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Prognostic significance of cell-adhesion molecules in histological variants of papillary thyroid carcinoma

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Abstract

The molecular structure of E-cadherin and its function are intimately related to β-catenin, their interactions ensuring the cell morphology and stability. Alterations of E-cadherin–β-catenin complex facilitate the tumor growth and spreading in the carcinogenic mechanism. We aimed to assess the E-cadherin and β-catenin immunoexpressions in different variants of papillary thyroid carcinoma (PTC), and the relationship of these markers with the clinicopathological prognostic factors. Our study group included 70 cases of PTC divided into two risk groups. The low-risk group comprised 45 cases diagnosed as conventional, follicular, oncocytic, macrofollicular, and clear cell variants, whereas the high-risk group consisted of 25 cases diagnosed as tall cell, follicular angioinvasive, cribriform-morular, hobnail, diffuse sclerosing, and solid subtype, respectively. Immunohistochemical exam was performed by using anti-E-cadherin and anti-β-catenin antibodies, and their expressions were semi-quantitatively evaluated. The association between E-cadherin and β-catenin, respectively, and clinicopathological prognostic factors was statistically analyzed. We noted statistically significant differences between membranous E-cadherin expression (low *versus* high) and tumor size, histological risk groups, tumor stage, lymph node metastases, vascular invasion and tumor relapse. We also found statistically significant correlation between membranous β-catenin expression (low *versus* high) and the risk groups, tumor size and tumor stage, but no associations of cytoplasmic β-catenin (low *versus* high) with the clinicopathological characteristics. Our study demonstrates that E-cadherin and β-catenin expressions differ in low- and high-risk groups of PTC. The aggressive behavior of the high-risk histological variants is associated with reduced membranous E-cadherin, and loss of membranous β-catenin followed by enhanced cytoplasmic expression. These results open large standpoints for a deeper characterization of the histological variants of PTC.

Keywords: papillary thyroid carcinoma, histological variants, E-cadherin, β-catenin.

Introduction

Papillary thyroid carcinoma (PTC) is the widespread type of thyroid carcinoma (TC) and represents 70–85% of this malignancy with an increased global incidence during the last years [1]. PTC is usually correlated with a good prognostic, but certain patients can have an aggressive behavior of disease, due to the occurrence of local or distant metastases; consequently, a worse prognostic of PTC cannot be excluded [2]. Although a solid panel of classical, clinicopathological prognostic factors is already defined [3], it seems that they are not enough to explain the variability of PTC progression. Therefore, the understanding of these different courses of PTC is far to be completed. As a consequence, the interest in carcinogenesis mechanism focuses on the study of new prognostic factors that could clarify the distinctive comportment of the tumor cells within the thyroid environment [4–7].

One challenging research topic address the molecular markers that include molecules involved in the cellular cycle regulation (*i.e.*, cyclin D1, p27, p53), or cell growth (Her2/neu), in cell adhesion (E-cadherin, β-catenin, claudin-1, tubulin), or in tumor microenvironment changes (fibronectin) [4, 8, 9]. Concurrently, the histological variants of PTC [1] sustain the stratification of PTC cases in high-risk or low-risk categories, starting from the time of diagnosis [10–14]. In spite of the strong attempt to validate new proposed prognostic factors, the diversity of pro and cons arguments sustains the actuality of this research.

E-cadherin, a calcium-dependent transmembrane cell adhesion molecule, is essential for the adhesion and normal function of epithelial cells [15]. In the adherens junctions, the E-cadherin intracytoplasmic domain links to β-catenin that in turn connects to α-catenin jointed to the actinic cytoskeleton [16, 17]. Besides the role in the intercellular stability [18], β-catenin acts as a signaling factor in the canonical Wnt pathway [19]. The decrease of E-cadherin expression is responsible for the loss of cell adhesion, tumor growth and proliferation, leading to metastasis [20, 21]. Low E-cadherin expression has been reported in several malignancies, in association with tumor advanced stages and disease progression [22–25]. Several studies sustain that loss of E-cadherin is a decisive step in dedifferentiation, progression, and metastatic spread of TC [26–28]. Thus, E-cadherin expression is maintained in differentiated or in minimally invasive TC, and completely absent in undifferentiated ones. On the other hand, the involvement of β-catenin in carcinogenesis is also documented in different types of tumors [29–33], but few reports focus on its expression in TC, in relationship with a poor prognostic [34–40].

Starting from this point, the aim of our study was to analyze the expression pattern of E-cadherin and β-catenin

in different subtypes of PTC and the relationship of these markers with the clinicopathological factors.

Patients, Materials and Methods

Patients

Our study group included 70 cases of PTC, diagnosed between 2006 and 2016 at the Laboratory of Pathology, "Sf. Spiridon" Emergency County Hospital, Iaşi, Romania. All cases were histopathologically reassessed by three pathologists for ascertain the PTC histological subtype and then divided into two risk groups [10–14]. The low-risk group comprised 45 cases diagnosed as conventional, follicular, oncocytic, macrofollicular, and clear cell variants, whereas the high-risk group consisted of 25 cases diagnosed as tall cell, follicular angioinvasive, cribriform-morular, hobnail, diffuse sclerosing, and solid subtype, respectively.

The study has been approved by the Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, based on the patients' informed consent.

Immunohistochemistry (IHC)

The sections obtained from the selected paraffin-embedded blocks corresponding to these cases were dewaxed in xylene, rehydrated in consecutive descending concentrations of ethanol (100%, 90%, 80%, and 70%), and rinsed in distillated water. The antigen retrieval was made by using Heat-Induced Epitope Retrieval (HIER) procedure, with an antigen retrieval solution with pH 6, in a microwave oven, for 30 minutes. After the endogenous peroxidase blocking with 3% hydrogen peroxide, the sections were incubated with the primary antibodies E-cadherin (clone EP700Y, 1:100 dilution, Thermo Scientific, USA) and *β*-catenin (clone *β*-catenin-1, 1:300 dilution, Agilent–Dako, USA). The reaction was amplified with UltraVision Quanto Detection System Horseradish peroxidase (HRP) DAB (Thermo Scientific, USA) and developed with 3.3'-diaminobenzidine (DAB) tetrahydrochloride chromogen (DakoCytomation, Carpinteria, USA). The counterstaining of the sections was done with Mayer's Hematoxylin. The normal thyroid tissue or the benign associated pathology were used as internal positive controls for the two antibodies.

Semi-quantitative assessment

The semi-quantitative assessment was done by using adapted scores based on the literature reports [36, 41] that took into account the staining intensity (I) and the percentage of positive cells (P).

For E-cadherin, we assessed the membranous expression, whereas the *β*-catenin expression was quantified both at membranous and cytoplasmic level. The intensity of the immunoreaction for the two markers was scored as: 0 – absent, 1 – weak, 2 – moderate, and 3 – strong. The percentage of E-cadherin membranous positive cells was scored as follows: 0 – <5%, 1 – 6–25%, 2 – 26–50%, 3 – 51–77%, and 4 – >75%. The E-cadherin final score was obtained by multiplying P by I. E-cadherin score values between 1–4 were considered low, and score values between 6–12 were considered high. The percentage of *β*-catenin membranous or cytoplasmic immunopositive cells was scored as: 0 – <10%, 1 – 10–30%, 2 – 31–50%, 3 – 51–70%, 4 – >70%. The *β*-catenin final score resulted by summation of P and I. Cases with values between 1–3 were considered with low score, and cases with score between 4–7 were considered with high score.

Statistical analysis

Statistical analysis was performed by using Statistical Package for the Social Sciences (SPSS) *v.* 19 program (SPSS Inc., IBM Corporation, Chicago, IL, USA) and the χ^2 (chi-square) test (Maximum-Likelihood, Yates, Mantel–Haenszel). Statistical significance was considered for $p<0.05$.

Results

Clinicopathological characteristics

In the whole group, 55 patients were females (more than two-thirds), and 15 patients were males. At the time of the diagnosis, the age of the patients ranged between 17 and 79 years old, with a median age of 49 years old. Surgical treatment consisted in total thyroidectomy with lymphadenectomy for 41 patients, and partial thyroidectomy for the remaining 29 cases. The histopathological exam revealed the tumor extrathyroidal extension in 49 cases, presence of lympho-vascular invasion in 46 cases, and multifocal tumors in 25 cases. The distribution of the PTC histological variants was as follows: conventional subtype – 16 cases, follicular subtype – nine cases, macrofollicular – six cases, clear cell – four cases, oncocytic variant – 10 cases, tall cell – eight cases, cribriform – five cases, hobnail – one case, diffuse sclerosing – three cases, solid – five cases, angioinvasive follicular – one case, conventional with dedifferentiation to squamous cell carcinoma – one case, oncocytic with undifferentiated solid areas – one case. Based on the *Classification of Malignant Tumors* (TNM), 18 cases were staged as pT1, 27 cases as pT2, and 25 cases as pT3. In nine patients, lymph node relapse was registered.

E-cadherin and *β*-catenin assessment

The intensity of E-cadherin membranous immunoexpression was predominantly moderate or low in PTC, compared to the adjacent benign or normal thyroid tissue, strongly positive. Moderate membranous expression was observed not only in conventional and follicular subtypes, but also in some cases with a more aggressive course, like tall cell, cribriform and diffuse sclerosing variants. Low E-cadherin expression was noticed in PTC cases with high tumor extent (pT3) and lymph node metastases. In these cases, E-cadherin expression was heterogeneous, with 20–60% positive cells present in tumor areas.

β-Catenin immunostaining pattern in normal thyroid tissue or in the adjacent Hashimoto thyroiditis and nodular goiter was strong membranous, either circumferential in the cuboidal and columnar cells, either basal in the hypofunctional areas. In PTC, *β*-catenin had a discontinuous membranous expression with the persistence of lateral membrane staining and the absence of immunostaining in apical and basal pole of the tumor cells. We also noted that in areas where the membranous staining was lacking, *β*-catenin was expressed in the cytoplasm of the tumor

cells with moderate or even high intensity. Some cases of conventional PTC preserved a strong, diffuse membranous staining, whereas in the follicular, tall cell and solid variants a large heterogeneity was observed. The nuclear staining of *β*-catenin was identified in PTC cribriform-morular variant in approximately 40% of the tumor cells together with moderate cytoplasmic expression and loss of the membranous one in more than 80% of the cells.

Figures 1–6 illustrate different immunoexpression patterns of E-cadherin and *β*-catenin.

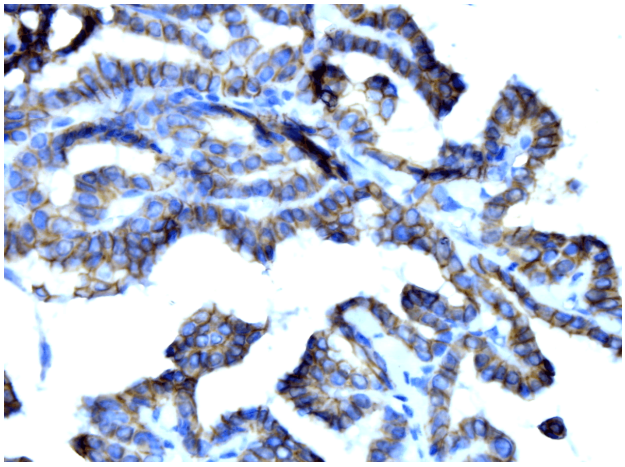

Figure 1 – ***PTC, conventional variant: membranous E-cadherin expression with strong intensity. IHC staining, anti-E-cadherin antibody, ×400.***

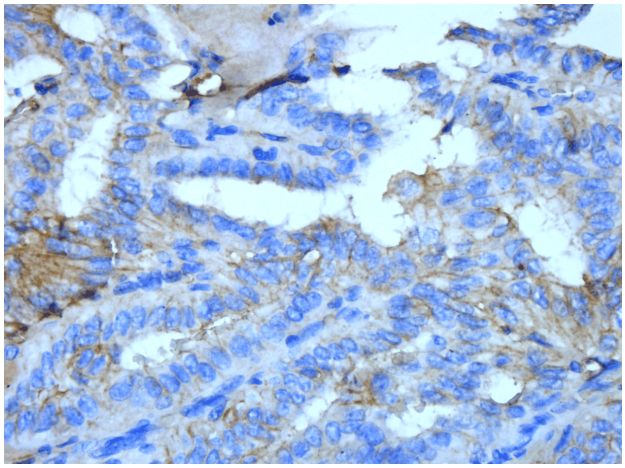

Figure 2 – ***PTC, tall cell variant: membranous E-cadherin expression with low intensity and heterogeneous pattern. IHC staining, anti-E-cadherin antibody, ×400.***

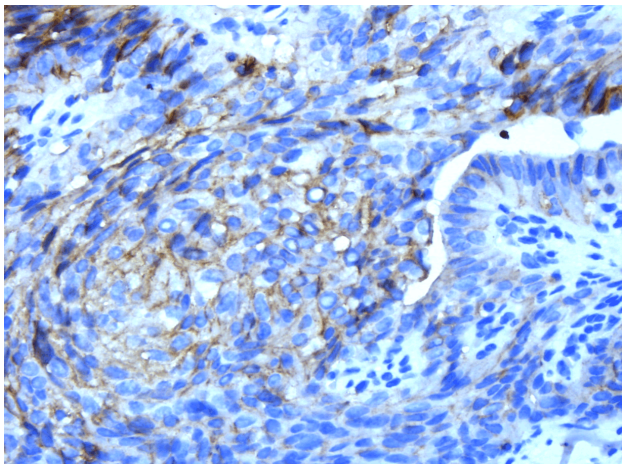

Figure 3 – ***PTC, cribriform-morular variant: membranous E-cadherin expression with low intensity and heterogeneous pattern. IHC staining, anti-E-cadherin antibody, ×400.***

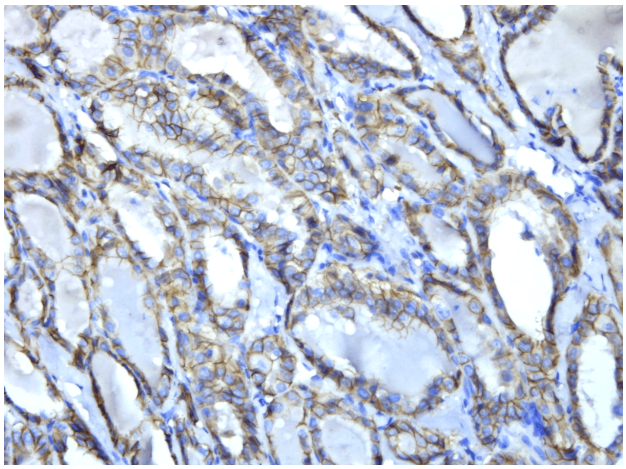

Figure 4 – ***PTC, follicular variant: membranous β-catenin expression with moderate intensity and homogenous pattern. IHC staining, anti-β-catenin antibody, ×100.***

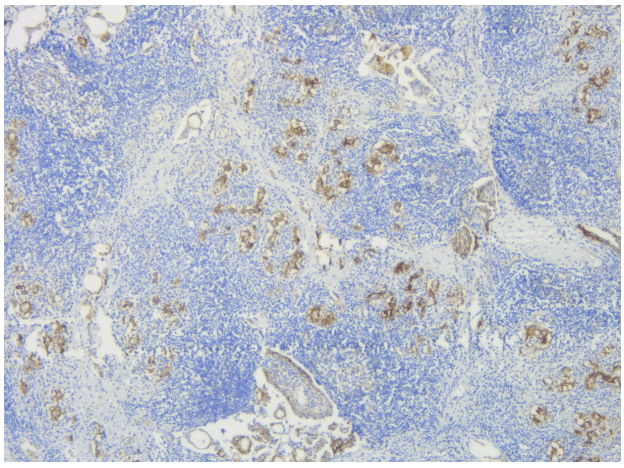

Figure 5 – ***PTC, diffuse sclerosing variant: cytoplasmic β-catenin expression with high intensity. IHC staining, anti-β-catenin antibody, ×50.***

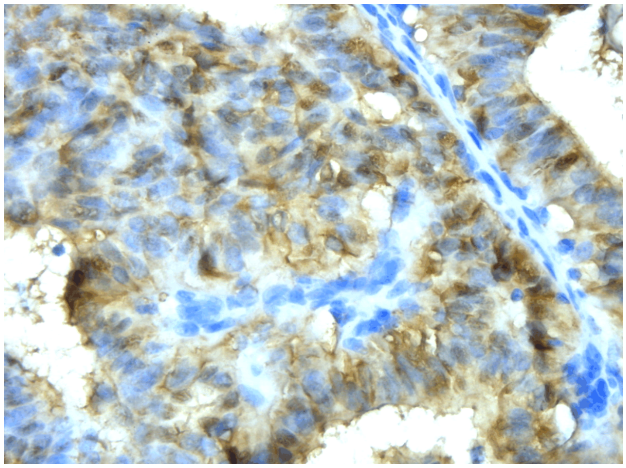

Figure 6 – ***PTC, cribriform-morular variant: cytoplasmic β-catenin expression with moderate intensity, associated with isolate nuclear β-catenin immunostaining. IHC staining, anti-β-catenin antibody, ×400.***

The results of the semi-quantitative assessment of E-cadherin and *β*-catenin expressions in accordance with the low and high score values, respectively, and the main clinicopathological characteristics were summarized in Tables 1 and 2.

The statistical analysis of E-cadherin expression and the clinicopathological factors showed significant differences between low *versus* high expression and tumor size (p=0.017), risk groups (p=0.003), tumor stage (p=0), lymph node metastases (p=0.001), vascular invasion (p=0) and tumor relapse (p=0.005). No correlation of E-cadherin expression with age, gender, multifocality and extrathyroidal invasion was found (Table 1). Membranous *β*-catenin expression (low *versus* high) was statistically significant correlated with the risk groups, tumor size and tumor stage (Table 2). No statistically significant differences were found between cytoplasmic *β*-catenin (low *versus* high) and the clinicopathological characteristics (Table 2).

Discussions

Even though the prognostic assessment of PTC relies, according to the *World Health Organization* (WHO), on the standard clinicomorphological factors, nowadays the pathologists look on the "candidate" prognosis markers and try to validate more sensitive criteria for the assessment of neoplastic evolution. These markers could be related to the carcinogenesis mechanism, their distinctive involvement controlling the subtle differences in prognostic. The endeavor of this approach could be the identification within the same histological phenotype of new subclasses of diagnosis (at present just predicted in an intuitive way, in relationship with the tumor behavior), characterized by molecular features.

Based on the expertise of the "Sf. Spiridon" Emergency County Hospital of Iaşi in the diagnosis and surgical treatment of thyroid tumor pathology, in the last years our scientific interest was pointed on the identification of a stratification algorithm applicable in PTC [40, 42–44]. This item comes out from the molecular classification of breast cancer [45] and, more recent, of lung cancer [46], that changed the traditional concept of diagnosis and treatment.

Table 1 – ***Relationship between E-cadherin expression and clinicopathological characteristics***

Clinicopathological characteristics	E-cadherin membranous expression		*p*-value from *chi*-square test
	Low (n=42)	***High (n=28)***	
Age at diagnosis			
<55 years old	15 (35.7%)	27 (64.3%)	1
≥55 years old	10 (35.7%)	18 (64.3%)	
Gender			
Female	20 (36.4%)	35 (63.6%)	0.828
Male	5 (33.3%)	10 (66.7%)	
Tumor size (median)			
<30 mm	6 (20%)	24 (80%)	**0.017**
≥30 mm	19 (47.5%)	21 (52.5%)	
Histopathological subtype			
Low-risk group	11 (22.7%)	34 (77.3%)	**0.003**
High-risk group	15 (57.7%)	10 (42.3%)	
Focality of the tumor			
Unifocal	15 (33.3%)	30 (66.7%)	0.577
Multifocal	10 (40%)	15 (60%)	
Tumor stage			
T1 + T2	8 (17.8%)	37 (82.2%)	**0**
T3	17 (68%)	8 (32%)	
Lymph node metastases			
N0	3 (15.8%)	16 (84.2%)	**0.001**
N1	15 (68.2%)	7 (31.8%)	
Lympho-vascular invasion			
Absent	1 (4.2%)	23 (95.8%)	**0**
Present	24 (52.2%)	22 (47.8%)	
Extrathyroidal invasion			
Absent	5 (23.8%)	16 (76.2%)	0.174
Present	20 (40.8%)	29 (59.2%)	
Tumor relapse			
Absent	18 (29.5%)	43 (70.5%)	**0.005**
Present	7 (77.8%)	2 (22.2%)	

Table 2 – ***Relationship between β-catenin expression and clinicopathological characteristics***

Clinicopathological characteristics	*β*-Catenin membranous expression		*p*-value from *chi*-square test	*β*-Catenin cytoplasmic expression		*p*-value from *chi*-square test
	Low (n=42)	***High (n=28)***		***Low (n=36)***	***High (n=34)***	
Age at diagnosis						
<55 years old	25 (59.5%)	17 (40.5%)	0.921	20 (47.6%)	22 (52.4%)	0.473
≥55 years old	17 (60.7%)	11 (39.3%)		16 (57.1%)	12 (42.9%)	
Gender						
Female	31 (56.4%)	24 (43.6%)	0.234	26 (47.3%)	29 (52.7%)	0.247
Male	11 (73.3%)	4 (26.7%)		10 (66.7%)	5 (33.3%)	
Tumor size (median)						
<30 mm	16 (47.1%)	18 (52.9%)	**0.032**	16 (47.1%)	18 (52.9%)	0.633
≥30 mm	26 (72.2%)	10 (27.8%)		20 (55.6%)	16 (44.4%)	
Histopathological subtype						
Low-risk group	23 (51.1%)	22 (48.9%)	**0.042**	26 (59.1%)	18 (40.9%)	0.095
High-risk group	19 (76%)	6 (24%)		10 (38.5%)	16 (61.5%)	
Focality of the tumor						
Unifocal	26 (54.2%)	22 (45.8%)	0.141	27 (56.3%)	21 (43.7%)	0.305
Multifocal	16 (72.7%)	6 (27.3%)		9 (40.9%)	13 (59.1%)	

Clinicopathological characteristics	β-Catenin membranous expression		p-value from *chi*-square test	β-Catenin cytoplasmic expression		p-value from *chi*-square test
	Low (n=42)	*High (n=28)*		*Low (n=36)*	*High (n=34)*	
Tumor stage						
T1 + T2	24 (51.1%)	23 (48.9%)	**0.029**	24 (51.1%)	23 (48.9%)	0.93
T3	18 (78.3%)	5 (21.7%)		12 (52.2%)	11 (48.8%)	
Lymph node metastases						
N0	14 (73.7%)	5 (26.3%)	0.121	12 (63.2%)	7 (36.8%)	0.257
N1	11 (50%)	11 (50%)		10 (45.5%)	12 (54.5%)	
Lympho-vascular invasion						
Absent	14 (51.9%)	13 (48.1%)	0.27	15 (55.6%)	12 (44.4%)	0.63
Present	28 (65.1%)	15 (34.9%)		21 (48.8%)	22 (51.2%)	
Extrathyroidal invasion						
Absent	12 (57.1%)	9 (42.9%)	0.749	9 (42.9%)	12 (57.1%)	0.437
Present	30 (61.2%)	19 (38.8%)		27 (55.1%)	22 (44.9%)	
Tumor relapse						
Absent	36 (59%)	25 (41%)	0.662	29 (47.5%)	32 (52.5%)	0.152
Present	6 (66.7%)	3 (33.3%)		7 (77.8%)	2 (22.2%)	

Within this context, the abnormalities of adhesion molecules could offer a more comprehensive understanding of the variances in tumor behavior. The molecular structure of E-cadherin and its function are intimately related to β-catenin, their interactions ensuring the normal cell morphology and stability [16–18]. Alterations in E-cadherin lead to the increase of cytoplasmic β-catenin expression and, subsequently, to the amplification of transcription [47]. This phenomenon, regarded as a sequence of the carcinogenic mechanisms, facilitates the tumor growth and spreading.

Starting from the '90, several studies focus on E-cadherin changes in PTC and follicular thyroid carcinoma (FTC) and show a significant loss of its membranous expression in poorly and undifferentiated forms, respectively [48–55]. Conversely, in benign thyroid lesions, a high expression of E-cadherin in thyroid cells is reported [28]. In PTC, the low E-cadherin expression is associated with tumor size, multifocality, capsular invasion, extrathyroidal extension, local recurrence and lymph nodes metastases [26, 27, 41, 56–59]. Consequently, the absence of E-cadherin could be regarded as a negative prognostic factor.

However, few papers analyze the E-cadherin profile in different histological subtypes of PTC, with limited results regarding conventional, follicular, tall cell and diffuse sclerosing variant, respectively [27, 36, 58, 60, 61]. These results indicate that the PTC variants associated with poor outcome present lower level of E-cadherin in comparison with the conventional and follicular subtypes, and also with minimally invasive FTC.

To the best of our knowledge, the differences in E-cadherin expression in all histological subtypes of PTC are still not established. Thus, our research brings new data on this topic, based on the particularities of the study group that includes a large variability of histological subtypes of PTC, divided in low- and high-risk groups. Our data show that the E-cadherin expression (low *versus* high) is significantly correlated with the histological risk groups. In our opinion, this assumption sustains the impact of the cellular pattern of PTC on the tumor behavior and the potential prognostic value of the histological variants. Moreover, the present study adds supplementary proofs that support the relationship between E-cadherin expression and tumor aggressiveness, reflected by tumor size, tumor stage, lymph node metastases, vascular invasion and tumor relapse, in concordance with the previous reported papers.

In TC, β-catenin is less studied than E-cadherin. A limited number of reports show a strong membranous β-catenin pattern in normal thyroid tissue or benign lesions, whereas in PTC, FTC and anaplastic TC the staining is heterogeneously positive in the plasma membrane, cytoplasm or nuclei [34–36, 38]. The main types of TC present a significantly lower membranous β-catenin expression in comparison with the normal or benign thyroid, correlated with the tumor stage, extra-thyroidal extension and distant metastasis [34, 35]. In poorly and undifferentiated TC, nuclear β-catenin appears concomitantly with the loss of membranous expression [35, 38, 39]. This fact represents the hallmark for the activation of Wnt/β-catenin signaling pathway, the nuclear β-catenin operating as a transcriptional activator [33]. Aberrant β-catenin expression or localization are also associated with a worse course in papillary thyroid micro-carcinomas [37]. Thus, the changes in β-catenin expression indicate a progressive loss of tumor differentiation with results in a poor prognostic [35, 37–39].

Strictly referring to PTC, β-catenin was analyzed in conventional, follicular, tall cell and diffuse sclerosing variants [36, 62], showing a predominant membranous expression in all subtypes and an infrequent dot-like cytoplasmic (paranuclear) expression in tall cell subtype; the nuclear expression, completely absent in these subtypes [62], is reported only in cribriform-morular variant [39, 63]. The scarcity of reports concerning the β-catenin expression in different histological subtypes of PTC justifies our work directed on this topic, with preliminary results communicated in 2018 at the *European Congress of Pathology* [40]. The novelty of our study consists in the twofold analysis of β-catenin (membranous- and cytoplasmic-oriented) in different histological variants of PTC, classified as high and low risk, and the comparison of these expression by referring to the clinicopathological factors. Our data reveal significant statistically differences between the membranous β-catenin expression in the two risk groups. Moreover, our results sustain the value

of the membranous *β*-catenin, assessed as high and low, in relationship with tumor size and tumor stage. Similar results prove, in PTC, the association of low membranous *β*-catenin pattern with an increased tumor size and distant metastases [34]. The absence of significant statistically differences between the *β*-catenin cytoplasmic expression and clinicopathological factors could be explained through an early stage of Wnt pathway activation, not necessarily reflected by the cytoplasmic (or nuclear) translocation of *β*-catenin.

Conclusions

Our study demonstrates that E-cadherin and *β*-catenin expressions differ in low- and high-risk groups of PTC. The aggressive behavior of the high-risk histological variants is associated with reduced membranous E-cadherin, and loss of membranous *β*-catenin followed by enhanced cytoplasmic expression. These results open large standpoints for a deeper characterization of the histological variants of PTC.

Conflict of interests

The authors declare no conflict of interests.

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