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RESEARCH ARTICLE

Distinct Morphological Features Predictive for Aggressiveness of Papillary Thyroid Microcarcinoma: a Study of 72 Cases and 80 Tumor Foci

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Introduction: In this study, we aimed to investigate the importance of some distinctive morphological parameters in predicting the extrathyroidal extension, as marker of aggressiveness, in a series of papillary thyroid microcarcinoma (PTMC) cases. **Material and methods:** All consecutive PTMC cases, sized ≥5mm, registered at the Department of Pathology, Tîrgu-Mureș Emergency County Hospital from January 2002 to December 2013 were re-evaluated. The following histological features were noted: the multifocality, the extrathyroidal extension, the histologic variant, the tumor's border (well circumscribed versus infiltrative), the PTC nuclear features (well developed versus subtle), the tumor associated stromal reaction (fibrosis/desmoplasia/sclerosis versus none of these changes), the presence of "plump pink" cells, psammoma bodies, intratumoral lymphocytic infiltrate, cystic change, back-to-back arrangement, intratumoral multinucleated giant cells and lymph node involvement. **Results**: Our study included 72 PTMC cases, summing up to a total of 80 PTMC foci. We have shown that extrathyroidal extension is significantly associated with the presence of "plump pink"cells (p=0.0019), well developed nuclear features of PTC (p=0.018) and tumor associated stromal reaction (fibrosis/dezmoplazia/sclerosis) (p<0.0001). Other parameters were more prevalent among PTMC foci with extrathyroidal extension, but did not reach statistical significance. **Conclusion**: Our results pointed out the importance of a distinct set of morphological microscopical parameters, predictive for extrathyroidal extension in PTMC cases ("plump pink" cells, well developed PTC nuclear features, tumor associated stromal reaction, infiltrative tumor borders and conventional PTC histology). All these parameters are important to be mentioned in the histopathological reports, as they might be associated with a more aggressive biological behaviour.

Keywords: thyroid, papillary microcarcinoma, extrathyroidal extension, plump cells

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Introduction

Papillary thyroid microcarcinoma (PTMC) is defined by the World Health Organization as a PTC incidentally discovered, measuring 1 cm or less [1].Worldwide, many studies have reported a significant increasing incidence of PTMC in the last four decades [2-8]. Moreover, its prevalence continues to rise. In fact, recent data suggest that PTMC represents the most common type of papillary thyroid cancer in patients older than 45 years [9]. Enhanced detection, due to the widespread use of thyroid ultrasonography and ultrasound-guided fine needle aspiration cytology, and better handling of thyroid specimens on the other hand, have likely contributed to this increase [10], but cannot fully explain it [11], suggesting that other causes may be involved.

Despite all this growing incidence, tumor-related mortality has remained very low, and reported to be 0.5% for PTMC [12]. Nevertheless, controversies do exist regarding the optimal management of patients with PTMC [13]. Although these tumors are generally considered clinically indolent, some might be associated with extrathyroidal extension, local recurrence or lymph node metastasis [12-15]. The identification of this "aggressive" subset of PTMC cases is critical to better triaging of the patients with regard to the need for additional therapy.

However, the risk factors associated with a more aggressive behavior of PTMC cases are not well defined. Age over 45 years-old, tumor size greater than 5 mm, male sex, multifocality, lymph node involvement, extrathyroidal extension and, more recently, genetic markers (*BRAFV600E* mutation) have been reported as valuable risk factors in PTMC cases by most of the studies [12,15]. Some microscopical morphological features have also been shown to predict potential aggressiveness in PTC, and in PTMC cases in particular. These include: the histological variant (conventional and tall cell *versus* follicular variant), infiltrative tumor borders, well developed nuclear features of PTC, tumor associated stromal reaction (including fibrosis, dezmolazia and/or sclerosis), focal polygonal eosinophilic ("plump pink") cells, psammoma bodies [16-19].

Some recent studies [16-18] have tried to associate these morphological features to the risk of aggressiveness of PTMC, as by example presence of *BRAFV600E* mutation,

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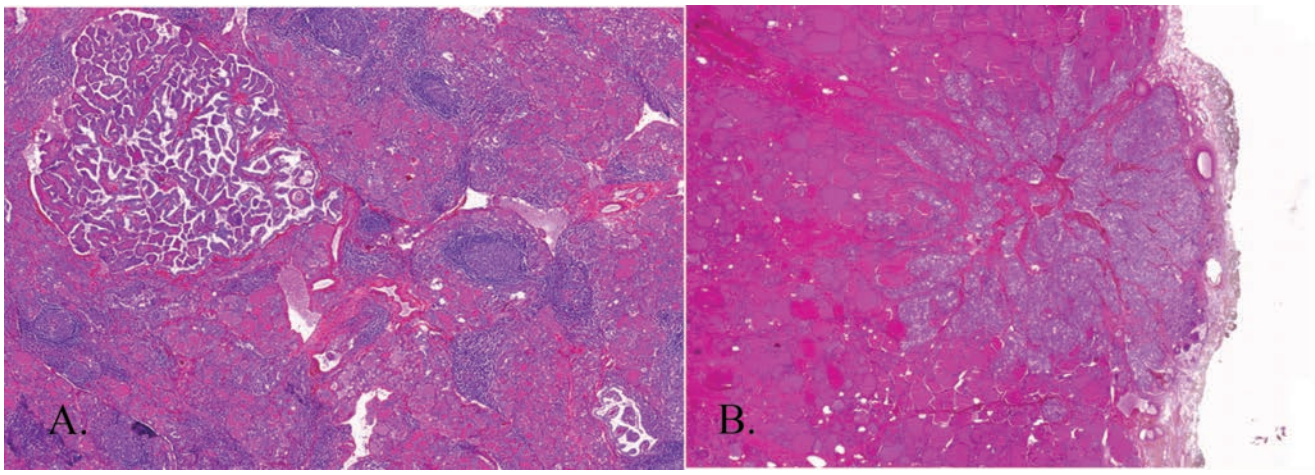

Fig. 1. Intrathyroidal versus extrathyroidal papillary thyroid microcarcinoma, HE stain, 4x: a multifocal, intrathyroidal conventional, papillary thyroid micrcarcinoma case (A); extrathyroidal extension, defined as tumor penetration into the adjacent adipose or musculartissues (B).

thought to be a genetic signature of poor prognosis, but their exact significance and the reproducibility in every day practice has to be defined.

In this study, we aimed to evaluate how these distinctive morphological features are correlated to the presence of extrathyroidal extension as mark of aggressiveness, in a series of PTMC cases.

Material and methods

All consecutive PTMC cases, sized ≥5mm, registered at the Department of Pathology, Tîrgu-Mureș Emergency County Hospital from January 2002 to December 2013 were re-evaluated. Criteria for inclusion were (1) a histopathological diagnosis consistent with PTMC (2) tumor size ≥5mm, as the morphologic features may not be adequately developed in smaller tumors, and thus, may be unreliable in predicting an aggressive behavior [17]. Tumors sized less than 5 mm at re-evaluation and cases where the histological slides were not available for evaluation were excluded

For all the cases, hematoxylin/eosine (HE)-stained slides were independently reviewed by two endocrine pathologists (ANB and NB). All cases with controversial features were then discussed and a consensus was reached using a double-headed microscope.

PTMCs were referred to PTCs incidentally found, that measured 1 cm or less [1] (Figure 1A). The diagnosis of PTC was exclusively based on nuclear features: enlargement, overlapping, irregularity of the nuclear contours, grooves, clearing or a ground glass appearance, and nuclear pseudoinclusions. The following histological features were evaluated: the size of the tumor, the multifocality, the extrathyroidal extension, the tumor location with respect to the thyroid surface (peripheral/subcapsular *versus* intrathyroidal), the histologic variant (conventional, follicular, tall cell, Wathin-like, oncocytic), the tumor interface with non-neoplastic thyroid tissue (well circumscribed, not encapsulated/encapsulated *versus* infiltrative), the characteristics of the tumor cells (the presence of "plump pink" cells), the nuclear features, the tumor associated stromal reaction (fri-

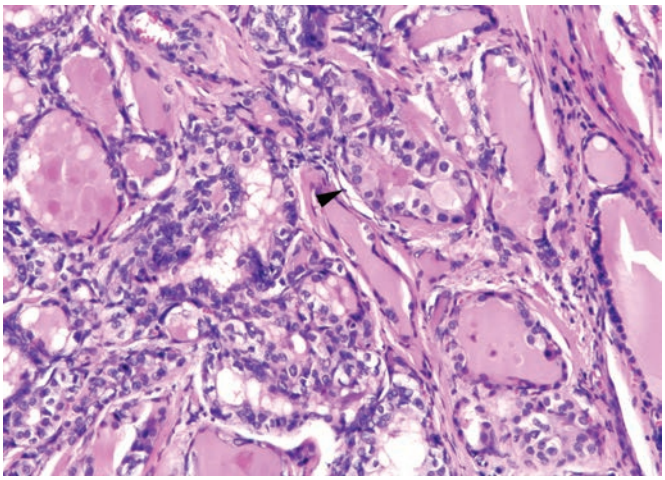

Fig. 2. "Plump cells" morphology, HE stain, 20x: polygonal tumor cells, with moderate to abundant, homogenous, eosinophilic cytoplasm, nuclear features of PTC, but not "tall" enough to be included in the tall cell variant (the cell's height was less than twice the width)

brosis/desmoplasia/sclerosis *versus* none of these changes), the presence of psammoma bodies, intratumoral lymphocytic infiltrate, cystic change, back-to-back arrangement, intratumoral multinucleated giant cells, and lymph node involvement (see Table I).

Extrathyroidal extension was defined as tumor penetration through the thyroid capsule into the adjacent adipose or muscular tissue (Figure 1B). Multifocality was referred to as the presence of at least two independent, isolated/non-contiguous tumor foci in the thyroid.

The diagnosis of the histological variant of PTMC was made in accordance to the WHO criteria [1]. Conventional PTCs had a characteristic papillary architecture that was pure or admixed with a variable proportion of follicles. The tumors defined as follicular variant of PTC were composed of small to medium sized, irregularly shaped follicles, with characteristic PTC nuclear changes in most of the cells lining these follicles and virtually no papillary structures. The diagnosis of other rare variants of PTC (tall cell, oncocytic, Warthin-like) was also in accordance to the WHO criteria.

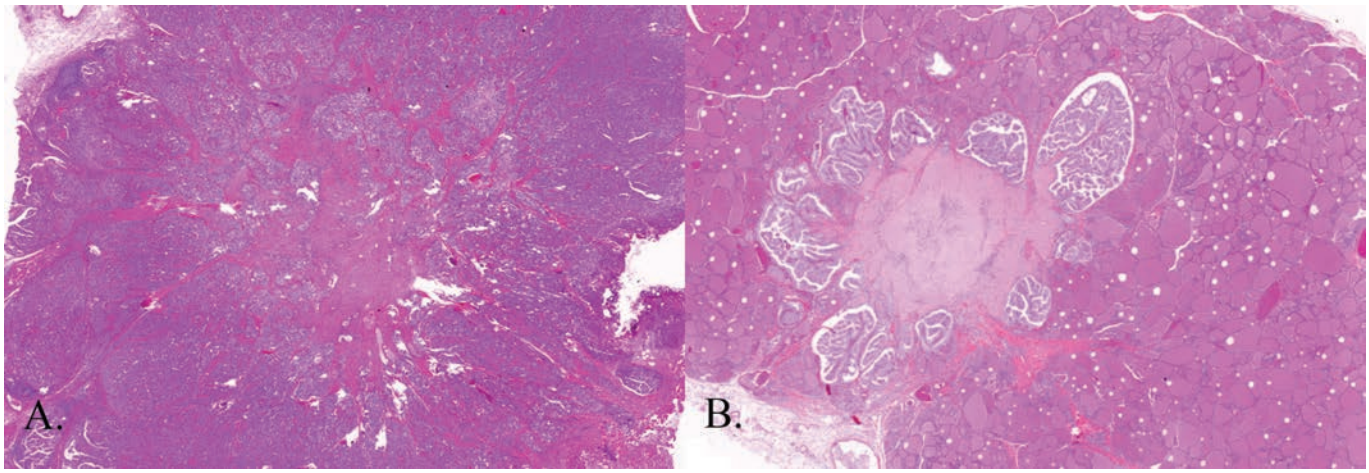

Fig. 3. Tumor associated stromal reaction, HE stain, 4x: desmoplasia was defined as the presence of proliferating fibroblasts in a myxoid stroma (A), and sclerosis as the presence of paucicellular, eosinophilic, dense bundles of collagen (B).

The tumor cell nuclei were assessed for six characteristic features of PTC: nuclear enlargement, overlapping, grooves, irregular nuclear membrane, chromatin clearing and pseudoinclusions. The nuclear features were considered well developed if at least five of six (≥5) features were present and subtle when fewer (≤4) features were present [17]. The presence of "plump pink", polygonal tumor cells, with moderate to abundant, homogenous, eosinophilic cytoplasm, nuclear features of PTC, but not "tall" enough to be included in the tall cell variant (the cell's height was less than twice the width) [16,17] was also noted (Figure 2).

Tumor associated stromal reaction was also documented. Fibrosis was defined as the presence of fibroblasts in a collagenous (non-mixoid) stroma, desmoplasia as the presence of proliferating fibroblasts in a myxoid stroma (Figure 3A), and sclerosis as the presence of paucicellular, eosinophilic, dense bundles of collagen [16,17] (Figure 3B).

The study was approved by the Ethical Committee of the University of Medicine and Pharmacy, Tîrgu-Mureş.

Statistical analysis

Descriptive statistics were used to summarize the study data. Morphologic features associated with extrathyroidal and intrathyroidal PTMC foci were compared using the Fisher exact test or the chi-squared test as appropriate. EpiInfo Software version 3.5.3 (CDC, Atlanta) was used for

Table I. Predictive morphological parameters for extrathyroidal extension in papillary thyroid microcarcinoma foci.

Characteristics	Extrathyroidal extension		p
	Present (n=32/80, 40%)	Absent (n=48/80, 60%)	
Size	7.12±1.64	6.85±1.71	0.89
Multifocality	9 (28.13%)	14 (29.17%)	0.91
Location			<0.0001
Peripheral, subcapsular	32 (100%)	19 (39.6%)	
Intrathyroidal	0	29 (60.4%)	
Histologic variant			0.0001
Conventional	27 (84.38%)	19 (39.6%)	
Follicular	0	26 (54.17%)	
Tall cell	1 (3.13%)	0	
Warthin-like	3 (9.38%)	3 (6.35%)	
Oncocitic	1 (3.13%)	0	
Tumor borders			<0.0001
Well circumscribed, not encapsulated	0	13 (27.1%)	
Encapsulated	0	15 (31.2%)	
Infiltrative	32 (100%)	20 (41.67%)	
Polygonal eosinophylic (plump pink) cells	11 (34.38%)	3 (6.25%)	0.0012
Nuclear features			
Well developed	32 (100%)	30 (62.5%)	0.0001
Subtle	0	18 (37.5%)	
Tumor associated stromal reaction			<0.0001
Fibrosis	10 (31.25%)	8 (16.67%)	
Dezmoplazia/Sclerosis	22 (68.75%)	10 (20.8%)	
None of these	0	30 (62.5%)	
Psammoma bodies	21 (65.6%)	25 (52.1%)	0.23
Tumor-associated inflammatory infiltrate	12 (37.5%)	18 (37.5%)	1
Cystic changes	0	1 (2.08%)	0.41
Back-to-back arrangement	11 (34.3%)	15 (31.2%)	0.77
Intratumoral multinulcleated giant cells	13 (40.6%)	16 (33.3%)	0.51
Lymph node involvement	1 (3.1%)	0	0.22

all statistical analysis, and a 2-tailed p-value < 0.05 was considered statistically significant.

Results

Ninety one consecutive ≥5mm sized PTMC cases were registered at the Department of Pathology, Tîrgu-Mureş Emergency County Hospital in the study period. Of these, 72 PTMC cases, summing up to a total of 80 PTMC foci, fulfilled all criteria and were finally included in the study. The rest of the cases were excluded because (1) the tumor foci were less than 5 mm at re-evaluation (*n*=7cases) (2) did not meet all the diagnostic criteria for PTMC (*n*=2 cases) or the histological slides were not available for evaluation (*n*=10 cases).The majority of cases occurred in women (*n*=68 cases, 95%), and the mean age at diagnosis was 50.3± 9.11 years-old.

Table I shows the histopathological features of PTMCs with extrathyroidal extension, compared to intrathyroidal foci. The size of the tumor foci and the multifocality rate were similar between the study categories (7.12±1.64 *versus* 6.85±1.71, p=0.89 and 9/32, 28.13% *versus* 14/48, 29.17%, p=0.91, respectively). The majority of PTMC foci with extrathyroidal extension corresponded to conventional PTMC, whereas more than half (54.17%) of the PTMC foci without extrathyroidal extension were consistent with a diagnosis of follicular variant of PTMC. Eight cases corresponding to rare variants of PTMC (tall cell, Warthin-like and oncocytic) were also included in the study and these variants were found to be more prevalent in cases with extrathyroidal extension.

We found polygonal, plump pink cells, characterized by abundant eosinophilic cytoplasm in 14/80 PTMC foci. This distinctive cell type was seen mainly in association with extrathyroidal extension, and the differences were statistically significant (0.0012). All PTMC foci with extrathyroidal extension revealed well developed PTC nuclear features, whereas only around half of the PTMC foci without extrathyroidal were associated to this morphological criteria of worst prognosis (p=0.0001) and a significant subset (18/48, 37.5%) revealed only subtle nuclear features of PTC.

Tumor associated stromal reaction (fibrosis/dezmoplazia/sclerosis) was significantly more prevalent in cases with extrathyroidal extension, compared to the rest of the tumors (p<0.0001).

The presence of psammoma bodies, tumor-associated inflammatory infiltrate, cystic change, back-to-back arrangement were not significantly associated to extrathyroidal extension (see Table I).

Only one PTMC case with extrathyroidal extension was associated with lymph node involvement.

Discussion

Extrathyroidal extension of PTC is known to be a predictive risk factor for local recurrence and lymph node metastasis, even in microcarcinomas [20]. Moreover, it has also been associated with the presence of *BRAFV600E* mutation, a T1799A point mutation in the *BRAF* gene, resulting in a valine-to-glutamic acid switch at codon 600 (V600E), that has emerged as a marker of aggressive behaviour in PTC [21]. Some studies have also suggested that this mutation may predict a more aggressive behaviour of PTMC [16,22]. This underlines its early driving role in thyroid tumorigenesis and its association with a more aggressive phenotype.

Recently, it has been pointed out the importance of morphology in predicting the presence of *BRAFV600E* mutation in PTCs, including PTMC cases [16-19,23]. Some architectural parameters (tumor associated stromal reaction, infiltrative tumor borders) and cell-specific parameters (well developed PTC nuclear features, polygonal, eosinophylic plump cell) have emerged as being highly predictive for the presence of *BRAFV600* mutation. This alternative morphological perspective has gain popularity as a possibly foreseeable aid in every day practice, in identifying those cases in which complementary treatment after surgery is needed [24].

In this study, we focused on some specific microscopical morphological details, highly predictive of *BRAFV600* mutation status in previous studies, and evaluated them in relation to the presence of extrathyroidal extension, a known marker of aggressiveness in PTMC cases. Our study included 72 PTMC cases, summing up to a total of 80 PTMC foci. We have shown that extrathyroidal extension is significantly associated with the presence of polygonal eosinophylic (plump pink) cells (p=0.0012), well developed nuclear features of PTC (p=0.0001) and tumor associated stromal reaction (fibrosis/dezmoplazia/sclerosis) (p<0.0001).

"Plump cells" were defined as large, polygonal tumor cells, with a cell height less than twice the width ("not tall enough"), that had features of squamoid metaplasia, with homogenous, eosinophilic, moderate to abundant cytoplasm, and shared nuclear features of PTC [16, 24]. The presence of these cell types, even focally, has been significantly associated with *BRAFV600E* mutation in the study performed by Virk RK et al [17]. In their analysis, the authors reported that 29/50 PTCs were characterised by these plump cells, with a 72% correlation with *BRAFV600E* mutation. The "plump" cell morphology has yield even more importance as a predictive parameter in thyroid fine-needle aspiration cytology (FNAC). In their study, Rossi ED et al. have found plump cells in all 47 *BRAFV600E* mutated, positive for malignancy (favouring PTC) cases (in 41 cases plump cells represented >20% of the tumor cells). In our study, plump cells, were significantly more prevalent in PTMC foci with extrathyroidal extension (0.0012), compared to PTMC foci without extrathyroidal extension, suggesting their possible role as marker of more aggressive behaviour.

Infiltrative tumor borders, conventional PTC histology and tumor associated stromal reaction (including fi-

brosis, dezmoplazia and sclerosis) have also been linked to the presence of *BRAFV600* mutation in previous studies [16-18]. In our study, the presence of desmoplasia/fibrosis/sclerosis was significantly associated to the extrathyroidal extension in PTMC cases (p<0.0001). Similarly, an infiltrative tumor border and conventional PTC histology were also found to be significantly more prevalent among PTMC cases with extrathyroidal extension, compared to the rest of the cases (p=0.0001).

Other morphological features, like psammoma bodies, tumor-associated inflammatory infiltrate, cystic change, back-to-back arrangement were not significantly associated to extrathyroidal extension in our study. Only one PTMC case with extrathyroidal extension was associated with lymph node involvement. One explanation could be the low rate of the prophylactic central neck dissection, performed by the surgeons in our institution.

Conclusion

In this study, we pointed out the importance of a distinct set of morphological parameters, predictive for extrathyroidal extension in PTMC cases. We have shown that extrathyroidal extension is significantly associated to the presence of polygonal eosinophylic ("plump pink") cells, well developed PTC nuclear features, tumor associated stromal reaction (fibrosis/dezmoplazia/sclerosis), infiltrative tumor borders and conventional PTC histology. All these parameters are important to be mentioned in the histopathological report, as they might be associated with a more aggressive biological behaviour and could play a role in the selection of patients in need for additional post-surgery treatment.

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