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Relevance of rosette patterns in variants of papillary thyroid carcinoma

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Abstract

Introduction: The detection of rosette-like clusters (RLC) of follicular cells in thyroid carcinoma has been reported mostly in the columnar cell variant of papillary thyroid carcinoma (PTC). Despite the fact that diagnosing variants of PTC is no longer encouraged by The Bethesda System for Reporting Thyroid Cytopathology, the identification of cytomorphological features such as RLC linked with these tumours might help reduce possible misinterpretation in thyroid fine needle aspiration (FNA) cytology. We accordingly investigated the potential correlation of architectural patterns including RLC with PTC variants.

Methods: We analysed 225 thyroid FNA cytology cases diagnosed as suspicious for malignancy (SFM) and positive for malignancy (M) over a 1-year time where all samples had corresponding histology. We also included 150 benign lesions from the same period. The presence of RLC vs similar appearing solid clusters, papillary structures and microfollicles were evaluated. We also performed immunocytochemistry and molecular testing for *BRAFV600E*.

Results: We included 100 (44.4%) SFM favouring PTC and 125 (55.6%) M cases with cyto-histological correlation. On histology, all SFM and M cases showed malignancy including 140 (62.2%) classic PTC and 85 (37.8%) PTC variants. The cytomorphological patterns in all FNA samples included solid (74%), papillary (89%), microfollicular (70%), and pseudo-RLC morphology (25.7%). We identified only pseudo-RLC in 33 FNA specimens from PTC variant cases that included tall cell variant (42.4%), hobnail variant (21.2%) and miscellaneous variants (36.3%) of PTC. No definitive RLC were detected in our series. Immunocytochemistry and *BRAFV600E* were not specifically linked with an RLC pattern.

Conclusions: These findings demonstrate that in our dataset the architectural pattern of RLC was not recognised within PTC variants. However, we did identify a pseudo-RLC pattern that was observed in association with tall cell variant and hobnail variant cases of PTC.

Liron Pantanowitz and Esther Diana Rossi share the senior authorship.

KEYWORDS
microfollicular pattern, papillary pattern, PTC variant, rosette pattern, thyroid, thyroid malignancy

1 | INTRODUCTION

Rosette and rosette-like patterns can be found in different tumours, particularly those from the nervous system.[1] Histologically, a typical rosette is characterised by a small round group or cluster of cells organised in a spoke-wheel or halo arrangement around a central lumen. The name is derived from their morphological resemblance to rose windows found in gothic churches, such as those found in the Cathedral of Notre Dame in Paris.[2-4] Whilst different authors have described rosette and rosette-like patterns as a manifestation of certain tumours, this architectural pattern alone is not pathognomonic of any specific tumour type.[1-2] Their presence, in fact, may be associated with various forms of tumour differentiation.[1-10] True rosettes are a characteristic finding in neurological tumours.[1] However, rosettes have also been identified in other neoplasms such as medullary thyroid carcinoma, osteosarcoma, fibromyxoid sarcoma, rhabdomyosarcoma, endometrial stromal sarcoma and some non-Hodgkin lymphomas.[1-11] Pseudorosettes tend to be associated with neuroblastomas, medulloblastomas, malignant melanoma, Merkel cell carcinoma, other neuroendocrine tumours of the skin, primitive neuroendocrine tumour, retinoblastoma, ependymoma, neurocytoma, glioblastoma, Wilm tumour and pheochromocytoma.[1-11]

To the best of our knowledge, the histological identification of a rosette pattern in thyroid histological samples has not been well published. However, rosettes have been previously documented in cytological samples from benign and malignant lesions such as adenomatous goiter, insular carcinoma and medullary thyroid carcinoma).[11-17] Sen et al also reported the presence of a rosette-like pattern in a fine needle aspiration cytology (FNAC) specimen of a well-differentiated thyroid follicular carcinoma diagnosed as columnar cell variant (CCV) of papillary thyroid carcinoma (PTC).[12] Only a few other papers have reported this finding associated with thyroid carcinoma in which a rosette-like pattern was linked with aggressive variants of PTC including the tall cell variant (TCV) of PTC.[13-17] Therefore, whether a rosette-like pattern is indeed associated with CCV-PTC and its potential diagnostic value in thyroid cytology remains unknown. To assess any possible correlation of rosette-like morphology with specific PTC variants, we accordingly examined a retrospective series of thyroid FNAC cases for such specific features.

2 | MATERIALS AND METHODS

We received institutional (Catholic University of the Sacred Heart) ethical approval for this study. A retrospective search was performed for all thyroid FNACs diagnosed as SFM and M over a 1-year period (January 2018 to December 2018) at the Catholic University "Agostino Gemelli" Hospital in Rome, Italy. Furthermore, to determine the presence of a rosette-like pattern in benign vs malignant thyroid entities, we included another 150 benign sequential lesions diagnosed during the same study period. The institutional electronic medical record system Armonia-Metafora, Italy (CU) was also searched for thyroidectomy specimens during the same study period. The patient's age, sex, FNAC diagnoses and follow-up information were recorded. All available pathology slides were reviewed. The majority of the thyroid nodules were evaluated and biopsied under ultrasound guidance by clinicians and radiologists.

2.1 | Thyroid FNAC specimens

All aspirations (with usually two passes performed for each lesion) were performed with 25- 27G needles. No rapid on-site assessment for adequacy of material was done. All patients consented to their procedure. All FNAC specimens were processed using a ThinPrep 5000^{TM} processor (Hologic Co). Prepared slides were fixed in 95% methanol and stained with a Papanicolaou stain. Any remaining material was stored in Preservcyt solution for potential ancillary studies.

Specimen adequacy was determined according to the Bethesda and British RCPath classification schemes.[18-19] The cytology cases were classified and diagnosed according to the new Italian Working Group SIAPEC-IAP classification.[20-21] All of the cases were re-evaluated and then re-classified according to The Bethesda System for Reporting Thyroid Cytology II (TBSRTC, 2017).[1936] For this retrospective study, analyses were conducted using TBSRTC terminology. This case series included the following distribution of diagnoses: 5.9% non-diagnostic including cystic cases; 77.8% benign lesions (BL); 3% atypia of undetermined significance/follicular lesion of undetermined significance; 6.1% follicular neoplasms; 2.2% SFM and 5% malignant (M) cases. All cytology and histology cases were reviewed by two cytopathologists whilst the re-classification according to TBSRTC was done by one cytopathologist (E.D.R.). Cases with an equivocal interpretation were subject to consensus review. The concordance between SIAPEC-IAP and TBSRTC classification systems was 95.9%.

According to the literature, rosettes are defined as a group of 15 or fewer follicular cells with a circular arrangement and central lumen that may/may not be filled with colloid.[18-19] Most of the cytoplasm of the cells in a rosette tends to be located towards the centre (i.e. nuclei are peripheral), implying that these cells are tall/columnar in shape. At our institution, we utilise liquid-based cytology (LBC) preparation vs the conventional slide method. Figure 1A,C and Table 1 compares the rosette-like clusters (RLC) as reported by Sen et al[12] to microfollicular and papillary structures. By comparison, a microfollicle is also comprised of a group of 15 or fewer follicular

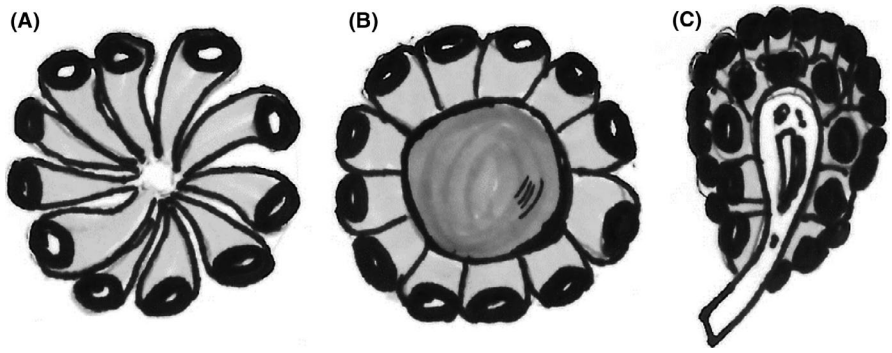

FIGURE 1 Comparison of papillary, rosette and microfollicular structures. Drawing of the different patterns in: (A) classic rosette structure; (B) cluster of follicular neoplastic cells; (C) papillary structure. Papanicolaou stain, 200×, liquid-based cytology

TABLE 1 Comparison of cytomorphological patterns

Follicular structure	Rosette pattern	Microfollicles	Papillary clusters
Definition	• Cells with circular arrangement • 15 or fewer cells • Cytoplasm merges toward centre • Most of the cytoplasm is located within centre (i.e. nuclei peripheral), implying the cells are tall/columnar • Central area can have a lumen (± filled with colloid)	• Cells with circular arrangement • 15 or fewer cells • Cytoplasm merges toward centre • Minimal cytoplasm/cell causes fewer to be located within centre • Cells shorter (cuboidal) • Central area usually has colloid	• Cells with more elongated arrangement • 15 or more cells • Cytoplasm need not merge toward centre • Minimal cytoplasm/cell • Cells shorter (cuboidal) • Central fibrovascular core

cells with a circular arrangement and central area usually filled with colloid. However, these follicular cells are shorter (cuboidal) and thus exhibit less cytoplasm in the microfollicle centre. A micropapillary cluster is comprised of 15 or more follicular cells arranged around a central fibrovascular core. The individual follicular cells in a papillary cluster are also shorter (more cuboidal) and have minimal cytoplasm that typically does not merge toward the center of the cellular cluster. Nonetheless, we only found a pseudorosette pattern in some cases. A pseudorosette pattern resembles rosettes, but does not exhibit the features depicted in Figure 1A and as described in the paper by Sen et al.[12] The presence/absence of these different follicular structures was recorded in our cohort of cases.

2.2 | Immunocytochemistry analysis

In our routine practice, we perform ancillary techniques including immunocytochemistry (ICC) and *BRAF*^{V600E} analysis in all suspicious and malignant thyroid cytological cases. Specifically, we perform ICC on all LBC samples. HBME-1 and galectin-3 ICC is performed using a protocol previously described by our group.[22-24] The minimal percentage of adequate lesional cells for the performance of ICC evaluation was defined at 30% in LBC samples. Lesional cells were interpreted to be positive when at least 50% of the cells demonstrated strong cytoplasmic staining. To avoid false negative and/or false positive yields, this 50% ICC cut-off value was also used for histological tissue sections. A case was considered to be overall positive for malignancy when there was concomitant expression of both immunomarkers. Adequate galectin-3 immunoreactivity was represented by cytoplasmic staining, and suitable HBME-1 staining was chiefly within the cytoplasm with accentuation on the cytoplasmic membrane and within the lumen. Positive controls included mesothelioma for HBME-1 (membranous positivity) and histiocytes for galectin-3 (cytoplasmic staining). Lymphocytes identified in the majority of the thyroid slides were used as a negative control.

2.2.1 | Molecular analysis for *BRAF* mutation

DNA was extracted from both LBC stored material and paraffin embedded tissues, with 100% concordance. *BRAF*^{V600E} mutational analysis was performed on DNA extracted from cytological and surgical specimens containing at least 70% tumour. Details of the protocol employed have been previously published by our group.[25-28]

2.3 | Histology specimens

All surgical specimens were fixed in 10% buffered formaldehyde, embedded in paraffin and 5-μm-thick sections then stained with haematoxylin-eosin. The diagnosis of classical variant of PTC was based on the presence of true papillary structures and distinctive nuclear features. The diagnosis of FVPTC relied upon the detection of follicular architecture coupled with the nuclear features of PTC in multiple foci. Encapsulated tumours with either lympho-vascular invasion (within the capsule or beyond) or capsular penetration were diagnosed as invasive FVPTC. All cases were classified according to the seventh edition of the tumour-node-metastasis-based staging system recommended by the American Joint Commission on Cancer.[29] The different PTC variants were classified according to

the World Health Organisation 2017.[30-31] For the definition of TCV of PTC, we adopted the standard definition of at least 30% of tall cell component. However, in our analysis we also included cases of PTC with a less than 30% TCV component. The histological diagnosis of non-invasive follicular thyroid neoplasm with papillary like nuclear features was rendered according to the criteria described by Nikiforov et al.[24] However, in our institution neoplasm with papillary like nuclear features terminology was used for follicular neoplasms without any overt papillary structures.

2.4 | Statistical analysis

Statistical analysis was performed using GraphPad-Prism 5 software (Graph Pad Software, San Diego, CA, USA) and MedCalc version 10.2.0.0 (MedCalc Software). Statistical comparison of continuous variables was performed using the Mann-Whitney *U*-test or paired *t*-test, as appropriate. Comparison of categorical variables was performed using the χ^2 statistic, and the Fisher's exact test. *P*-values <.05 were considered as statistically significant.

3 | RESULTS

Table 2 summarises the clinical-pathological features for our retrospective series. To validate the search for a rosette-like pattern in malignant lesions as reported by Sen et al,[12] we selected SFM and M cases with a with cyto-histological correlation resulting in a histological diagnosis of PTC and its variants. Furthermore, to determine if there was a difference of a rosette-like pattern for benign and malignant thyroid entities, we also included 150 sequential benign lesions diagnosed during the same study period. The entire series was composed of 239 female and 136 male patients with an age range of 9 to 82 years (mean: 36.5 years). The size of thyroid lesions subject to FNAC ranged from 5 mm to 65 mm in greatest dimension (median size = 14 mm). All sub-cm lesions were discovered during radiological screening for causes unrelated to thyroid disease. There was no significant difference in the size of these thyroid nodules among the diagnostic entities. The cytological series included 150 BL (40%), 100 SFM (26.6%) and 125 M (33.3%). Only 20 (13.3%) out of 150 BL had histological follow-up, showing 15 goitre and five follicular adenoma cases. The histological diagnoses for SFM and positive for malignant cases included: 140 classical PTC (cPTC-62.2%) and 85 PTC variants (37.8%).

The distribution of the PTC variants, analysed in Table 3, was characterised by 140 classic PTC, 19 pure TCV, 27 PTC with a less than 30% TCV component, 13 hobnail PTC variant, eight follicular variant of PTC (FVPTC), six Warthin-like PTC, five columnar cell variant of PTC (CCV-PTC), four cases with both TCV plus CCV-PTC, and three solid variant of PTC (Table 3). For the TCV cases, we included both pure TCV (19 cases) and also PTC cases with a TCV component (27 cases, 58.7%) that contained less than 30% tall cells. In Table 3 we further evaluated the 85 PTC variants distributed according to their cytology diagnosis of SFM (33 cases) and positive for malignancy (52 cases). On histology, the 33 SFM were diagnosed as six pure TCV and 14 PTC with a less than 30% TCV component, six hobnail PTC variant, one Warthin-like PTC, four FVPTC and two CCV-PTC. The 52 malignant cases were diagnosed as 13 TCV and 13 PTC with less than 30% TCV, seven hobnail PTC variant, five Warthin-like PTC, four FVPTC, three CCV-PTC, four combined TCV and CCV, and three as solid variant of PTC (Table 3).

TABLE 2 Clinicopathological features of study cases

Parameter	Quantity (n = 375)
Female	239 (63.7%)
Male	136 (36.2%)
Thyroid nodule size	6-65 mm
Cytological diagnosis	BL = 150 (40%) SFM = 100 (26.6%) PM = 125 (33.3%)
Histological diagnosis (245 cases)	15 goitre (6.1%) 5 FA (2%) cPTC = 140 (48.9%) PTC variants = 85(34.6%)
ICC*	
H+/G+	209 (92.8%)
H+/G−	16 (7.1%)
H−/G+	0
H−/G−	0
BRAF^{V600E*}	47(21%)

Abbreviations: BL, benign lesions; cPTC, classical PTC; FA, follicular adenoma; G, galectin-3; H, HBME-1; ICC, Immunocytochemistry; PM, positive for malignancy; PTC, papillary thyroid carcinoma; SFM, suspicious for malignancy.

*ICC and *BRAF*^{V600E} only in SFM and M categories

Tables 4 and 5 compare the distribution of the different follicular architectural patterns encountered in the different samples according to FNAC and final histopathology diagnoses, respectively. In the 150 benign cases we confirmed the presence of large sheets of follicular cells, few follicular clusters, and lack of any papillary, rosette or pseudorosette patterns. Regardless of the FNAC diagnostic category, Table 4 shows that solid (without either a rosette or a pseudorosette pattern) clusters were present in all PTC cases. The different cytological architectural patterns (in order of decreasing frequency) showed that there were 225 cases with solid clusters of neoplastic cells (100%), isolated follicular cells in 201 cases (89.3%), papillary structures in 200 cases (88.8%), microfollicular clusters in 154 cases (68.4%). None of our cases had an unequivocal RLC pattern. Nonetheless, we did identify a pseudorosette pattern in 33 cases (14.6%, Figure 2A-C and Figure 3A-B). Comparing these different cytomorphological patterns stratified according to the FNAC diagnosis of SFM and positive for malignancy, we found that the number of cases with pseudorosettes was documented in 21 cases with M and 12 in SFM without any significant statistical correlation ($P > .001$). The other cytomorphological patterns had a similar distribution in both SFM and malignant cases. In Table 5 we analysed these patterns

TABLE 3 Distribution of variants of PTC in the suspicious for malignancy and positive for malignancy cytological categories

Histology Cytology	CCV-PTC	FVPTC	Hobnail-PTC	Classic PTC	Solid-PTC	TCV*	TCV + CCV	Warthin-like PTC
SFM	2	4	6	44	0	6 (14)	0	1
PM	3	4	7	96	3	13 (13)	4	5

Note: Including pure TCV and in brackets, cases of PTC with TCV component <30%.

Abbreviations: CCV-PTC, columnar cell variant of PTC; FVPTC, follicular variant of PTC; PM, positive for malignant; PTC, papillary thyroid carcinoma; SFM, suspicious for malignancy; TCV, tall cell variant of PTC.

TABLE 4 Distribution of cases according to follicular architectural pattern based upon FNAC diagnoses

Growth pattern	BL (n = 150)	SFM (n = 100)	PM (n = 125)	SFM + PM (n = 225)
Solid clusters	150 (100%)	100 (100%)	125 (100%)	225 (100%)
Papillary	0	88 (88%)	112 (89.6%)	200 (88.8%)
Microfollicular	0	56 (56%)	98 (78.4%)	154 (68.4%)
Isolated follicular cells	0	94 (94%)	107 (85.6%)	201 (89.3%)
Pseudorosette-like clusters	O	12 (12%)	21 (16.8%)	33 (14.6%)

Abbreviations: BL benign lesions; PM, positive for malignancy; SFM, suspicious for malignancy.

TABLE 5 Distribution of cases according to follicular architectural pattern stratified by histological diagnoses

Growth pattern	CCV-PTC (n = 5)	FVPTC (n = 8)	H-PTC (n = 13)	S-PTC (n = 3)	TCV (n = 46)	TCV + CCV-PTC (n = 4)	W-PTC (n = 6)	cPTC (n = 140)
Solid clusters	2 (40%)	8 (100%)	13 (100%)	3 (100%)	27 (58.6%)	4 (100%)	6 (100%)	140 (100%)
Papillary	2 (40%)	2 (25%)	8 (61.5%)	2 (66.6%)	29 (63%)	4 (100%)	6 (100%)	130 (92.8%)
Microfollicles	2 (40%)	3 (37.5%)	10 (76.9%)	2 (66.6%)	20 (43.4%)	4 (100%)	0 (%)	126 (90%)
Isolated cells	2 (40%)	8 (100%)	12 (92.3%)	3 (100%)	27 (58.6%)	4 (100%)	6 (100%)	140 (100%)
Pseudorosette-like clusters	3 (60%)	3 (25%)	7 (53.8%)	2 (33.3%)	14 (30.4%)	2 (50%)	2 (33.%)	0

Abbreviations: CCV-PTC, columnar cell variant of PTC; cPTC, classic PTC; FVPTC, follicular variant of PTC; H-PTC, Hobnail variant PTC; PTC, papillary thyroid carcinoma; S-PTC, Solid variant PTC; TCV, tall cell variant of PTC; W-PTC, Warthin-like PTC.

specifically according to the different histological variants of PTC. A pseudorosette pattern was found in cases with a histological diagnosis of pure TCV (14 cases, 42.4%) and hobnail variant (seven cases, 21.2%). Further, seven out of nine cases with a CCV component (including three pure CCV-PTC and four mixed TCV and CCV-PTC) had a focal pseudorosette pattern. None of our cPTC cases showed an RLC component or a pseudorosette pattern. Of note, we did not find any pseudorosette cluster within the histological samples of our series.

None of our BL cases had been diagnosed with the support of ancillary techniques. ICC stain results are reported in Table 6. ICC results showed that the majority of our SFM and M cases (209 cases, 92.8%) had a concordant positive immunopanel for HBME-1 and galectin-3 with only 16 cases (7.1%) showing positivity for a single immunomarker (i.e. HBME-1). For cases grouped in the SFM category, ICC showed nine cases with negative staining for galectin-3 and revealed 100% positivity for HBME-1. The cases interpreted as being positive for malignancy had positive ICC staining in all cases except for seven that only showed immunopositivity for HBME-1. None of our malignant lesions had a negative concordant panel.

The different cytomorphological patterns did not correlate with any of the analysed immunomarkers. $BRAF^{V600E}$ was expressed in 47 cases out of 225 malignancies (21%). We found a 100% concordance between cytology and histology for $BRAF^{V600E}$ expression. The results of molecular testing for $BRAF^{V600E}$ demonstrated a lack of any statistical correlation between $BRAF^{V600E}$ and architectural patterns. In fact, we found that 88 cases (70.4%) were of $BRAF^{V600E}$ wild type phenotype whilst 37 M cases (29.6%) had a *BRAF* mutation (Table 6). Among the $BRAF^{V600E}$ mutated cases, we found 14 cPTC, SEVEN hobnail PTC and 16 TCV. Of note, we did not find any specific significant statistical correlation between *BRAF* and the pseudorosette pattern ($P > .001$).

4 | DISCUSSION

It is well established that specific morphological patterns may be characteristic of particular tumours.[1-2] Such patterns often play an important role in the pathological identification of these

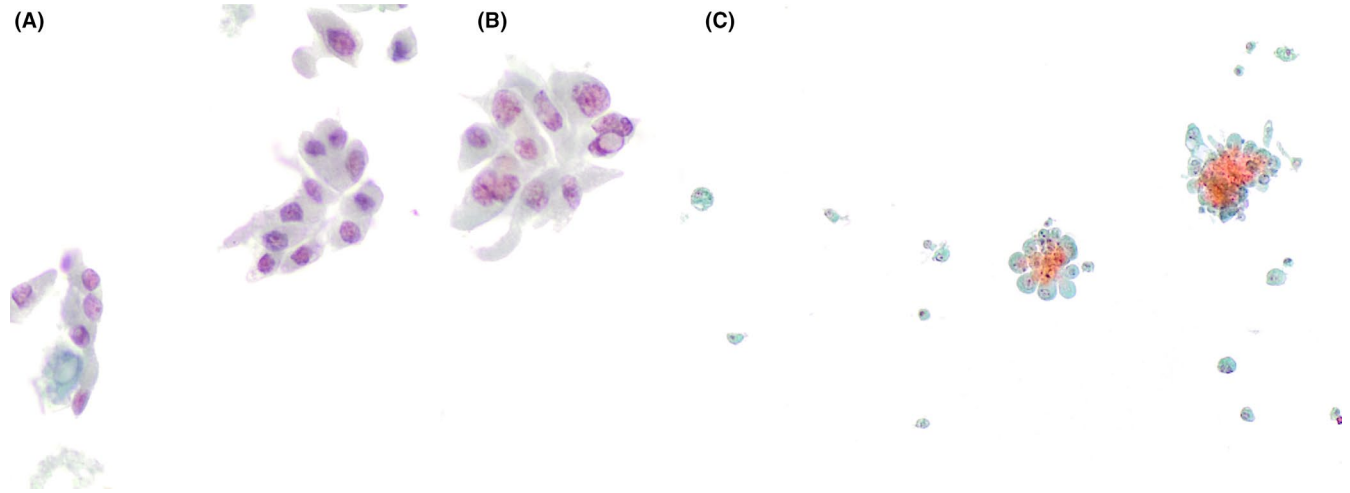

FIGURE 2 (A, B) Follicular cells in a pseudorosette-like pattern from a case of tall cell variant of papillary thyroid carcinoma (Papanicolaou stain, 200×, liquid-based cytology); (C) details of another pseudorosette-like patter from the same case (Papanicolaou stain, 400×, liquid-based cytology)

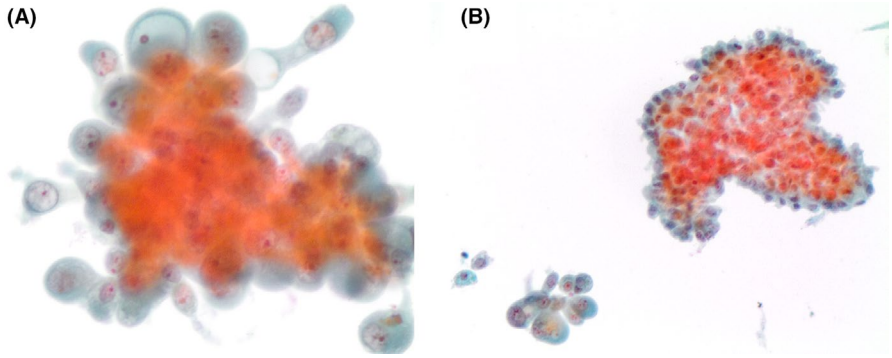

FIGURE 3 (A, B), Follicular cells in a pseudorosette-like pattern from a case of hobnail cell variant of papillary thyroid carcinoma (Papanicolaou stain, 400× liquid-based cytology); (B) details from the same case with a pseudorosette-like cluster in the left bottom and a neoplastic cluster in the upper right (Pap stain, 200× liquid-based cytology)

TABLE 6 Correlation between diagnostic categories and ancillary techniques

Cytology diagnosis	H+	G+	*BRAF*^{V600E}
SFM (100 cases)	100 (100%)	91 (91%)	10 (10%)
PM (125 cases)	125 (100%)	118 (94.4%)	37 (29.6%)

Abbreviations: G, galectin-3; H, HBME-1; PM, Positive for malignancy; SFM, suspicious for malignancy.

specific entities. However, they are usually not pathognomonic of any one tumour. One such pattern observed in various tumours is the formation of rosettes or rosette-like structures. The term *rosette* has been attributed to a pattern resembling a flower, characterised by cells radially organised around a centre. Four main kinds of rosette have been described with different morphological features including: (a) Homer Wright rosette; (b) Flexner-Wintersteiner rosette; (c) ependymal rosette; and (d) perivascular pseudorosettes.[1-10] Whilst these rosettes may not always be seen, their recognition is often helpful in the diagnosis of neural entities such as medulloblastoma/ primitive neuroendocrine tumour, retinoblastoma, ependymoma, central neurocytoma and pineocytoma.

To the best of our knowledge, the definition of a rosette pattern has not been reported in thyroid lesions based on histological samples. Nevertheless, whilst a few books and atlases have mentioned the possibility of focal rosette-like features, this has not been clearly linked with either a specific entity or PTC variant. Of note, a few papers have described the presence of a rosette-like component representing a minor or focal feature in cytological samples from both benign and malignant thyroid lesions, even though they lacked pictures demonstrating this pattern.[11-17] In our series of 150 BL we did not find any true rosette or pseudorosette follicular structures, confirming that this pattern is likely linked more with malignant thyroid nodules. The case report by Sen et al is, to the best of our knowledge, the only paper reporting the presence of rosettes in a thyroid FNAC with CCV-PTC, which triggered the reason to undertake our study.[12] To the best of our knowledge, no prior large series has corroborated a correlation of a specific PTC variant with an RLC pattern. A few authors previously described microfollicles as *rosettes* in cases of insular thyroid carcinoma and/or benign adenomatous lesions (14-18). However, their *rosette-like pattern* did not meet its strict definition.[17]

Since the first edition of TBSRTC, several new variants of thyroid carcinoma have been described.[32-33] The revised second edition of TBSRTC even included a detailed definition with particular criteria

for some of these PTC variants.[33] However, there was no mention of an RLC pattern in any of these PTC variants. Analysing published data from the literature, we found that very few authors mentioned the presence of rosettes and/or a pseudorosette architectural pattern in association with thyroid carcinoma.[31] Importantly, these patterns were not linked with specific diagnostic or prognostic features. Honambirajan et al, however, did emphasise that rosettes may superficially resemble the microfollicles of FVPTC and that their presence should thus raise suspicion for an aggressive PTC variant.[13]

According to TBSRTC, the recognition of PTC variants is generally unnecessary on FNAC given that precise subtyping is rarely possible or reliable due to difficulties in sampling the predominant pattern, due to the rarity of some PTC variants, and because of the morphological overlap with conventional PTC.[32-33] Nevertheless, the recognition of certain PTC variants (e.g. TCV, CCV-PTC and hobnail variant) that manifest with an aggressive behavior may warrant recognising them on FNAC because they often require more aggressive therapy. In a recent review article, Rossi et al discussed the morphological features that may be associated with PTC variants.[31] However, there were no details discussed regarding the relevance of rosettes in PTC variants.[31] By definition, variants of PTC must have some of the essential nuclear features of typical PTC including nuclear groves, clearing and pseudoinclusions. In contrast to the relatively easy identification of TCV findings on FNAC, the recognition of CCV-PTC is more elusive since the typical features of PTC are not consistently found in this variant. TBSRTC defines CCV-PTC as a variant characterised by columnar cells with hyperchromatic, oval and pseudostratified nuclei with supranuclear or subnuclear cytoplasmic vacuoles resembling colonic epithelium or secretory-type endometrium. With regard to the architectural patterns seen in CCV-PTC, TBSRTC mentions that neoplastic follicular cells may be arranged as papillae, clusters, flat sheets, and sometimes, small tubular structures but not RLC as observed by Sen and colleagues.[12] As reported by several authors, our LBC series confirms some morphological differences with conventional slides. In particular, we confirmed a lack of unequivocal features of rosette clusters. However, we did appreciate that a few of the cases had a pseudorosette pattern.

Our series, involving a review of 1 year's worth of thyroid FNAC cases diagnosed as SFM or malignant, found that a few pseudorosette clusters were present in 14 TCV and 7 hobnail variant cases of PTC. Apart from the case report by Sen et al,[12] we did not uncover articles mentioning an association with rosettes and either the TCV or hobnail variant. Such pseudorosette pattern may be attributed to peculiar morphological features in these variants (tall cells and columnar features of neoplastic cells), and as such when detected could suggest a possible aggressive variant. Concerning CCV-PTC, we did not find a obvious RLC pattern but did document a few pseudorosette clusters in seven out of nine cases, including four cases where there was a combination of TCV and CCV-PTC. Furthermore, a few pseudorosette clusters were also recognised in a minority of the other PTC variants including one solid variant of PTC, two FVPTC and two Warthin-like variant cases. None of the conventional cPTC cases had either an RLC or pseudorosette pattern identified, but did frequently contain papillary and microfollicular cell clusters. While ICC, especially HBME-1, demonstrated strong and diffuse positivity in all cases of PTC supporting this diagnosis, such staining did not show any correlation with RLC. For $BRAF^{V600E}$, we found an association with the aggressive variants of PTC but no correlation with cytomorphological pattern. Compared to the literature, we found a low number of $BRAF^{V600E}$ mutated cases 47 (21% for SFM plus M) which might be attributed to the fact that cPTC and several of the PTC variants are frequently wild type $BRAF^{V600E}$.

In conclusion, our analysis confirmed the presence of an RLC pattern in several PTC variants, and that such *rosettes* were not associated with cPTC cases. Our data show that a pseudorosette pattern is possibly identified in the TCV, hobnail variant of PTC and CCV-PTC. Furthermore, none of our BL cases had a rosette or pseudorosette pattern, suggesting that their presence is most likely to be associated with malignant thyroid lesions. Another interesting point is the absence of pseudorosette clusters in histological samples, confirming that this architectural pattern is mostly seen in cytological samples. The detection of a pseudorosette architectural pattern combined with the typical cytological findings of classical nuclear and cellular features of conventional PTC might offer an additional feature in those cases of thyroid FNAC with an equivocal interpretation or scant neoplastic component.

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CONFLICTS OF INTERESTS

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The Data used for this paper can be shared.

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