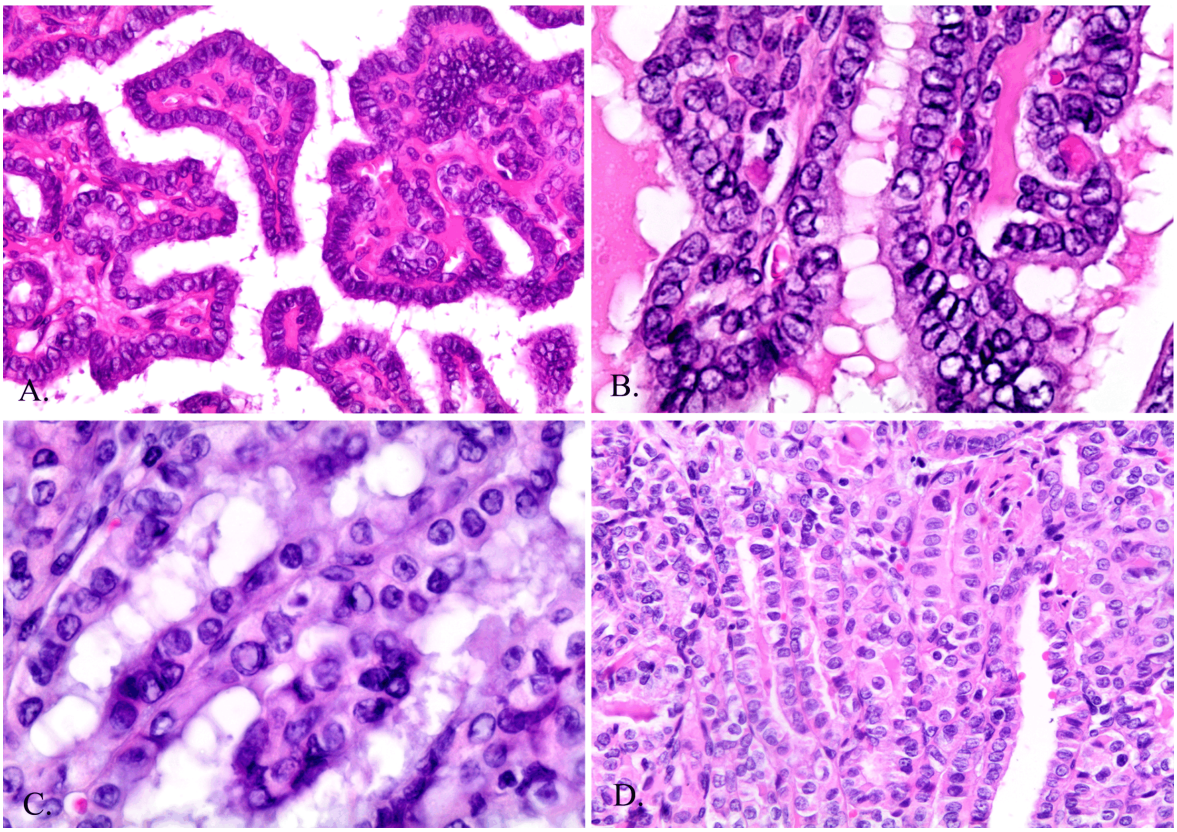

Figure 2 – ***Papillary thyroid microcarcinoma, characteristic nuclear features: nuclear enlargement, overlapping, irregularity of the nuclear contours, grooves, clearing or ground glass appearance and nuclear pseudoinclusions (A–C); characteristic nuclear features in the setting of "plump" polygonal, tumor cells, with moderate to abundant, homogenous, eosinophilic cytoplasm (D). HE staining: (A and D) ×200; (B and C) ×400.***

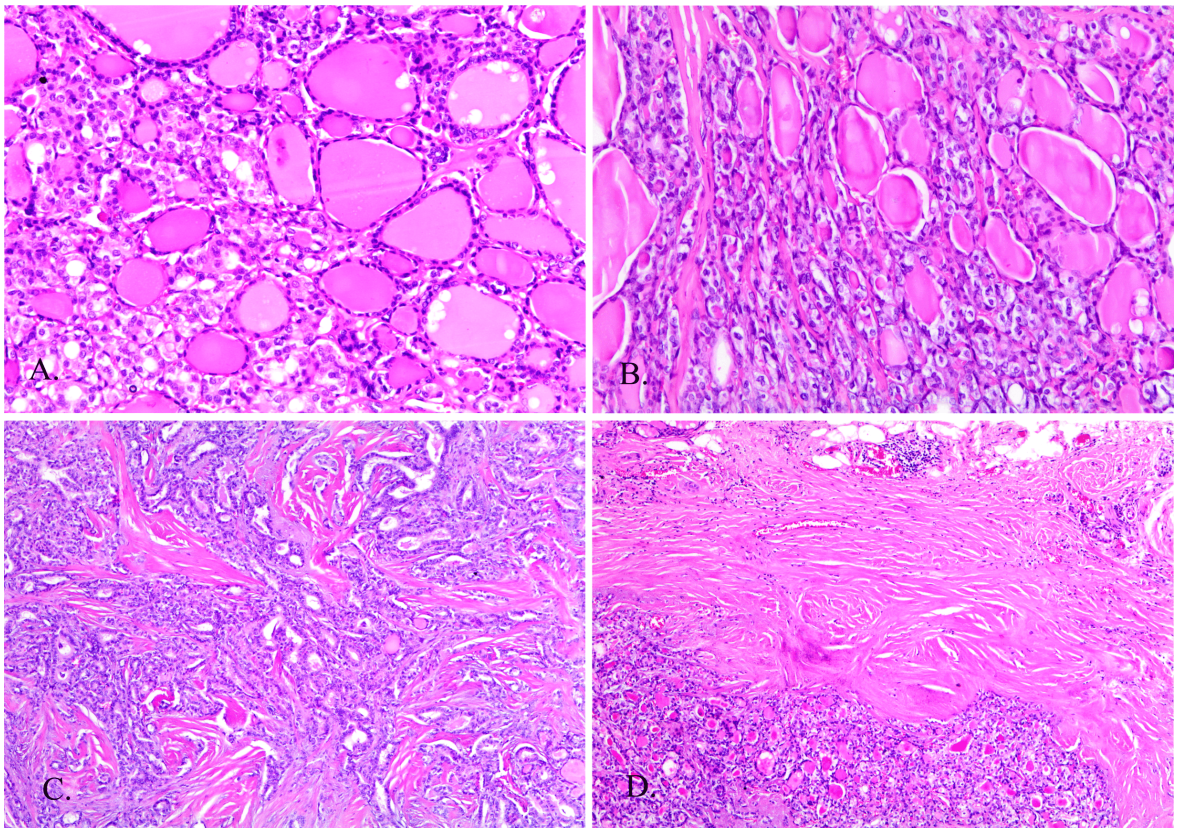

Figure 3 – ***Assessment of the tumor-associated stromal fibrosis: absence of fibrosis (A); fibrosis grade 1, defined as mild fibrosis, with the presence of few inconspicuous, delicate fibrous areas within or at the periphery of the tumor nodule (B); fibrosis grade 2+, defined as moderate (C)/extensive (D) fibrosis that was clearly recognizable, with multiple fibrotic bands within and at the periphery of the tumor nodule. HE staining: (A–D) ×40.***

Table 1 – *Microscopic appearance of papillary thyroid microcarcinoma foci and* **BRAF^{V600E}** *status*

Morphological features No. of positive cases (%)	*BRAF*^{V600E} mutation		*p*
	Positive, n=9 (%)	*Negative, n=16 (%)*	
Peripheral, subcapsular location, *n*=14 (56)	8/9 (88.9)	6/16 (37.5)	**0.013**
Extrathyroidal extension, *n*=2 (8)	0/9 (0)	2/16 (12.5)	0.269
Multifocality, *n*=11 (44)	2/9 (22.2)	9/16 (56.3)	0.1
Histological variant:			
▪ Conventional, *n*=11 (44)	7/9 (77.8)	4/16 (25)	**0.05**
▪ Follicular *n*=11 (44)	1/9 (11.1)	10/16 (62.5)	
▪ Tall cell *n*=2 (8)	1/9 (11.1)	1/16 (6.2)	
▪ Oncocytic, *n*=1 (4)	0/9	1/16 (6.2)	
Positive resection margin, *n*=4 (16)	1/9 (11.1)	3/16 (18.8)	0.617
Tumor's border:			
▪ Well-delimited, non-encapsulated, *n*=3 (12)	0/9 (0)	3/16 (18.7)	0.065
▪ Encapsulated, *n*=4 (16)	0/9 (0)	4/16 (25)	
▪ Infiltrative, *n*=18 (72)	9/9 (100)	9/16 (56.2)	
Characteristic PTC nuclear features:			
▪ Nuclear enlargement, *n*=25 (100)	9/9 (100)	16/16 (100)	
▪ Overlapping, *n*=25 (100)	9/9 (100)	16/16 (100)	
▪ Grooves, *n*=25 (100)	9/9 (100)	16/16 (100)	
▪ Irregular nuclear membrane, *n*=25 (100)	9/9 (100)	16/16 (100)	
▪ Chromatin clearing, *n*=21 (84)	9/9 (100)	12/16 (75)	0.102
▪ Pseudoinclusions, *n*=13 (52)	8/9 (88.9)	5/16 (31.3)	**0.006**
▪ "Sickle"-shaped, *n*=9 (36)	4/9 (44.4)	5/16 (31.3)	0.509
▪ Poorly developed PTC nuclei	0/9	4/16 (25)	0.101
▪ Well-developed PTC nuclei	9/9 (100)	12/16 (75)	
Tumor-associated stromal reaction:			
▪ None, *n*=7 (28)	0/9	7/16 (43.7)	
▪ Fibrosis 1+, *n*=4 (16)	1/9 (11.1)	3/16 (33.3)	
▪ Fibrosis 2+, *n*=14 (56)	8/9 (88.9)	6/16 (37.5)	**0.032**
"Plump", pink cells, *n*=14 (56)	5/9 (55.6)	9/16 (56.3)	0.973
Intratumoral lymphocytic infiltrate, *n*=1 (4)	0/9	1/16 (6.3)	0.444
Back-to back arrangement, *n*=1 (4)	0/9	1/16 (6.3)	0.444
Intratumoral multinucleated giant cells, *n*=9 (36)	4/9 (44.4)	5/16 (31.2)	0.509
Psammoma bodies, *n*=4 (16)	2/9 (12.5)	2/16 (22.2)	0.524
Stromal calcification, *n*=2 (4)	1/9 (11.1)	1/16 (6.3)	0.667

BRAF: B-Raf proto-oncogene serine/threonine kinase; PTC: Papillary thyroid carcinoma.

The histological subtype of PTMC was established according to the *WHO* criteria [1]. The diagnosis of conventional PTC was based on the nuclear features and evidence of papillary architecture that was pure or mixed papillary and follicular architecture (Figure 1C). The diagnosis of follicular variant of PTC was made when the tumor consisted exclusively of small to medium sized, irregularly shaped follicles, lined by cells with characteristic PTC nuclear features and no papillary structures (Figure 1D).

"Plump" tumor cells were defined as pink, polygonal large tumor cells, with abundant, eosinophilic cytoplasm and well developed PTC nuclear features. Not to be included in the "tall cell" variant, the cell's height needed to be less than twice the width (not "tall" enough) [15, 16] (Figure 2D).

The tumor cell nuclei were assessed for seven characteristic PTC features, grouped into three categories: (*i*) changes in the nuclear size and shape (enlargement and overlapping), (*ii*) irregularities of the nuclear membrane (irregularity of the nuclear contours, grooves and pseudo-inclusions) and (*iii*) chromatin characteristics (clearing and ground glass appearance) (Figure 2, A–D). "Sickle" nuclei were defined as smaller, eccentrically located nuclei, with a particular sickle shape [18]. An interpretation of well-developed PTC nuclear features was rendered when ≥5 nuclear features were present, whereas subtle PTC nuclear features were consisted when fewer (≤4) nuclear features were present.

Tumor-associated stromal reaction was also documented and was scored as: *absence of fibrosis*, *fibrosis grade 1*+ and *fibrosis grade 2*+ (Figure 3). A *fibrosis grade 1*+ was consistent with the presence of mild fibrosis features (only few, inconspicuous, delicate bundles of collagen fibers at the periphery or within the tumor nodule). *Fibrosis grade 2*+, on the other hand, was defined as moderate/ extensive fibrosis, with clear evidence of thick, multiple fibrotic bands within and at the periphery of the tumor nodule [19].

The pathological evaluation was performed without prior knowledge of the *BRAF*^{V600E} mutational status of the PTMC foci.

DNA extraction and *BRAF*^{V600E} mutation analysis

The area of interest (the tumor area) was marked on

the HE staining prior to analysis. Using the HE staining as a guide and a standard microscope, we performed a manual macrodissection of the marked area. For each case, five 4-μm thick sections were used. The DNA material was scraped from the preselected area and transferred to an Eppendorf tube for DNA isolation using the Master Pure™ DNA purification kit (Epicentre, Madison, WI, USA), as previously described [20]. A spectrophotometer (Eppendorf BioSpectrometer, Hamburg, Germany) was used to determine both the yield (A260) and purity (A260/A280 ratio) of isolated DNA in each case.

All PTMCs foci were then subject to real-time polymerase chain reaction (RT-PCR) amplification targeting the *BRAF* gene. Exon 15 of the *BRAF* gene was amplified using a pair of selected primers (forward and reverse) in order to flank the $BRAF^{V600E}$ point mutation [20]. The presence of the mutation was assessed by high resolution melting (HRM) analysis. The samples revealed as positive for $BRAF^{V600E}$ mutation following HRM analysis (with a slight deviation of the melting curve) were then further analyzed for confirmation by Sanger sequencing.

Statistical analysis

The statistical analysis was performed using the EpiInfo Software version 3.5.3 (CDC, Atlanta, USA). Morphological features associated with $BRAF^{V600E}$ mutation-positive and $BRAF^{V600E}$ mutation-negative PTMCs were compared using the Fisher's exact test. The level of statistical significance was set at $p<0.05$.

Results

Nineteen PTMC cases with analysis of 25 tumor foci were included in the study. Seventeen patients were women and two men, aged between 24 to 79 years old. Multifocality and extrathyroidal extension were described in 11 (44%) and two (8%) PTMC cases, respectively. None of the PTMCs had lymph node metastases.

More than half of the tumor foci (n=14, 56%) had a peripheral, subcapsular location and only four (16%) were associated with a positive resection margin. The distribution of the histological subtypes of PTMC foci was as follows: 11 conventional, 11 follicular variant of PTC, two tall-cell and one oncocytic variant.

Out of the 25 PTMC foci, 16 (64%) were $BRAF^{V600E}$ negative, whereas nine (36%) were $BRAF^{V600E}$ positive, revealing the $BRAF^{V600E}$ mutation following both HRM analysis and Sanger sequencing. The association of the tumor morphology and $BRAF^{V600E}$ mutation is shown in Table 1.

Most of the $BRAF^{V600E}$ positive PTMC foci were conventional PTC (7/9, 77.8%), whereas only one follicular variant of PTMC foci was associated with the mutation (1/9, 11.1%) (p=0.05). An infiltrating tumor border was more prevalent among $BRAF^{V600E}$ positive PTMC foci (9/9, 100%), compared to $BRAF^{V600E}$ negative ones (9/16, 56.2%), but the difference did not reach statistical significance (p=0.65).

Our data showed that subcapsular (superficial) localization of the tumor was significantly associated with the mutation (p=0.013). Moreover, tumor-associated stromal reaction (moderate/extensive fibrosis corresponding to fibrosis grade 2+) was also significantly more prevalent among $BRAF^{V600E}$ positive (8/9, 88.9%), compared to $BRAF^{V600E}$ negative (6/16, 37.5%) PTMC foci (p=0.032).

Characteristic PTC nuclear features did not differ significantly among the study groups, except for the nuclear pseudoinclusions, present in 8/9 (88.9%) $BRAF^{V600E}$ positive, compared to only 5/16 (31.2%) $BRAF^{V600E}$ negative PTMC foci (p=0.006). "Sickle"-shaped nuclei were identified in nine (36%) PTMC foci, with almost equal distribution among $BRAF^{V600E}$ positive (n=4, 44.4%), and $BRAF^{V600E}$ negative (n=5, 31.2%) cases (p=0.509). Well-developed PTC nuclear features were present in all $BRAF^{V600E}$ positive PTMC foci (9/9, 100%), compared to 12/16 $BRAF^{V600E}$ negative PTMC foci, but the difference did not reach statistical significance.

"Plump", pink tumor cells were identified in 14 (56%) cases, with almost equal distribution among $BRAF^{V600E}$ positive and $BRAF^{V600E}$ negative PTMC foci (55.6% *versus* 56.3%, p=0.973). Intratumoral multinucleated giant cells, on the other hand, were slightly more prevalent among $BRAF^{V600E}$ positive PTMC foci (44.4% *versus* 31.2%, p=0.509). Other morphological features (intratumoral lymphocytic infiltrate, back-to-back arrangement, psammoma bodies and stromal calcification) were only sparsely present (one, one, four and two cases, respectively), with no statistically significant differences according to the $BRAF^{V600E}$ mutation status (Table 1).

Discussions

The $BRAF^{V600E}$ mutation represents a very specific marker for PTC [21, 22]. As this mutation seems to be involved in PTC tumorigenesis, it has been suggested that it might also serve as a prognostic factor for PTC. However, literature data is yet controversial. Some studies have found only little or no evidence towards this association [23–25], including the 2015 *American Thyroid Association* (ATA) guidelines [26]. Many other recent studies, on the other hand, have linked the mutation to aggressive features of PTC (including PTMC) [14, 27–32], such as extrathyroidal extension, lymph node metastasis, tumor recurrence, or even advanced Tumor-Node-Metastasis (TNM) stage, according to some authors [30]. Thus, the precise landscape of the $BRAF^{V600E}$ mutation and its hallmark upon the patient's outcome are far from being completely elucidated.

Recently, several studies have highlighted the importance of morphology in predicting the $BRAF^{V600E}$ mutation status in PTC cases, including PTMCs [15, 16, 18, 19, 33]. PTCs with and without $BRAF^{V600E}$ mutation appear to be morphologically distinct as demonstrated by Virk *et al.* [16]. Thus, the morphological criteria could be an extremely valuable diagnostic tool, moreover since assessing the $BRAF^{V600E}$ mutation in all PTCs is expensive and not available to all pathology laboratories. The $BRAF^{V600E}$ mutated PTC cases are more likely to have tumor-associated stromal fibrosis, infiltrative, poorly-delimited tumor margins, conventional or tall-cell histology, subcapsular (superficial) tumor location, multifocality/intraglandular tumor spread, polygonal, eosinophilic ("plump cells") or well-developed nuclear features of PTC [16, 19]. These differences were also noted in PTMCs [15, 28].

This morphological perspective appears as a possible complementary tool in selecting those PTMC cases

(*BRAF*^{V600E} mutated) in which additional, post-surgery treatment would be justified [33] or, by contrast, tumors that have negligible clinical risk (*BRAF*^{V600E} wild type) [28].

In the present study, we aimed to identify those morphological features that could predict the presence of the *BRAF*^{V600E} mutation in a series of PTMCs in our Department. Our results have shown that subcapsular (superficial) localization (p=0.013), conventional histological type (p=0.005), tumor-associated stromal reaction (p=0.003) and evidence of nuclear pseudoinclusions (p=0.006) are significantly associated with the presence of the *BRAF*^{V600E} mutation in PTMCs. Our results are in accordance with previously published data [15, 19, 28, 30]. Virk *et al.* have also demonstrated that *BRAF*^{V600E} positive PTMCs are significantly associated with tumor-associated fibrosis and desmoplasia, infiltrating tumor borders, cystic changes and classic nuclear features of PTC [15]. Regarding the tumor borders, in our study, an infiltrating pattern was also more prevalent among *BRAF*^{V600E} positive PTMC foci, compared to *BRAF*^{V600E} negative ones, but the difference did not reach statistical significance (p=0.65).This might be related to the low number of PTMC foci that we have tested for the presence of the *BRAF*^{V600E} mutation, which represents a limitation of our study. However, despite this limitation, we have performed a complete morphological characterrization for all the cases included in the study (19 PTMC cases, with analysis of 25 PTMC foci) and succeeded to highlight the importance of some simple, easy to evaluate morphological parameters, highly predictive for the presence of the *BRAF*^{V600E} mutation in PTMCs, as demonstrated by other studies as well.

A combined molecular-pathological scoring system for risk stratification in PTMC was recently proposed by Niemeier *et al.* [19]. The following factors were included in this score: superficial tumor location, tumor-associated stromal fibrosis, multifocality/intraglandular tumor spread and *BRAF*^{V600E} mutation. We also found superficial tumor location and tumor-associated stromal fibrosis to be present together in *BRAF*^{V600E} mutated PTMC foci.

BRAF^{V600E} has also been reported as associated with lymph node involvement [28]. Our data showed that none of the PTMC cases included in the study had lymph node metastasis. One explanation could be the low rate of lymph node dissection performed by the surgeons in our Institution, in echographically suspicious cases only.

Conclusions

To sum up, our results have pointed out the value of several morphological features (subcapsular localization, conventional histological type, tumor-associated stromal reaction and nuclear pseudoinclusions) in predicting a *BRAF*^{V600E} mutation profile in PTMCs. All these parameters should be documented in the histopathological report as they seem to be associated with the presence of the *BRAF*^{V600E} mutation and could serve as a risk stratification tool in the selection of patients in need for adjuvant post-surgery therapy.

Conflict of interests

The authors declare no conflict of interests.

Acknowledgments

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References

[1] Delellis RA, Williams ED. Tumours of the thyroid and parathyroid. In: DeLellis RA, Lloyd RV, Heitz PU, Eng C (eds). Pathology and genetics of tumours of endocrine organs. World Health Organization (WHO) Classification of Tumours, International Agency on Cancer Research (IARC) Press, Lyon, 2004, 49–66.

[2] Cătană R, Boilă A, Borda A. Thyroid cancer profile in Mures County: a 20 years study. Rom J Morphol Embryol, 2012, 53(4):1007–1012.

[3] Davies L, Welch HG. Current thyroid cancer trends in the United States. JAMA Otolaryngol Head Neck Surg, 2014, 140(4):317–322.

[4] Enewold L, Zhu K, Ron E, Marrogi AJ, Stojadinovic A, Peoples GE, Devesa SS. Rising thyroid cancer incidence in the United States by demographic and tumor characteristics, 1980–2005. Cancer Epidemiol Biomarkers Prev, 2009, 18(3): 784–791.

[5] Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer, 2010, 127(12):2893–2917.

[6] Yu XM, Wan Y, Sippel RS, Chen H. Should all papillary thyroid microcarcinomas be aggressively treated? An analysis of 18,445 cases. Ann Surg, 2011, 254(4):653–660.

[7] Pacini F. Management of papillary thyroid microcarcinoma: *primum non nocere*! J Clin Endocrinol Metab, 2013, 98(4): 1391–1393.

[8] Garnett MJ, Marais R. Guilty as charged: *B-RAF* is a human oncogene. Cancer Cell, 2004, 6(4):313–319.

[9] Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S, Hawes R, Hughes J, Kosmidou V, Menzies A, Mould C, Parker A, Stevens C, Watt S, Hooper S, Wilson R, Jayatilake H, Gusterson BA, Cooper C, Shipley J, Hargrave D, Pritchard-Jones K, Maitland N, Chenevix-Trench G, Riggins GJ, Bigner DD, Palmieri G, Cossu A, Flanagan A, Nicholson A, Ho JW, Leung SY, Yuen ST, Weber BL, Seigler HF, Darrow TL, Paterson H, Marais R, Marshall CJ, Wooster R, Stratton MR, Futreal PA. Mutations of the *BRAF* gene in human cancer. Nature, 2002, 417(6892):949–954.

[10] Hilger RA, Scheulen ME, Strumberg D. The Ras–Raf–MEK–ERK pathway in the treatment of cancer. Onkologie, 2002, 25(6):511–518.

[11] Xing M, Alzahrani AS, Carson KA, Shong YK, Kim TY, Viola D, Elisei R, Bendlová B, Yip L, Mian C, Vianello F, Tuttle RM, Robenshtok E, Fagin JA, Puxeddu E, Fugazzola L, Czarniecka A, Jarzab B, O'Neill CJ, Sywak MS, Lam AK, Riesco-Eizaguirre G, Santisteban P, Nakayama H, Clifton-Bligh R, Tallini G, Holt EH, Sýkorová V. Association between *BRAF* V600E mutation and recurrence of papillary thyroid cancer. J Clin Oncol, 2015, 33(1):42–50.

[12] Moon HJ, Kim EK, Chung WY, Shin DY, Kwak JY. *BRAF* mutation in fine-needle aspiration specimens as a potential predictor for persistence/recurrence in patients with classical papillary thyroid carcinoma larger than 10 mm at a *BRAF* mutation prevalent area. Head Neck, 2015, 37(10):1432–1438.

[13] Xing M. *BRAF* mutation in thyroid cancer. Endocr Relat Cancer, 2005, 12(2):245–262.

[14] Chen Y, Sadow PM, Suh H, Lee KE, Choi JY, Suh YJ, Wang TS, Lubitz CC. *BRAF*^{V600E} is correlated with recurrence of papillary thyroid microcarcinoma: a systematic review, multi-institutional primary data analysis, and meta-analysis. Thyroid, 2016, 26(2):248–255.

[15] Virk RK, Van Dyke AL, Finkelstein A, Prasad A, Gibson J, Hui P, Theoharis CG, Carling T, Roman SA, Sosa JA, Udelsman R, Prasad ML. *BRAF*^{V600E} mutation in papillary thyroid microcarcinoma: a genotype–phenotype correlation. Mod Pathol, 2013, 26(1):62–70.

[16] Virk RK, Theoharis CG, Prasad A, Chhieng D, Prasad ML. Morphology predicts *BRAF*^{V600E} mutation in papillary thyroid carcinoma: an interobserver reproducibility study. Virchows Arch, 2014, 464(4):435–442.

[17] Tuttle RM, Morris LF, Haugen BR, Shah JP, Sosa JA, Rohren E, Subramaniam RM, Hunt JL, Perrier ND. Thyroid-differentiated and anaplastic carcinoma. In: Amin MB, Edge S, Greene F, Byrd DR, Brookland RK, Washington MK, Gershenwald JE, Compton CC, Hess KR, Sullivan DC, Jessup JM, Brierley JD, Gaspar LE, Schilsky RL, Balch CM, Winchester DP, Asare EA, Madera M, Gress DM, Meyer LR (eds). American Joint Committee on Cancer (AJCC) Cancer Staging Manual. 8th edition, American College of Surgeons, Springer, Chicago, 2016, 873–890.

[18] Rossi ED, Bizzarro T, Martini M, Capodimonti S, Fadda G, Larocca LM, Schmitt F. Morphological parameters able to predict *BRAF*^{V600E}-mutated malignancies on thyroid fine-needle aspiration cytology: our institutional experience. Cancer Cytopathol, 2014, 122(12):883–891.

[19] Niemeier LA, Kuffner Akatsu H, Song C, Carty SE, Hodak SP, Yip L, Ferris RL, Tseng GC, Seethala RR, Lebeau SO, Stang MT, Coyne C, Johnson JT, Stewart AF, Nikiforov YE. A combined molecular-pathologic score improves risk stratification of thyroid papillary microcarcinoma. Cancer, 2012, 118(8):2069–2077.

[20] Nechifor-Boilă A, Loghin A, Descotes F, Decaussin-Petrucci M, Borda A. Evaluation of a DNA extraction and purification protocol using archived formalin-fixed paraffin-embedded tissues for *BRAF* mutations analysis in papillary thyroid microcarcinomas. Appl Immunohistochem Mol Morphol, 2017, May 25.

[21] Cancer Genome Atlas Research Network. Integrated genomic characterization of papillary thyroid carcinoma. Cell, 2014, 159(3):676–690.

[22] Costa V, Esposito R, Pallante P, Ciccodicola A, Fusco A. The "next-generation" knowledge of papillary thyroid carcinoma. Cell Cycle, 2015, 14(13):2018–2021.

[23] Choi SY, Park H, Kang MK, Lee DK, Lee KD, Lee HS, Kim SW, Lee EN, Hong JC. The relationship between the *BRAF*^{V600E} mutation in papillary thyroid microcarcinoma and clinicopathologic factors. World J Surg Oncol, 2013, 11:291.

[24] Eloy C, Santos J, Soares P, Sobrinho-Simões M. The preeminence of growth pattern and invasiveness and the limited influence of *BRAF* and *RAS* mutations in the occurrence of papillary thyroid carcinoma lymph node metastases. Virchows Arch, 2011, 459(3):265–276.

[25] Ito Y, Yoshida H, Maruo R, Morita S, Takano T, Hirokawa M, Yabuta T, Fukushima M, Inoue H, Tomoda C, Kihara M, Uruno T, Higashiyama T, Takamura Y, Miya A, Kobayashi K, Matsuzuka F, Miyauchi A. *BRAF* mutation in papillary thyroid carcinoma in a Japanese population: its lack of correlation with high-risk clinicopathological features and disease-free survival of patients. Endocr J, 2009, 56(1):89–97.

[26] Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, Pacini F, Randolph GW, Sawka AM, Schlumberger M, Schuff KG, Sherman SI, Sosa JA, Steward DL, Tuttle RM, Wartofsky L. 2015 American Thyroid Association Management Guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: The American Thyroid Association Guidelines Task Force on thyroid nodules and differentiated thyroid cancer. Thyroid, 2016, 26(1):1–133.

[27] Kim SJ, Lee KE, Myong JP, Park JH, Jeon YK, Min HS, Park SY, Jung KC, Koo DH, Youn YK. *BRAF*^{V600E} mutation is associated with tumor aggressiveness in papillary thyroid cancer. World J Surg, 2012, 36(2):310–317.

[28] Tallini G, de Biase D, Durante C, Acquaviva G, Bisceglia M, Bruno R, Bacchi Reggiani ML, Casadei GP, Costante G, Cremonini N, Lamartina L, Meringolo D, Nardi F, Pession A, Rhoden KJ, Ronga G, Torlontano M, Verrienti A, Visani M, Filetti S. *BRAF* V600E and risk stratification of thyroid microcarcinoma: a multicenter pathological and clinical study. Mod Pathol, 2015, 28(10):1343–1359.

[29] Xing M, Westra WH, Tufano RP, Cohen Y, Rosenbaum E, Rhoden KJ, Carson KA, Vasko V, Larin A, Tallini G, Tolaney S, Holt EH, Hui P, Umbricht CB, Basaria S, Ewertz M, Tufaro AP, Califano JA, Ringel MD, Zeiger MA, Sidransky D, Ladenson PW. *BRAF* mutation predicts a poorer clinical prognosis for papillary thyroid cancer. J Clin Endocrinol Metab, 2005, 90(12):6373–6379.

[30] Lu HZ, Qiu T, Ying JM, Lyn N. Association between *BRAF*^{V600E} mutation and the clinicopathological features of solitary papillary thyroid microcarcinoma. Oncol Lett, 2017, 13(3): 1595–1600.

[31] Kim TH, Park YJ, Lim JA, Ahn HY, Lee EK, Lee YJ, Kim KW, Hahn SK, Youn YK, Kim KH, Cho BY, Park DJ. The association of the *BRAF*^{V600E} mutation with prognostic factors and poor clinical outcome in papillary thyroid cancer: a meta-analysis. Cancer, 2012, 118(7):1764–1773.

[32] Lupi C, Giannini R, Ugolini C, Proietti A, Berti P, Minuto M, Materazzi G, Elisei R, Santoro M, Miccoli P, Basolo F. Association of *BRAF*^{V600E} mutation with poor clinicopathological outcomes in 500 consecutive cases of papillary thyroid carcinoma. J Clin Endocrinol Metab, 2007, 92(11):4085–4090.

[33] Finkelstein A, Levy GH, Hui P, Prasad A, Virk R, Chhieng DC, Carling T, Roman SA, Sosa JA, Udelsman R, Theoharis CG, Prasad ML. Papillary thyroid carcinomas with and without *BRAF*^{V600E} mutations are morphologically distinct. Histopathology, 2012, 60(7):1052–1059.

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