Original paper

Potential significance of podoplanin immunohistochemical expression in papillary thyroid carcinoma

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Podoplanin (PDPN) is a lymphatic endothelial marker expressed by a range of human malignancies in which it has been shown to contribute to tumor progression and metastasis. However, there is a lack of the studies, examining the function of PDPN in thyroid cancer. The current study was performed to explore the possible diagnostic value of PDPN expression in papillary thyroid cancer (PTC) and to evaluate the marker's potential for prediction of regional lymph node metastasis. Lymphatic vascular density (LVD) and the stromal/cancer-associated fibroblasts (CAFs), labeled by PDPN, were examined in PTC compared to the other thyroid lesions. The current study included 50 cases of PTC and 50 cases of non-PTC thyroid lesions. Immunohistochemical staining was performed using monoclonal PDPN antibodies. Podoplanin expression was scored as positive and negative. Podoplanin expression was found in 36% of PTC cases, but it was not found in benign, low risk (borderline), or malignant lesions other than PTC. Furthermore, lymph node metastasis was significantly correlated with PDPN expression, LVD and CAFs (p-values < 0.00001, < 0.001 and 0.0002 respectively). These findings support the diagnostic utility of PDPN expression in PTC and its predictive value for LN metastasis.

Key words: cancer-associated fibroblasts, lymphatic vessel density, papillary thyroid carcinoma, predictive value, podoplanin, diagnostic role.

Introduction

Papillary thyroid carcinoma (PTC) is the most common thyroid cancer, accounting for 80-85% of all thyroid cancer cases with a rising incidence due to the improved screening [1-3]. Although PTC has an overall favorable prognosis with an expected indolent behavior, lymph node metastases occur in about 50% of cases. In the new era of personalized medicine, distinguishing between indolent PTC cases and those with a propensity for a more aggressive behavior may make it possible to tailor the clinical management protocols and avoid aggressive therapeutic approaches [4, 5].

The differentiation of PTC from its diagnostic mimics depends fundamentally on a meticulous histopathological examination. However, immunohistochemical studies are sometimes warranted for resolving diagnostic controversy and evaluation of prognostic parameters in certain thyroid lesions [6-9].

Numerous reliable diagnostic markers are currently employed for confirming the diagnosis of PTC; these include HBME-1 (Hector Battifora mesothelial-1), GAL-3 (galectin-3), CK19 (cytokeratin 19), CD117, CD56, and claudin-1 [6, 7]. However, there is continuous research to identify new markers that might achieve higher sensitivity and specificity

in diagnosing PTC to overcome the limitations of currently available markers. Podoplanin (PDPN) is considered a promising novel marker with diagnostic and prognostic value in PTC [10]. Podoplanin is a 38-kDa type I mucin-like transmembrane sialoglycoprotein that has utility as a specific lymphatic endothelial marker [11].

Previous studies have reported increased expression of PDPN in human squamous cell carcinomas of the lung, larynx, cervix, skin, and esophagus [12]. Podoplanin has also been shown to contribute to cancer-associated thrombosis, epithelial mesenchymal transition, cancer cell invasion, and metastasis [13].

So, PDPN may have a potential role in prediction of lymph node metastasis in several malignancies through assessment of lymphatic vessel density (LVD) [14, 15]. Moreover, solid malignancies containing PDPN-expressing cancer-associated fibroblasts (CAFs) have been reported to have poor prognosis with frequent regional lymph node (LN) metastasis [16].

Accordingly, we aimed to determine the utility of PDPN in discriminating PTC from other thyroid lesions and to assess its predictive value for LN metastasis. Hence, we compared PTC and other thyroid lesions in terms of PDPN labelled lymphatic vessel density (LVD) and CAFs to reveal their association with regional LN metastasis and high T stage in PTC. According to these incentives, we hope that using PDPN immunohistochemical evaluation could improve preoperative planning and treatment strategies in cases of PTC.

Material and methods

Tissue specimen

This retrospective cohort study was performed at the pathology department of Ain Shams University Hospitals, Cairo, Egypt. A total of 100 formalin fixed paraffin blocks of total thyroidectomy specimens in the period between 2017 and 2020 were identified according to the ethical and scientific research council of the Faculty of Medicine at Ain Shams University in Cairo, Egypt (permission number: FWA 000017585; date of approval: 2/2020). Archival slides of all cases were reexamined to verify the diagnosis in accordance with the most recent WHO classification [17]. Cases of thyroiditis and/or any other immunological diseases are excluded so as not to affect the immunohistochemical results.

Inter-observer differences were further evaluated to reach a consensus decision. The selected cases were finally segregated as two main groups: PTC (50 cases) and non-PTC (50 cases). Further subgrouping of the non-PTC cases into benign, borderline/low risk and malignant lesions other than PTC was done. Benign lesions included follicular adenoma, hyperplastic nodules, and MNG (multinodular goiter). Non-invasive follicular thyroid neoplasms with papillary-like nuclear characteristics (NIFTP) and well-differentiated thyroid tumor with uncertain malignant potential (WDT-UMP) were representative of borderline/low risk tumors. Follicular carcinoma, medullary thyroid carcinoma, and anaplastic carcinoma were among the malignant lesions. In the PTC cases, the histologic subtype, tumor focality, tumor stage, and lymph node metastasis were reported among the histologic variables according to the latest thyroid cancer TNM staging (8th edition, 2017) [18].

Immunohistochemical staining

Paraffin sections of 4 μm thickness were prepared and stained using a Benchmark Ventana (GX) automated immunostainer using ultraview peroxidase substrate diaminobenzidine (DAB). Slides were incubated with primary anti-podoplanin antibodies (mouse monoclonal Ig, prediluted 7 ml clone IHC570) for 32 minutes at 37ºC. Slides were removed from the instrument and rinsed in wash buffer when the staining run was complete. Hematoxylin was used as a counterstain on all samples.

Positive control sections of human tonsil and healthy thyroid tissues were included by preparation along the exact same protocol of tested sections. Human tonsil follicular dendritic cells, basal cells of the overlaying squamous epithelium, and endothelial cells lining the lymphatic channels in normal thyroid tissue were considered reliable control only when they displayed positive PDPN cytoplasmic and/or membranous staining which was the case in all tested sections [19].

The expression of PDPN in epithelial cells, lymphatic vessels, and CAFs was carefully evaluated. For the epithelial cells, percentage positive scores were assigned according to the scale: 0 (0%), 1 (1-10%), 2 (11-30%), 3 (31-50%), 4 (51-80%) and 5 (81-100%). Staining intensity was scored semi-quantitatively as follows: 0 (none), 1 (mild), 2 (moderate) and 3 (intense). A final immunoreactivity score (IRS) was then calculated by multiplying the intensity score by the percentage score [19]. LVD was assessed by manually counting the number of lymphatic vessels labeled by PDPN in five high-power fields. Then, the average of them was recorded [20]. Podoplanin expression in stromal fibroblasts or CAFs was considered when observing PDPN staining in more than 5% of the connective tissue stroma [21].

Statistical analysis

Podoplanin IHC score data were analyzed using an IBM-PC compatible computer running SPSS Statistics (version 25, IBM, USA).

Descriptive statistics were used to describe the study groups. The χ^2 test, Fisher's exact test, Student's t test, and the one-way ANOVA test were implemented to examine the correlations of PDPN expression, LVD, and CAFs with the different histopathological variables.

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated to evaluate the clinical utility of PDPN and PDPN labelled CAFs in the diagnosis of PTC and prediction of lymph node metastasis. Receiver operator characteristics curve (ROC curve) was plotted to evaluate the diagnostic accuracy of LVD in diagnosis of PTC and prediction of lymph node metastasis. The cut-off points for PDPN labelled LVD and area under the curve (AUC) were calculated.

Results

The demographic and histopathological features of this retrospective cohort study are summarized in (Table I).

Table I. The demographic and histopathological features of the study groups

Variables	Study groups (N = 100)
Age	
Mean ±SD	48 ±14
Gender	
Female	83 (83)
Male	17 (17)
Tumor type	
PTC group	50 (50)
Classic subtype	28 (56)
Follicular subtype	11 (22)
Micropapillary subtype	9 (18)
Solid subtype	1 (2)
Tall cell subtype	1 (2)
Non-PTC group	50 (50)
Hyperplastic nodules	14 (28)
Follicular adenoma	14 (28)
Multinodular goiter	8 (16)
NIFTP	3 (6)
WDT-UMP	4 (8)
Follicular carcinoma	5 (10)
Medullary carcinoma	1 (2)
Anaplastic carcinoma	1 (2)
T stage in PTC	
T1/T2	38 (76)
T3/T4	12 (24)
Tumor focality in PTC	
Unifocal	39 (78)
Bifocal	4 (8)
Multifocal	7 (14)
LN metastasis in PTC	
LN positive	20 (40)
LN negative	30 (60)

SD – standard deviation, N – number, PTC – papillary thyroid carcinoma, NIFTP – non-invasive follicular thyroid neoplasm with papillary like nuclear features, WDT-UMP – well differentiated tumor of uncertain malignant potential, LN – lymph node

Epithelial podoplanin expression in papillary thyroid carcinoma

Podoplanin was expressed in 36% (18/50) of PTC group, but none of the non-PTC group (0/50) (Fig. 1A-C). 16% of PTC cases had an IRS ≥ 7, whereas all the non-PTC group had an IRS of zero. Accordingly, there were significant differences in PDPN expression and IRS between the two groups ($p = 0.0001$).

Neither PDPN expression nor its IRS was significantly correlated with PTC subtype or the tumor focality ($p = 0.07$ and $p = 0.2$, respectively).

Moreover, there was no discernible association between PDPN expression and T stage in the PTC group ($p = 0.06$). However, the IRS was higher among T3/T4 PTC cases ($p = 0.02$).

Podoplanin expression and its IRS were higher among PTC cases with lymph node metastasis ($p < 0.0001$) (Fig. 2A-D). Detailed results are illustrated in Table II.

In terms of diagnosing PTC, PDPN had a sensitivity of 36%, specificity of 100%, PPV of 100%, and NPV of 100%. Its predictive value for LN metastasis had a sensitivity of 70%, specificity of 86.7%, PPV of 77.8%, and NPV of 81.3% (Table III).

Podoplanin-expressing lymphatic vessel density in papillary thyroid carcinoma

Podoplanin-expressing lymphatic vessel density (LVD) (mean ±SD) was significantly higher in PTC compared to non-PTC group (2.35 ±2.06 vs. 0.22 ±0.04, $p < 0.0001$; Fig. 3A-D).

Moreover, PTC subtype, T stage, and LN metastasis had a significantly positive correlation with LVD ($p = 0.01$, $p = 0.01$, and $p < 0.001$, respectively); however, no significant correlation was demonstrated between LVD and tumor focality ($p = 0.64$; Table IV).

The clinical utility of PDPN-expressing LVD, in diagnosis of PTC and prediction of LN metastasis, was assessed by ROC curve analysis. With a positive cut-off value ≥ 0.9, LVD in PTC vs. non-PTC displayed a sensitivity of 94%, specificity of 56.5%, PPV of 82.5%,

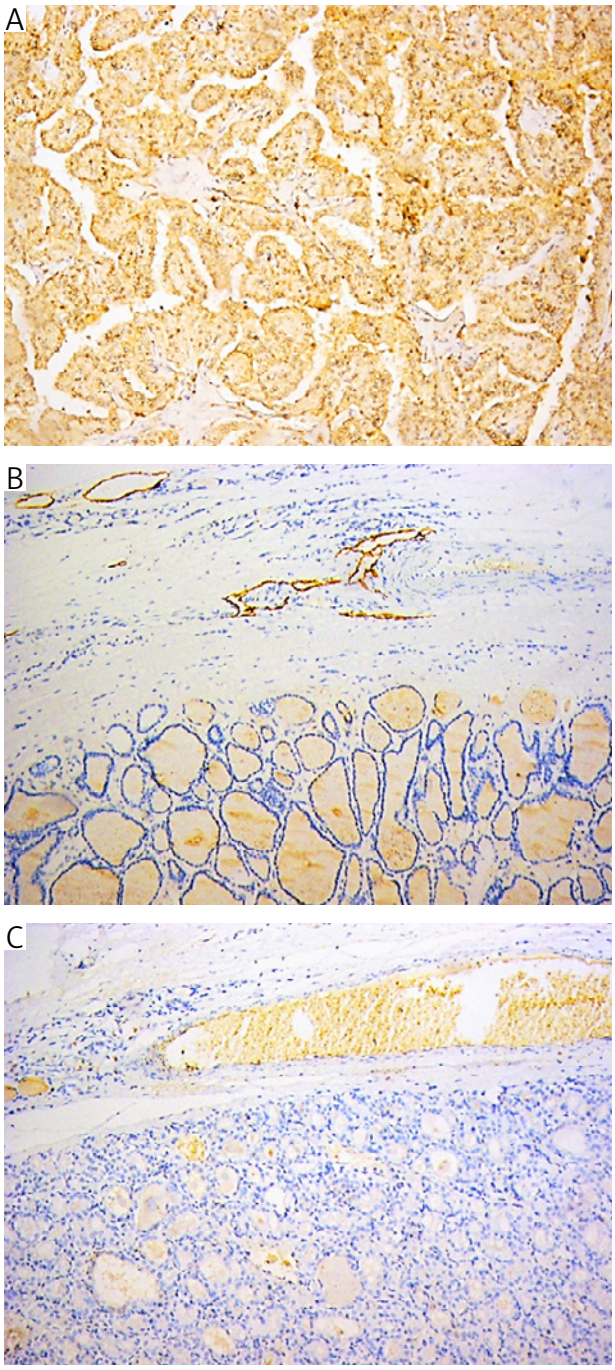

Fig. 1. Podoplanin expression in PTC vs. non-PTC. **A**) Representative case of PTC with moderate diffuse expression of PDPN in tumor cells ×100. **B**) Representative case of a hyperplastic nodule demonstrating negative PDPN expression in tumor cells with PDPN expression in nearby lymphatic vessels that serve as positive internal control ×100. **C**) Representative case of noninvasive follicular thyroid neoplasm with papillary-like nuclear features (low-risk/borderline lesion) demonstrated negative PDPN expression in tumor cells with low LVD in the peritumoral regions ×100

and NPV of 81.3%. Regarding its predictive value for LN metastasis, it showed a sensitivity of 89.7%, specificity of 84.6%, PPV of 81.3%, and NPV of 91.7% with a positive cut-off value ≥ 1.9 (Fig. 4A, B).

Podoplanin-expressing CAFs in papillary thyroid carcinoma

Podoplanin-expressing CAFs were significantly different between PTC and non- PTC groups (16% vs. 0%, p = 0.003; Fig. 5A, B). Furthermore, they were significantly associated with tumor stage and LN metastasis in the PTC group (p = 0.001 and p = 0.0002, respectively; Table V). On the other hand, the correlation between them and PTC subtype or tumor focality was not significant (p = 0.11 and p = 0.15, respectively; Table V). Podoplanin-expressing CAFs in PTC vs. non-PTC showed sensitivity of 16%, specificity of 100%, PPV of 100%, and NPV of 45.7%. For prediction of LN metastasis, CAFs manifested sensitivity of 40%, specificity of 100%, PPV of 100%, and NPV of 71.4%. Furthermore, they showed sensitivity of 50%, specificity of 94.7%, PPV of 75%, and of NPV 85.7% in low T stage vs. high T stage PTC (Table VI).

Discussion

Podoplanin has been proposed as a marker of thyroid pathology that has pathological roles in tumor cell migration, invasion, and metastasis [22]. It was first described as a specific lymphatic endothelial marker useful for determining lymph node metastases and evaluating tumor LVD [23]. Although previous studies suggested that PDPN contributes to carcinogenesis, tumor progression, metastasis, and poor survival [24, 25], more recent research suggested that it may play an antitumoral role in squamous cell lung cancer and lip cancer [26, 27]. Due to this debate and the remarkably scarce research, working on the role of PDPN in thyroid cancer, particularly PTC, we are motivated to explore the diagnostic utility of PDPN expression in PTC as well as its role in predicting LN metastasis and high T stage.

In the current study, a significant difference in PDPN expression and the corresponding IRS was found between the PTC and non-PTC groups. These results are supported by others [23, 28, 29] as they found significantly higher PDPN expression in PTC compared to the normal, benign and other malignant thyroid lesions. So, PDPN might have a substantial utility in the differential diagnosis of PTC from potential histologic mimics.

The IRS and PDPN expression of various PTC subtypes was not significantly different, in our research. These findings agree with Rudzinska *et al.* [29]. There were not many studies exploring the significance of PDPN in differentiating the subtypes

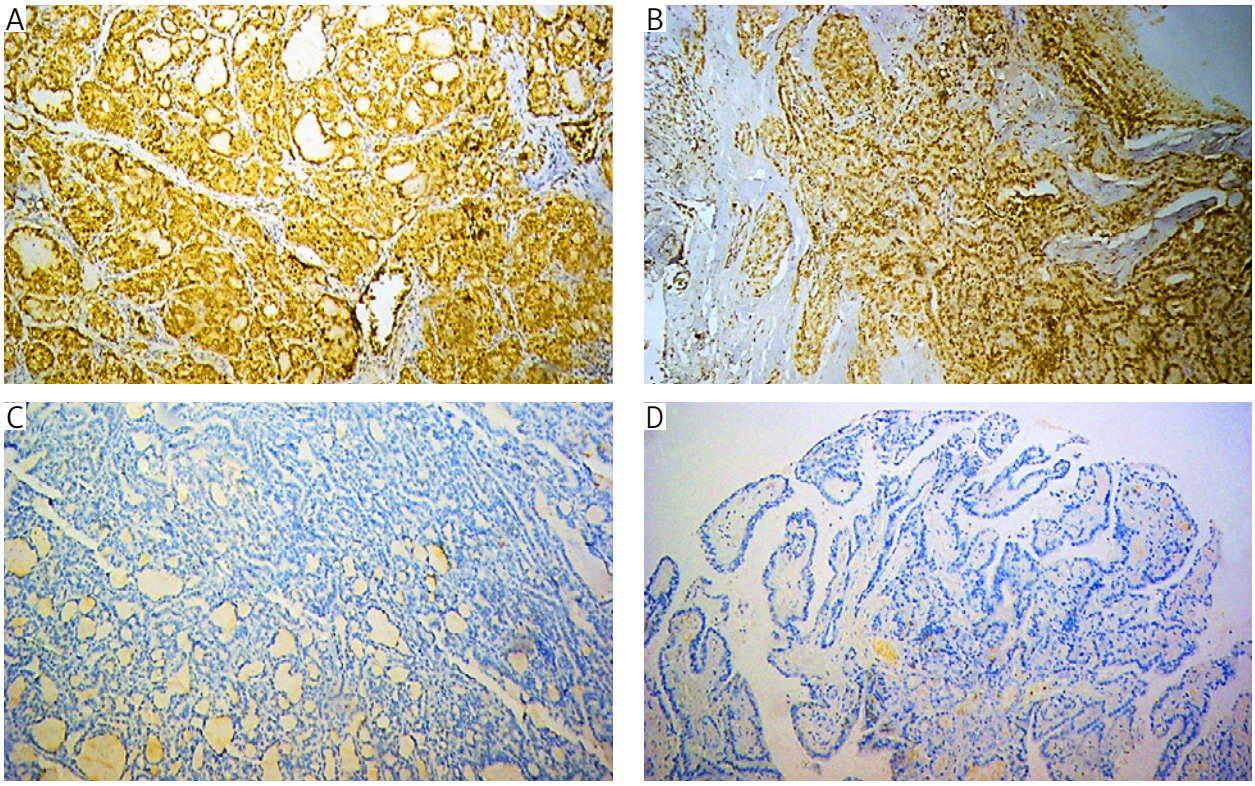

Fig. 2. Podoplanin expression in PTC with LN metastasis vs. PTC with no LN metastasis. **A**, **B**) Two representative cases of follicular subtype PTC with LN metastasis demonstrating diffuse expression of PDPN in tumor cells ×100. **C**, **D**) Two representative cases of PTC with no LN metastasis demonstrating negative PDPN expression in tumor cells ×100

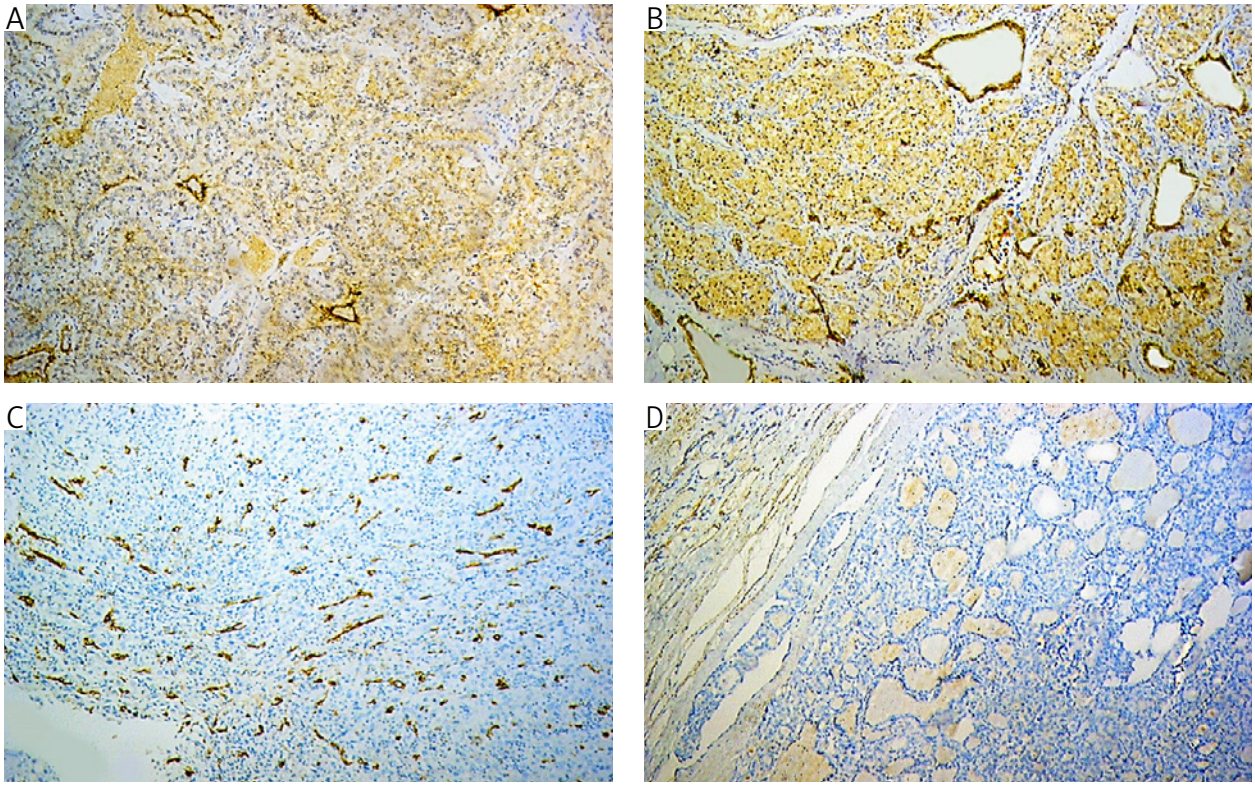

Fig. 3. Podoplanin-expressing LVD in PTC (with LN metastasis) vs. non-PTC. **A**) Representative case of PTC with weak expression of PDPN in tumor cells and strong expression of PDPN in lymphatic vessels ×100. **B**) Representative case of PTC with moderate expression of PDPN in tumor cells and strong expression of PDPN in lymphatic vessels ×100. **C**) Representative case of PTC with negative PDPN expression in tumor cells and high PDPN expression highlighting areas of high LVD ×100. **D**) Representative case of follicular carcinoma with blood vessel invasion demonstrating negative PDPN expression in tumor cells and blood vessel walls with tumor invasion ×100

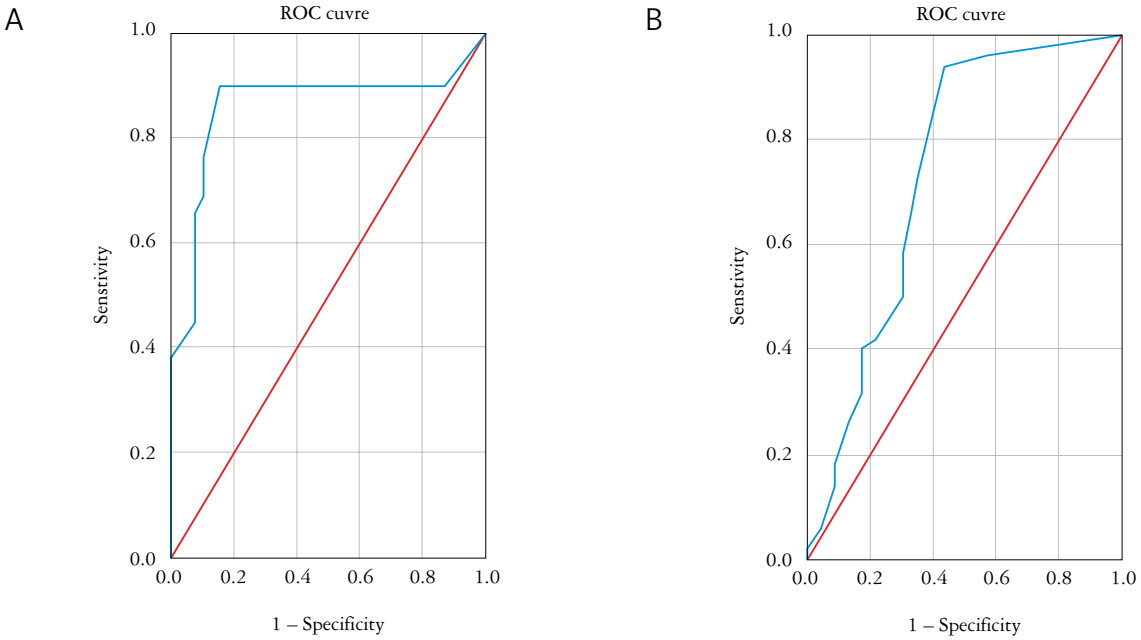

Fig. 4. The clinical utility of PDPN-expressing LVD in PTC. **A**) For diagnosis of PTC, area under the curve: 0.737; standard error: 0.072; asymptotic significance 0.01 and confidence interval: 0.596-0.877. **B**) For prediction of LN metastasis, area under the curve: 0.857; standard error: 0.056; asymptotic significance 0.0 and confidence interval: 0.748-0.966

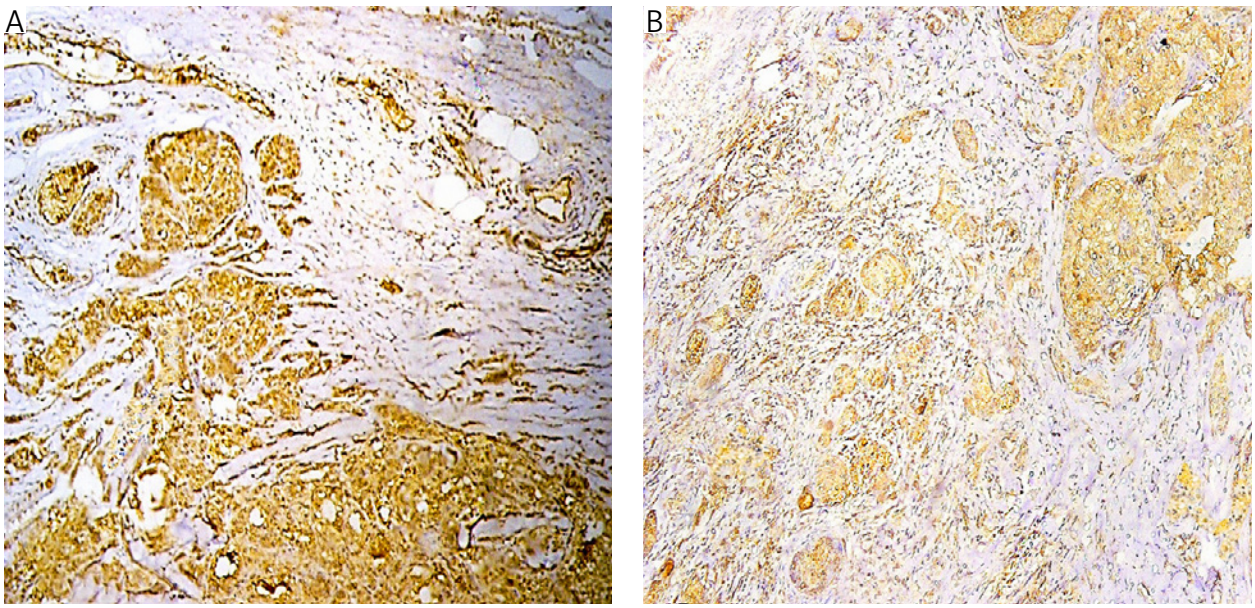

Fig. 5. Podoplanin-expressing CAFs in PTC with LN metastasis. **A, B**) Two representative cases of PTC with LN metastasis demonstrating strong IHC expression of PDPN in both tumor cells and CAFs ×100

of PTC. Additionally, we didn't find enough number of different subtypes; thus, our finding warrants further verification by testing in larger scale studies.

There was no significant association between PDPN expression and tumor focality, and T stage in PTC. However, the IRS was higher among high T stage PTC cases. This is concordant with Rudzinska *et al.* [29]. On the other hand, we identified a significant correlation between PDPN expression and lymph node metastasis in the PTC group. Similar results were reported by Gong *et al.* and Mello *et al.* [23, 30]. These results highlight the potential value of PDPN in tumor invasiveness and progression, so it can be of predictive value.

The LVD has been frequently correlated with tumor recurrence and poor prognosis. Moreover, it has a potential role as a predictive marker for lymph node metastasis in colorectal cancer, breast cancer, and

Table II. PDPN and IRS correlations with different pathological criteria

Study groups	N (%)	PDPN expression			χ^{2*} (p-value)
		IRS 1-6 n (%)	IRS ≥ 7 n (%)	IRS 0 n (%)	
PTC group	50 (50)	10 (20)	8 (16)	32 (64)	18.9 (<0.0001)
Non-PTC group	50 (50)	0	0	50 (100)	
Classic PTC	28 (56)	6 (12)	7 (14)	15 (30)	12.2 (0.07)
Follicular PTC	11(22)	3 (6)	1 (2)	7 (14)	
Micropapillary PTC	9 (18)	0	0	9 (18)	
Tall cell PTC	1(2)	1 (2)	0	0	
Solid PTC	1 (2)	0	0	1 (2)	
Unifocal PTC	39 (78)	7 (14)	6 (12)	6 (12)	3.3 (0.2)
Bifocal PTC	4 (8)	2 (4)	1 (2)	1(2)	
Multifocal PTC	7 (14)	1 (2)	1 (2)	5 (10)	
T1/T2 PTC	38 (76)	8 (16)	3 (6)	27 (54)	7.8 (0.06)
T3/T4 PTC	12 (24)	2 (4)	5 (10)	5 (10)	
LN positive PTC	20 (40)	7 (14)	7 (14)	6 (12)	17.3 (<0.00001)
LN negative PTC	30 (60)	3 (6)	1(2)	26 (52)	

PDPN – podoplanin; PTC – papillary thyroid carcinoma; IRS – immunoreactivity score; LN – lymph node

** χ^2 test was used to explore the significance of PDPN in differentiating PTC from non-PTC and to examine the correlation of PDPN expression with LN metastasis in PTC group, however, Fisher exact test was used to find the correlations of PDPN expression with PTC subtype, focality and T stage in PTC. P-value ≤ 0.05 is significant*

head and neck squamous cell carcinoma (HNSCC) [31, 32], so we assessed it using PDPN and we found that PDPN labelled LVD is higher in PTC than in non-PTC groups. This is in keeping with Gong *et al.* and Giorgadze *et al.* [23, 33]. Moreover, the LVD in PTC subtypes was assessed, and a significant difference was found between them. A conflict was found among studies in this aspect [34, 35]. This can be attributed to the different assessment and analytical methods used. Also, the limited number of cases cannot validate such results necessitating further research.

Podoplanin-expressing CAFs have been implicated in tumor migration, invasiveness, lymphangiogenesis, and metastasis in a number of malignancies, including thyroid carcinoma [36, 37]. The protumorigenic effects of PDPN-positive CAFs can probably be ascribed to induction of Rho A activity, which causes ezrin phosphorylation and actin rearrangement, so it enhances tumor cell movement and metastasis, and promotes epithelial mesenchymal transition [12, 38, 39]. CAFs also have immunosuppressive impact on the microenvironment by blunting CD8+T cell responses and secreting immunosuppressive cytokines such as TGF-1 and IL-10 [40]. Undoubtedly, producing any novel insights, regarding the carcinogenic attributes of CAFs, can open promising horizons in launching contemporary, efficient, immune-targeted therapies [41].

Table III. The clinical utility of PDPN expression in PTC

Value of PDPN expression		95% CI
Diagnosis of PTC		
Sensitivity	36%	22.92-50.81%
Specificity	100%	92.89-100%
PPV	100%	-
NPV	60%	55.93-65.8%
Prediction of LN metastasis in PTC group		
Sensitivity	70%	45.72-88.11%
Specificity	86.7%	69.28-96.24%
PPV	77.8%	57.36-90.11%
NPV	81.3%	68.62-89.57%

PPV – positive predictive value; NPV – negative predictive value; CI – confidence interval; PTC – papillary thyroid carcinoma, LN – lymph node

It is clear that there have been few research studies on the expression of PDPN and other CAF-associated proteins in thyroid carcinoma, and our study is innovative in comparing PDPN-expressing CAFs between PTC and non-PTC cases. Our findings showed that PDPN-expressing CAFs were significantly higher among PTC cases. Accordingly, our preliminary results emphasize that PDPN has a distinct expression profile in PTC that might distinguish it from other mimics.

Table IV. Correlations of LVD with different pathological criteria

Study group	N (%)	PDPN-expressing LVD	Test value (p-value)
		Mean ±SD	
PTC	50 (50)	2.35 ±2.06	10.4* (<0.0001)
Non-PTC	50 (50)	0.22 ±0.04	
Classic PTC	28 (56)	2.99 ±1.46	
Follicular PTC	11 (22)	1.71 ±1.18	
Micropapillary PTC	9 (18)	1.27 ±0.28	4.23** (0.01)
Tall cell PTC	1 (2)	1.8	
Solid PTC	1 (2)	1.8	
Unifocal PTC	39 (78)	2.22 ±1.30	
Bifocal PTC	4 (8)	2.68 ±2.23	0.46** (0.64)
Multifocal PTC	7 (14)	1.89 ±1.76	
T1/T2 PTC	38 (76)	1.82 ±1.16	2.99* (0.01)
T3/T4 PTC	12 (24)	3.11 ±1.73	
LN positive PTC	20 (40)	3.7 ±1.1	7.89* (<0.001)
LN negative PTC	30 (60)	1.45 ±0.74	

SD – standard deviation; LVD – lymphatic vessel density, PDPN – podoplanin; LN – lymph node

**Student t-test was used to assess the significance of LVD in differentiating PTC from non-PTC groups and to explore its correlation with T stage and LN metastasis*

*** one way Anova test was used to assess LVD correlation with PTC subtype and focality of PTC. P-value ≤ 0.05 is significant*

Table V. Correlations of PDPN expressing stromal/CAFs with different pathological criteria

Study group	N (%)	PDPN expressing stromal/CAFs		(p-value) χ^2
		Present N (%)	Absent N (%)	
PTC	50 (50)	8 (8)	42 (42)	8.7* (<0.003)
Non-PTC	50 (50)	0	50 (50)	
Classic PTC	28 (56)	8 (28.6)	20 (71.4)	
Follicular PTC	11 (22)	0	11 (100)	
Micropapillary PTC	9 (18)	0	9 (100)	7.5** (0.11)
Tall cell PTC	1 (2)	0	1 (100)	
Solid PTC	1 (2)	0	1 (100)	
Unifocal PTC	39 (78)	5 (12.8)	34 (87.2)	3.7* (0.15)
Bifocal PTC	4 (8)	2 (50.0)	2 (50)	
Multifocal PTC	7 (14)	1 (14.3)	6 (85.7)	
T1/T2 PTC	38 (76)	2 (5.3)	36 (94.7)	10.5* (0.001)
T3/T4 PTC	12 (24)	6 (50)	6 (50)	
LN positive PTC	20 (40)	8 (20)	12 (60)	14.2** (0.0002)
LN negative PTC	30 (60)	0	30 (100)	

CAFs – cancer associated fibroblasts; PDPN – podoplanin; LN – lymph node

** χ^2 test was used to compare CAFs among PTC and non-PTC groups and to explore their correlations with tumor focality and T stage*

*** Fisher exact test was used to explore their correlations with PTC subtype and LN metastasis. P-value ≤ 0.05 is significant*

Although numerous approaches for predicting LN metastasis in patients with thyroid cancer have been established, the rate of LN metastasis remains high following total thyroidectomy, even after the use of radioactive iodine ablation. Consequently, there is an urgent clinical need for novel prognostic indicators of LN metastases [42]. So, the LVD and CAFs were evaluated in the current study in relation to LN metastasis to explore their potential prognostic significance for LN metastasis. As a result, a significant

association between LVD and CAFs, and LN metastases was discovered. This is concordant with previous studies [23, 43, 44]. Thus, PDPN labelled LVD and CAFs can be considered as potential predictive markers for the regional LN metastasis in PTC. Such results pave the way for further specified research work in this aspect.

Also, we assessed the LVD and CAFs in PTC in relation to the tumor focality and T stage. There was no statistically significant correlation between tumor focality and LVD in the PTC group in accordance with Lei *et al.* [45]. This finding would suggest that the multiple foci seen in our cases might represent synchronous tumors rather than metachronous tumors with intra-thyroid dissemination.

On the other hand, our findings and others showed a significant correlation between LVD and T stage [46]. Nonetheless, some previous studies, have found no significant association between LVD and tumor stage [35, 47]. These contradictory findings are mostly attributed to the inconsistent methodologies employed to assess LVD [48]. We believe that our finding is likely objective, as we implemented PDPN, a specific lymphatic endothelial marker, for reliable LVD detection; however, larger scale studies are needed to validate this proposal. Additionally, we found a significant correlation between PDPN-expressing CAFs and T stage. This is harmonious with many other reports [16, 44, 49]. BRAFV600E mutation, an aggressive finding, has been more prevalent in PTC with higher PDPN-expressing CAFs [50, 51]. Moreover, previous studies have reported significant correlation between CAFs and high T stage in breast carcinoma, lung adenocarcinoma; and head and neck carcinoma [52-54].

Conclusions

Podoplanin expression in PTC (i) carries both diagnostic value and potential utility as a predictor of regional LN metastasis; (ii) evaluates LVD and CAFs which are both significantly associated with LN metastasis; (iii) shows high specificity, as revealed in our study, which qualifies it to be included in the current diagnostic IHC panels to refine their accuracy in diagnosis of equivocal lesions; (iv) may facilitate the development of novel targeted pharmaceutical therapies which can generally improve efficacy of many cancer therapy protocols. The drawback in our study is that PDPN expression (i) has low sensitivity in diagnosis of PTC; (ii) cannot differentiate between PTC subtypes; (ii) has no significant association with neither tumor focality nor T stage. These restrictions might be imposed by the limited representative numbers of these cases in our study, so our results cannot be considered conclusive until tested in further research.

Table VI. The clinical utility of PDPN-expressing CAFs in PTC

Value of PDPN expressing CAFs		95% CI
Diagnosis of PTC		
Sensitivity	16%	7.17-29.11%
Specificity	100%	92.89-100%
PPV	100%	-
NPV	45.7%	51.33-57.33%
Prediction of LN metastasis in PTC group		
Sensitivity	40%	19.12-63.95%
Specificity	100%	88.34-100%
PPV	100%	-
NPV	71.4%	63.61-78.14%
Prediction of high T stage in PTC group		
Sensitivity	50%	21.09-78.91%
Specificity	94.7%	82.25-99.36%
PPV	75%	40.99-92.83%
NPV	85.7%	77.22-91.39%

CAFs – cancer associated fibroblasts; PDPN – podoplanin; LN – lymph node; PTC – papillary thyroid carcinoma; PPV – positive predictive value; NPV – negative predictive value; CI – confidence interval

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The authors declare no conflict of interest.

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