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DOI 10.1016/j.prp.2025.156030

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TITLE:

Usefulness of CD56, *BRAF* and CD15 in the differential diagnosis of thyroid lesions with nuclear features of papillary carcinoma

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Key words: Thyroid, papillary carcinoma, tall cell subtype, NIFTP.

Word count: 3154 words

Abbreviations:

TCS: tall cell subtype

CS: classic subtype

NIFTP: noninvasive follicular thyroid neoplasm with papillary-like nuclear features

Compliance with Ethical Standards:

Funding: This study was funded by the CIR2022026 grant from the 24th call for the Beques Taulí de Recerca i Innovació, awarded by the Fundació Parc Taulí, valued at €5500. This grant has been used to finance consumables and laboratory expenses necessary for the project.

Conflict of Interest: The authors have no relevant financial or non-financial interests to disclose.

Ethical approval: This project has been approved by the Ethics Committee of the center where it has been conducted (reference 2023/5012).

Acknowledgments:

This work has been carried out within the Doctorat Program of Surgery and Morphological Sciences at the Universitat Autònoma de Barcelona.

We thank the technical and administrative staff of the Department of Pathology at the Hospital Universitari Parc Taulí for their collaboration in the execution of the project.

Author statement

Ricard Onieva: Writing – original draft, conceptualization, investigation. Maria Rosa Bella: Writing – original draft, conceptualization, supervision. Joan Carles Ferreres: Writing – review and editing, resources. Marta Alcalà, Ismael Capel, Albert Cano, Berta Bella, Francisco Javier Guirao, Víctor Pérez, Maria Rosa Escoda, Santiago Barcons, Roser Monmany, Khalid El Hamshari and Catalina Padilla: Investigation, Writing – review and editing.

Usefulness of CD56, *BRAF* and CD15 in the differential diagnosis of thyroid lesions with nuclear features of papillary carcinoma

ABSTRACT

Introduction: Differential diagnosis of thyroid lesions with papillary carcinoma-like nuclear features includes low-risk entities, such as follicular thyroid neoplasia with papillary-like nuclear features (NIFTP), and high-risk entities, such as the tall cell subtype of papillary thyroid carcinoma (TCS). Morphological criteria are essential to distinguish them, but having immunohistochemical techniques to discriminate between these entities would be desirable.

Materials and methods: 8 TCS, 27 papillary carcinomas of classic subtype (CS) and 20 NIFTP were selected. Immunohistochemical staining for *BRAF*, CD56, B-Catenin, CD15, Muc-1 and Napsin A was performed for each case and correlated with the histological diagnosis.

Results: All TCS cases were positive for *BRAF* and CD15 ($p < 0.05$ compared to CS) and none showed positivity for CD56. In the comparison of papillary carcinoma (TCS and CS) and NIFTP, immunostaining for *BRAF*, CD56 and CD15 showed statistically significant differences.

Conclusions: *BRAF*, CD56 and CD15 immunostaining are useful to differentiate between NIFTP, CS and TCS, making it possible to identify a group of tumours with the combination CD56 negative, *BRAF* positive and CD15 positive that includes 100% of TCS and 35% of CS and a group of CD56 positive, *BRAF* negative and CD15 negative tumours that includes 52% of NIFTP and 7% of CS, facilitating the diagnosis.

Key words: Thyroid, papillary carcinoma, tall cell subtype, NIFTP.

HIGHLIGHTS

BRAF, CD56 and CD15 immunostainings are useful in thyroid papillary carcinoma.

BRAF, CD56 and CD15 can discern between NIFTP and papillary carcinoma.

BRAF, and CD15 can discern between classic subtype and tall cell subtype.

INTRODUCTION

In the diagnosis of papillary thyroid carcinoma, histological or cytological evaluation of the nuclear characteristics of tumour cells is essential. However, since the introduction of follicular thyroid neoplasia with papillary-like nuclear features (NIFTP), the presence of these nuclear characteristics no longer indicates unequivocally malignant neoplasm (1,2). While it is true that the treatment of NIFTP is surgical, as it is for papillary carcinoma, the type of surgery may not be the same. A lobectomy seems to be sufficient in the case of NIFTP (2), whereas a total thyroidectomy followed by radioiodine ablation is required for aggressive subtypes of papillary carcinoma, such as the tall cell subtype (TCS), the most common aggressive subtype of papillary carcinoma (3).

Currently, the diagnosis of NIFTP or papillary carcinoma subtypes is almost always made through histological examination of the surgical specimen. This is due to two main reasons: first, despite efforts made to date, there are no cytological criteria that allow for the definitive diagnosis of NIFTP (4) or TCS (5–7) since they all have papillary-like nuclear features; and second no conclusive immunohistochemical or molecular profiles have been established to improve diagnostic precision, either in surgical or cytological samples for this differential diagnosis.

Several studies have reported the use of different immunohistochemical markers to facilitate the diagnosis of papillary carcinoma or its subtypes, with limited success. Some of the markers used include CD56 (8–13), Cytokeratin 19, galectin-3, and HBME-1 (8,12,13), as well as others such as CD15 (14), Muc-1 (15,16), and beta-catenin (17,18). A differential expression of Napsin A between CS and TCS has also been observed (22).

Given the improvement in therapeutic planning that would result from determining, prior to surgery, whether a lesion with nuclear features of papillary carcinoma is more likely to be a NIFTP or an aggressive subtype, we have conducted a retrospective observational study to evaluate immunohistochemical markers in surgical specimens from thyroidectomy. Our goal is to establish differences in the expression of *BRAF*, CD56, CD15, beta-Catenin, Muc-1, and Napsin A between the classical subtype of papillary carcinoma (CS), as the most common subtype, TCS as the most frequent aggressive subtype, and NIFTP, as a lesion classified as a low-risk neoplasm. Identifying differences in immunohistochemical expression could contribute to more accurate pre-surgical diagnosis.

MATERIALS AND METHODS

To conduct this retrospective observational study of cases and controls, approval was obtained from the Ethics Committee of our institution. All cases of papillary thyroid carcinoma presenting more than 30% of TCS component and all cases of NIFTP from the archives of the Department of Pathology of our hospital, diagnosed between January 1, 2000, and December 31, 2017, were selected for the study. For this selection, all cases (368 tumours) from the study period were histologically reviewed by an expert pathologist to confirm or update the diagnosis according to the 5th edition criteria of the WHO classification of endocrine and neuroendocrine tumours. Three cases of CS were randomly selected as controls for each TCS. Three additional cases of CS were randomly added in anticipation of possible issues during the immunohistochemical tests. A total of 55 cases were selected (8 TCS papillary carcinomas, 27 CS papillary carcinomas, and 20 NIFTP).

Tumour histopathology was reviewed again to confirm the diagnosis of each selected case, and a representative block from each tumour was selected to perform immunohistochemical studies for *BRAF* V600E, CD56, beta-catenin, CD15, Muc-1, and Napsin A.

Immunohistochemical staining was performed using the BenchMark Ultra system. For the *BRAF* technique (VE1, Ventana), an antigen retrieval time of 64 minutes at 100°C was used, followed by incubation at 36°C for 24 minutes. For CD56 (MRQ-42, Cell Marque), an antigen retrieval time of 36 minutes at 75°C was employed, followed by 52 minutes of incubation at 36°C. Beta-Catenin (14, Cell Marque) was retrieved at 95°C for 52 minutes and incubated for 52 minutes at 36°C. CD15 (MMA, Ventana) was retrieved at 95°C for 52 minutes and incubated

for 52 minutes at 36°C. Muc-1 (H23, Ventana) was retrieved for 64 minutes at 95°C and incubated for 24 minutes at 36°C. Napsin A (MRQ-60, Cell Marque) was retrieved for 36 minutes at 95°C and incubated for 8 minutes at 36°C.

The interpretation of the immunohistochemical techniques was carried out according to the criteria outlined in Table 1. For CD15, four patterns were determined, as shown in Figure 1. For the purposes of result interpretation, CD15 expression was considered negative in cases with the absence of membrane staining and cases with isolated positive cells, and positive in cases with focal or diffuse positivity. Cytoplasmic staining was not considered.

Clinical histories of the included cases were reviewed to obtain the patient's sex, age at diagnosis, tumour size, lymph node involvement, distant metastasis, and presence of recurrence at 5 years from surgery. Persistent disease after surgery was not considered recurrence.

For statistical analysis, descriptive methods were applied, calculating mean and standard deviation for quantitative variables, and frequencies and proportions for qualitative variables. To assess associations, chi-square or Fisher's exact test was used depending on the sample characteristics. The IBM SPSS Statistics software version 29.0.0.0 was used for inferential statistics.

RESULTS

The immunohistochemical study was conducted on 55 cases, 53 of which had evaluable results. One case, corresponding to the classical subtype, did not show tumour representation in the immunohistochemical stains and was excluded. A case initially classified as NIFTP was also excluded from the analysis, as it showed positivity for the *BRAF* mutation, which is incompatible with the diagnosis of NIFTP. For the data analysis, two comparisons were made: CS vs TCS and papillary carcinoma (CS and TCS) vs NIFTP.

CS VS TCS

Table 2 shows the clinical data and immunohistochemical results from the comparison of the 26 cases of CS versus the 8 cases of TCS. It displays the statistically significant differences obtained with the staining for *BRAF* and CD15. Notably, in two cases of TCS, with *BRAF* and CD15 stains evaluated as positive, the tumour was heterogeneous. Both tumours showed two clearly differentiated components: one component consisting of cells three times taller than wide, with positive staining for *BRAF* and CD15, and a second component consisting of cells less than three times taller than wide, negative for both stains (Figure 2).

In the analysis of the results, it is noteworthy that no case with mutated *BRAF* was positive for CD56. Additionally, it is striking that all TCS cases were positive for *BRAF* and CD15, and negative for CD56. Staining for beta-catenin, Muc-1, and Napsin A did not show utility in distinguishing CS from TCS.

An analysis was also conducted to determine whether any of the immunohistochemical stains could predict recurrence, but no statistically significant results were obtained.

PAPILLARY CARCINOMA VS NIFTP

Table 3 shows the comparison between papillary carcinoma (including CS and TCS) and NIFTP. As can be seen, the stains for CD56, CD15, and *BRAF* showed statistically significant differences between the two entities, although *BRAF* should always be negative in NIFTP according to the current WHO diagnostic criteria.

In the evaluation of the immunohistochemical stains, a case classified as NIFTP showed heterogeneous staining. This case showed positivity for CD56 throughout the tumour, except in a small well-defined intratumoural nodular region, which corresponded to a focus of CD15 expression. Following this finding, the hematoxylin-eosin preparation was reviewed, but no morphological differences were found between the two areas with different immunohistochemical expression.

DISCUSSION

The main conclusion of our study is the contribution of CD15 and *BRAF* to the diagnosis of TCS, and the contribution of CD56, *BRAF*, and CD15 to the diagnosis of NIFTP. This is relevant because, currently, the differential diagnosis between these entities relies on morphological and architectural criteria (1), which inevitably means that it cannot be conclusively applied to cytological samples. The capability to establish immunohistochemical differences between these entities is essential in the path toward a pre-surgical diagnosis, based on cytological samples, allowing to decide the most appropriate surgical approach for each patient based on a more precise diagnosis.

In the analysis of the immunohistochemical results on TCS, only *BRAF* and CD15 were found to be useful for distinguishing them from CS. Regarding *BRAF*, its association to TCS is well documented in the literature (19,20), with some studies even indicating a 100% prevalence, as in our series, of *BRAF* mutation in TCS (17,21). This makes it a highly sensitive marker, although not a distinctive feature, as it is also found in 54% of the CS cases in our study, and therefore has low specificity. A similar situation occurs with CD15, which was positive at least focally in all TCS cases, but also in some CS cases. In a literature review, we only found one other study that examined CD15 in TCS and CS, conducted by *Ostrowski et al.* (14). In that study, all TCS cases (11/11) were positive for CD15, while only 1/16 was positive in the CS group. Although our results are not as conclusive, they seem to show a similar trend in the TCS cases, even though the two studies are not directly comparable, as they used different diagnostic criteria: *Ostrowski et al.* define TCS as tumours composed of more than 70% of cells more than twice as high as wide, while the current definition is more than 30% of cells three times as high as wide (1,14), which is the criterion used in this study.

Our results show that CD56 loses its expression in TCS and in the vast majority of CS cases. This loss of expression aligns with the literature, which describes the loss of CD56 in thyroid malignant lesions (10). However, two studies by *Scarpino et al.* (11) and *Cho et al.* (12) propose that TCS may regain CD56 expression, a finding that has not been confirmed in our series (9,11).

Additionally, the results of the markers Napsin A, Muc-1, and beta-Catenin are noteworthy. For Napsin A, only one previous study by *Bishop et al.* (22) is available. In that study, 38 papillary

carcinomas were examined, of which 4 were TCS. In that study only two cases were positive, both TCS. These results differ from those observed in our sample, with one TCS case positive and 9 CS positive cases. However, since the study by *Bishop et al.* was performed using microarrays, the limited tumour representation could explain the negativity in cases of heterogeneous antigen expression (22).

Muc-1 does not generate consensus in the literature. On one hand, two studies suggest that its expression is higher in TCS than in CS (15,16), one of which even associates Muc-1 expression with decreased disease-free survival (15). Another study questioned this higher expression of Muc-1 in TCS, stating that 87.9% of CS cases express Muc-1, compared to 70% of TCS cases (23). In our study, Muc-1 seems to be expressed more in TCS than in CS, according with most previous studies, although the difference in expression is not significant.

Regarding beta-Catenin, a previous study by *Min et al.* (17) suggested that TCS might exhibit increased expression, along with a cytoplasmic dot pattern. This pattern has also been described in another study (18). However, in our analysis, these findings were not confirmed, as no cases of cytoplasmic or nuclear "dot" expression were found.

It is important to note that when comparing results from different studies, the disparity in diagnostic criteria used for diagnosing TCS over time must be taken into account. This is relevant because, although cases diagnosed as TCS are compared, the diagnostic criteria may not be the same. Furthermore, in larger studies, it would be important to examine the behaviour of cases that present a cellular height close to the diagnostic limits, in order to better understand the effects of different criteria and the compatibility between studies, as well as the best cut-off point for prognostic purposes.

Concerning NIFTP, it cannot be positive for BRAF, according to the diagnostic criteria currently accepted by the WHO classification (1). This diagnostic criteria led to the exclusion of a case from our series that, morphologically, appeared to be an NIFTP but presented the BRAF mutation by immunohistochemistry. After obtaining this result, the tumour's histology was reviewed, confirming that it was a well-defined lesion with a follicular architecture, with follicles of varying sizes and papillary carcinoma-like nuclei. No psammoma bodies, vascular invasions, papillary structures, or other features were identified that would contradict the NIFTP diagnosis. Therefore, in this case, the BRAF mutation study was essential for an accurate diagnosis. This finding raises the need to always consider BRAF mutation testing in the diagnosis of NIFTP, as in some cases this analysis is essential for the correct diagnosis.

The use of CD56 in NIFTP has been explored in other studies, with contradictory results, reporting positivity rates between 15% and 82.4% (4,8,12,13), with our results falling in an intermediate range.

To date, the immunohistochemical pattern in NIFTP for Muc-1, Napsin A, or CD15 has not been studied, making our work the first to explore these markers. CD15 shows significant results, unlike Muc-1 and Napsin A. However, due to the low number of cases in our series, it would be interesting to expand the study, especially considering that the results for Muc-1 are close to statistical significance.

An unexpected finding in the evaluation of immunohistochemical techniques was the presence of tumours with distinct components, which also suggests that the expression of certain antigens could be related to one another, although we cannot fully understand the etiopathogenic mechanism.

Two TCS tumours presented the same pattern: the first tumour component corresponded to CS and did not show the *BRAF* mutation or positivity for CD15, while the second component, morphologically TCS, exhibited the *BRAF* mutation and also expressed CD15. Although the description of a morphologically CS case without the *BRAF* mutation presenting a TCS area with the *BRAF* mutation is not new in the literature (24), its identification in 2 out of 8 cases in our series could indicate that this is a relatively frequent phenomenon. It is important to note that the potential relationship between CD15 and the *BRAF* mutation has not been described in the literature. This association could also suggest that these two tumours were originally CS, which, upon accumulating mutations, including the *BRAF* mutation, acquired CD15 expression. In our study, all TCS cases simultaneously presented the *BRAF* mutation and CD15 expression; however, in CS cases, the expression of these markers seems to be independent. This implies that one is likely not a direct consequence of the other, and other factors are probably involved.

Among the NIFTP cases, a tumour with two populations was also found based on the immunohistochemical results: a major component with intense CD56 expression and negativity for CD15, and a minor component with expression of CD15 and loss of CD56 expression. Unlike the TCS cases, this case did not show any morphological differences between the areas with different immunohistochemical expression. This finding raises the possibility that NIFTP may evolve into more aggressive entities, as previously proposed by Nikiforov (2).

The main objective of this study, beyond establishing differential immunohistochemical markers between CS, TCS, and NIFTP, is to facilitate the distinction between these three entities using immunohistochemistry, which is applicable to cytological samples. Successfully distinguishing these three entities preoperatively, in cytological samples, would allow for better surgical planning, as TCS, by itself, is already a risk criterion and requires total thyroidectomy to administer radioiodine (3), whereas NIFTP can be safely treated with a hemithyroidectomy (2). In other words, over-treatment could be reduced, and reoperations for completing thyroidectomy could be avoided.

In our analysis, we did not find any marker that was sufficiently sensitive and specific to definitively differentiate between these three entities, but better accuracy could be achieved by combining immunohistochemical stains for CD56, *BRAF*, and CD15, following an algorithm, which is presented as a flowchart in Figure 3. First, CD56 should be assessed. If positive, it should be confirmed that *BRAF* is negative, and CD15 should be evaluated. If CD15 is negative, the probability that the definitive diagnosis is NIFTP should be considered. If CD15 is positive, it could also be an NIFTP, but with a higher probability of being CS. If CD56 is negative in the initial assessment, then *BRAF* should be evaluated, and if positive, CD15 should be checked. If CD15 is also positive, this could indicate a TCS case. In the combination of CD56 negative and *BRAF* negative, the most likely diagnosis is CS, although NIFTP cannot be definitively ruled out. We did not find any cases in our series that showed simultaneous positivity for both CD56 and *BRAF*. The combination of CD56 negative, *BRAF* positive, and CD15 negative seems to delineate a group that includes only CS. This could suggest that CD15 is highly useful to exclude a diagnosis of TCS in the group of CD56 negative and *BRAF* positive tumours.

Applying this algorithm to our case sample, 100% of TCS cases and 35% of CS cases would fall into the group formed by the combination of CD56 negative, *BRAF* positive, and CD15 positive. The combination of CD56 positive, *BRAF* negative, and CD15 negative would include 52% of NIFTP cases and 7% of CS cases. The remaining combinations found in our series, taken together, would group 57% of CS cases and 47% of NIFTP cases. It is important to note that this

group of combined results does not include any case positive for *BRAF*, as our series did not include any CD56 positive cases with *BRAF* mutation. If a case with this combination was found, the most likely diagnosis would be CS, as NIFTP cannot be diagnosed in cases with a positive *BRAF*.

With these results, we suggest that the diagnostic algorithm using CD56, *BRAF*, and CD15 is useful for distinguishing NIFTP, CS, and TCS, although this study has a limited number of cases and would require validation with a larger sample size. The main strength of the algorithm lies in its ability to separate TCS cases, which are the most aggressive. Furthermore, it also has some capacity to identify NIFTP, which are considered low-risk neoplasms, unlike papillary carcinomas, which are considered malignant neoplasms (1). We want to emphasize that this algorithm is highly discriminative between NIFTP and TCS, as it relies on *BRAF*, CD56, and CD15, the three most differential markers between them, which is crucial for its preoperative application. However, it should be noted that the application of this algorithm does not provide unequivocal results. Therefore, although it can be a diagnostic aid, it should not be used without considering morphological diagnostic criteria.

CONCLUSIONS

The main conclusion of our study is the demonstration of the utility of *BRAF* and CD15 for the diagnosis of TCS. Furthermore, by using CD56, *BRAF*, and CD15, an algorithm can be built to help distinguishing TCS, CS, and NIFTP, which could be useful if applied to preoperative cytological studies, improving the surgical management of patients. We consider of interest for our results to be replicated and applied to cytological samples to assess their utility in daily clinical practice.

REFERENCES

1. Baloch ZW, Asa SL, Barletta JA, Ghossein RA, Juhlin CC, Jung CK, et al. Overview of the 2022 WHO Classification of Thyroid Neoplasms. *Endocr Pathol*. 2022;33(1):27-63. doi: 10.1007/s12022-022-09707-3.
2. Nikiforov YE, Seethala RR, Tallini G, Baloch ZW, Basolo F, Thompson LDR, et al. Nomenclature Revision for Encapsulated Follicular Variant of Papillary Thyroid Carcinoma. *JAMA Oncol*. 2016;2(8):1023. doi: 10.1001/jamaoncol.2016.0386.
3. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, et al. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. *Thyroid*. 2016;26(1):1-133. doi: 10.1089/thy.2015.0020.
4. Maletta F, Massa F, Torregrossa L, Duregon E, Casadei GP, Basolo F, et al. Cytological features of “noninvasive follicular thyroid neoplasm with papillary-like nuclear features” and their correlation with tumour histology. *Hum Pathol*. 2016;54:134-42. doi: 10.1016/j.humpath.2016.03.014.

5. Baum JE, Soong L, Scognamiglio T, Margolskee EM, Hoda RS, Rao R. Cytological diagnosis of papillary thyroid carcinoma with tall cells on ThinPrep liquid-based cytology. *Diagn Cytopathol*. 2019;47(6):541–6. doi: 10.1002/dc.24146.
6. Ahn J, Jin M, Kim WG, Kim TY, Kim WB, Shong YK, et al. Limitations of fine-needle aspiration and core needle biopsies in the diagnosis of tall cell variant of papillary thyroid carcinoma. *Clin Endocrinol (Oxf)*. 2023;98(1):110–6. doi: 10.1111/cen.14735.
7. Tanaka A, Hirokawa M, Higuchi M, Suzuki A, Yamao N, Hayashi T, et al. Diagnostic clues indicating tall cell variants of papillary thyroid carcinoma in fine needle aspiration. *Diagn Cytopathol*. 2019;47(5):452–7. doi: : 10.1002/dc.24122.
8. Tastekin E, Keskin E, Can N, Canberk S, Mut A, Erdogan E, et al. CD56, CD57, HBME1, CK19, Galectin-3 and p63 immunohistochemical stains in differentiating diagnosis of thyroid benign/malign lesions and NIFTP. *Polish J Pathol*. 2019;70(4):286–94. doi: 10.5114/pjp.2019.93131
9. Cho U, Kim Y, Jeon S, Jung CK. CD56 Expression in Papillary Thyroid Carcinoma Is Highly Dependent on the Histologic Subtype: A Potential Diagnostic Pitfall. *Appl Immunohistochem Mol Morphol*. 2022;30(5):389–396. doi: 10.1097/PAI.0000000000001015.
10. Pyo JS, Kim DH, Yang J. Diagnostic value of CD56 immunohistochemistry in thyroid lesions. *Int J Biol Markers*. 2018;33(2):161–7. doi: 10.1177/1724600817748538.
11. Scarpino S, Di Napoli A, Melotti F, Talerico C, Cancrini A, Ruco L. Papillary carcinoma of the thyroid: low expression of NCAM (CD56) is associated with downregulation of VEGF-D production by tumour cells. *J Pathol*. 2007;212(4):411–9. doi: 10.1002/path.2183.
12. Cho H, Kim JY, Oh YL. Diagnostic value of HBME-1, CK19, Galectin 3, and CD56 in the subtypes of follicular variant of papillary thyroid carcinoma. *Pathol Int*. 2018;68(11):605–13. doi: 10.1111/pin.12729.
13. Chuang HW, Wang JS, Tsai JW, Hsu CT, Lin KJ. Immunohistochemistry helps to distinguish noninvasive follicular thyroid neoplasm with papillary-like nuclear features/noninvasive encapsulated follicular variant of papillary thyroid carcinoma with other follicular thyroid lesions. *Med*. 2021;57(11). doi: 10.3390/medicina57111246.
14. Ostrowski ML, Merino MJ. Tall cell variant of papillary thyroid carcinoma: A reassessment and immunohistochemical study with comparison to the usual type of papillary carcinoma of the thyroid. *Am J Surg Pathol*. 1996;20(8):964–74. doi: 10.1097/00000478-199608000-00005.
15. Wreesmann VB, Sieczka EM, Socci ND, Hezel M, Belbin TJ, Childs G, et al. Genome-wide profiling of papillary thyroid cancer identities MUC1 as an independent prognostic marker. *Cancer Res*. 2004;64(11):3780–9. doi: 10.1158/0008-5472.CAN-03-1460.
16. Abrosimov A, Saenko V, Meirmanov S, Nakashima M, Rogounovitch T, Shkurko O, et al. The cytoplasmic expression of MUC1 in papillary thyroid carcinoma of different histological variants and its correlation with cyclin D1 overexpression. *Endocr Pathol*. 2007;18(2):68–75. doi: 10.1007/s12022-007-0012-x.

17. Min HS, Lee C, Jung KC. Correlation of immunohistochemical markers and BRAF mutation status with histological variants of papillary thyroid carcinoma in the Korean population. *J Korean Med Sci.* 2013;28(4):534–41. doi: 10.3346/jkms.2013.28.4.534.
18. Andriescu EC, Căruntu ID, Giușcă SE, Lozneau L, Apostol DGC. Prognostic significance of cell-adhesion molecules in histological variants of papillary thyroid carcinoma. *Rom J Morphol Embryol.* 2018;59(3):721–7.
19. Li C, Lee KC, Schneider EB, Zeiger MA. BRAF V600E mutation and its association with clinicopathological features of papillary thyroid cancer: A meta-analysis. *J Clin Endocrinol Metab.* 2012;97(12):4559–70. doi: 10.1210/jc.2012-2104.
20. Liu Z, Zeng W, Chen T, Guo Y, Zhang C, Liu C, et al. A comparison of the clinicopathological features and prognoses of the classical and the tall cell variant of papillary thyroid cancer: a meta-analysis. *Oncotarget.* 2017 Jan 24;8(4):6222-6232. doi: 10.18632/oncotarget.14055. Erratum in: *Oncotarget.* 2018 Mar 23;9(22):16271. doi: 10.18632/oncotarget.24998.
21. Lee SH, Jung CK, Bae JS, Jung SL, Choi YJ, Kang CS. Liquid-based cytology improves preoperative diagnostic accuracy of the tall cell variant of papillary thyroid carcinoma. *Diagn Cytopathol.* 2014 Jan;42(1):11–7. doi: 10.1002/dc.23007.
22. Bishop JA, Sharma R, Illei PB. Napsin A and thyroid transcription factor-1 expression in carcinomas of the lung, breast, pancreas, colon, kidney, thyroid, and malignant mesothelioma. *Hum Pathol.* 2010;41(1):20–5. doi: 10.1016/j.humpath.2009.06.014.
23. Morari EC, Silva JR, Guilhen ACT, Cunha LL, Marcello MA, Soares FA, et al. Muc-1 expression may help characterize thyroid nodules but does not predict patients' outcome. *Endocr Pathol.* 2010;21(4):242–9. doi: 10.1007/s12022-010-9137-4.
24. Abrosimov A, Saenko V, Rogounovitch T, Namba H, Lushnikov E, Mitsutake N, et al. Different structural components of conventional papillary thyroid carcinoma display mostly identical BRAF status. *Int J Cancer.* 2007;120(1):196–200. doi: Abrosimov A, Saenko V, Rogounovitch T, Namba H, Lushnikov E, Mitsutake N, et al. Different structural components of conventional papillary thyroid carcinoma display mostly identical BRAF status. *Int J Cancer.* 2007;120(1):196–200.

TABLES:

Table 1: Criteria for evaluating immunohistochemical stains

Immunohistochemistry	Positive	Focal	Negative
<i>BRAF</i>	Cytoplasmic staining, any percentage or intensity	---	Absence of cytoplasmic staining
CD56	>90% of tumour cells with membranous staining	10-90% of tumour cells with membranous staining	<10% of tumour cells with membranous staining
CD15	Intense and diffuse membranous positivity	Groups of consecutive cells with membranous staining, any percentage	Isolated cells with membranous staining or absence of staining
Muc-1	>50% of tumour cells with cytoplasmic staining	1-50% of tumour cells with cytoplasmic staining	<1% of tumour cells with cytoplasmic staining
Napsin A	>50% of tumour cells with cytoplasmic staining	1-50% of tumour cells with cytoplasmic staining	<1% of tumour cells with cytoplasmic staining
B-Catenin	Membranous staining, any percentage or intensity	---	Absence of membranous staining

Table 2: Comparison of classic and tall cell subtypes of papillary carcinoma.

		Classic subtype (26)	Tall cell subtype (8)
Mean age (years)		45.3	55.5
Sex	Male	6 (23%)	3 (38%)
	Female	20 (77%)	5 (63%)
Stage T (AJCC 8 ^a Ed)	T1a	8 (31%)	2 (25%)
	T1b	10 (38%)	1 (13%)
	T2	4 (15%)	3 (38%)
	T3a	1 (4%)	0 (0%)
	T3b	3 (12%)	2 (25%)
Stage N (AJCC 8 ^a Ed)	N0	10 (38%)	4 (50%)
	N1a	7 (27%)	0 (0%)
	N1b	8 (31%)	4 (50%)
	NX	1 (4%)	0 (0%)
Stage M (AJCC 8 ^a Ed)	M0	25 (96%)	8 (100%)
	M1	1 (4%)	0 (0%)
Recurrence at 5 years	Yes	5 (19%)	4 (50%)
	No	21 (81%)	4 (50%)
<i>BRAF</i> p 0.030	Positive	14 (54%)	8 (100%)
	Negative	12 (46%)	0 (0%)
CD56 p NS	Positive	3 (12%)	0 (0%)
	Negative or focal	23 (88%)	8 (100%)
B-Catenin p NS	Membranous	24 (92%)	8 (100%)
	Cytoplasm or negative	2 (8%)	0 (0%)
Muc-1 p NS	Positive	3 (12%)	3 (38%)
	Negative or focal	23 (88%)	5 (63%)
CD15 p 0.005	Positive (diffuse or focal)	11 (42%)	8 (100%)
	Negative (negative or isolated cells)	15 (58%)	0 (0%)
Napsin A p NS	Positive or focal	9 (35%)	1 (13%)
	Negative	17 (65%)	7 (88%)

p-value calculated with Fisher's exact test. NS (not significant).

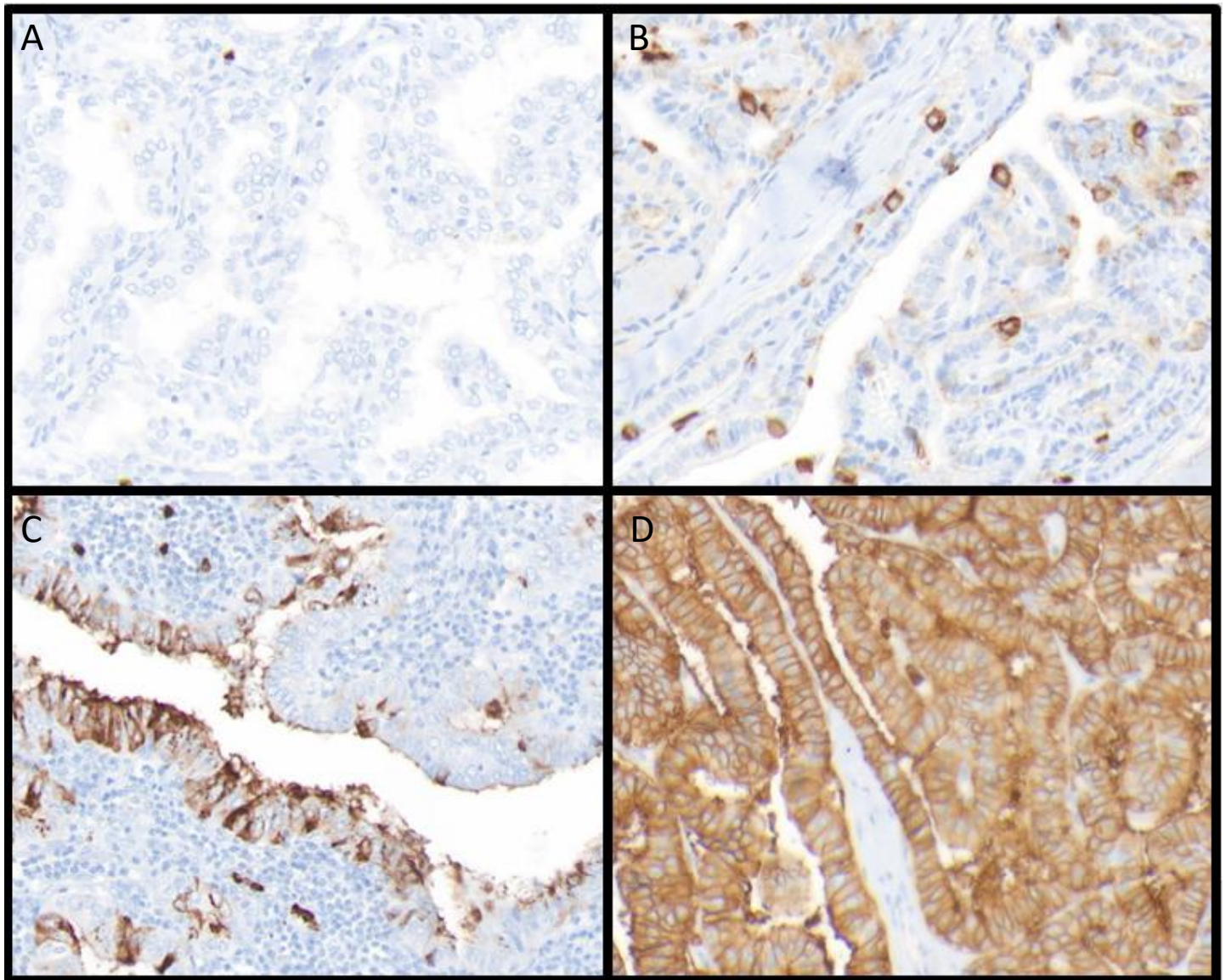
Table 3: Comparison of papillary carcinoma and NIFTP

		Papillary carcinoma, includes classic and tall cell subtype (34)	NIFTP (19)
Mean age (years)		47.7	48.4
Sex	Male	9 (26%)	3 (16%)
	Female	25 (74%)	16 (84%)
<i>BRAF</i> p 0.001	Positive	22 (65%)	0 (0%)
	Negative	12 (35%)	19 (100%)
CD56 p <0.001	Positive	3 (9%)	12 (63%)
	Negative or focal	31 (91%)	7 (37%)
B-Catenin p NS	Membranous	32 (94%)	19 (100%)
	Cytoplasm or negative	2 (6%)	0 (0%)
Muc-1 p 0.077	Positive	6 (18%)	0 (0%)
	Negative or focal	28 (82%)	19 (100%)
CD15 p 0.001	Positive (diffuse or focal)	19 (56%)	2 (11%)
	Negative (negative or isolated cells)	15 (44%)	17 (89%)
Napsin A p NS	Positive or focal	10 (29%)	2 (11%)
	Negative	24 (71%)	17 (89%)

p-value calculated with Fisher's exact test. NS (not significant).

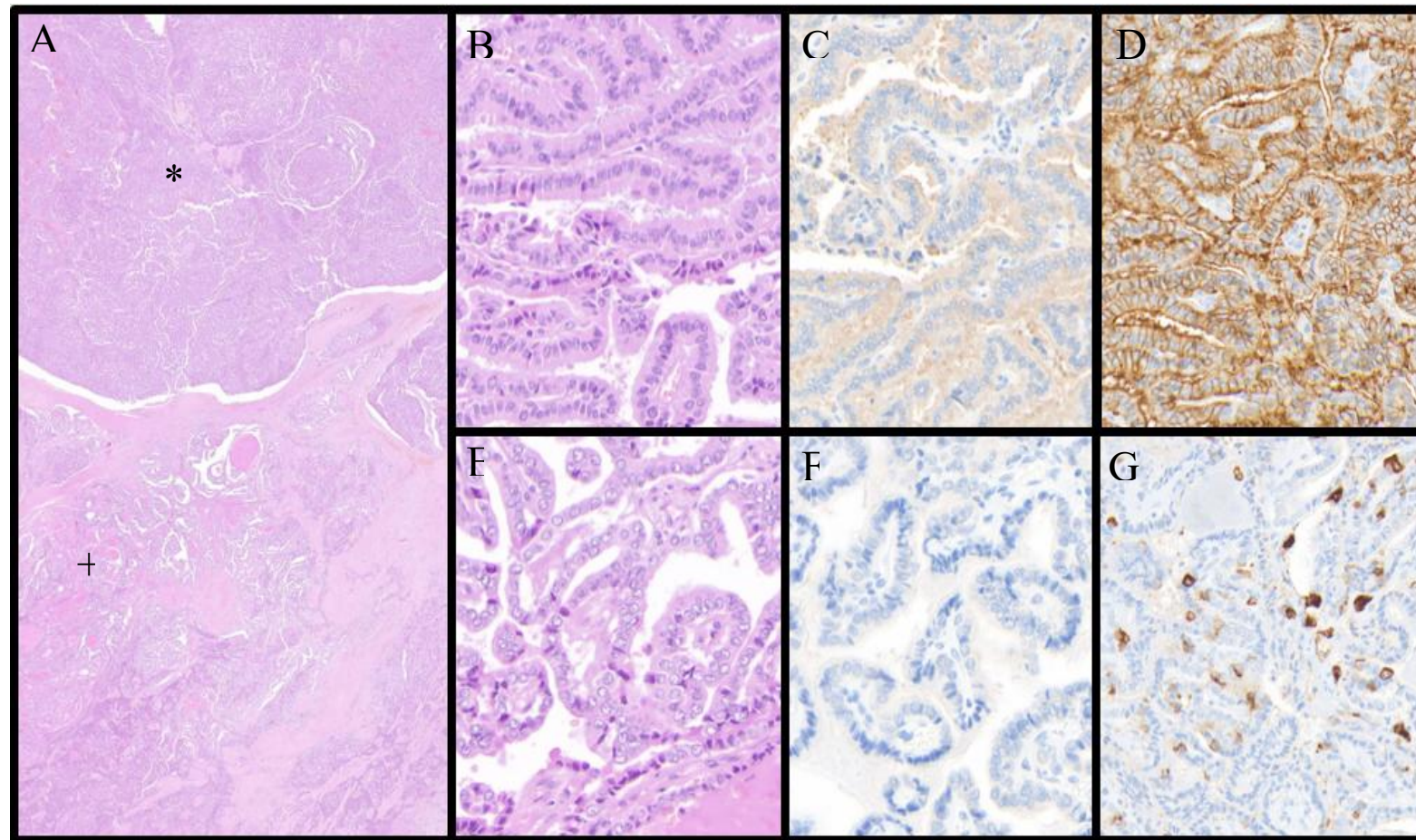
FIGURES

Figure 1



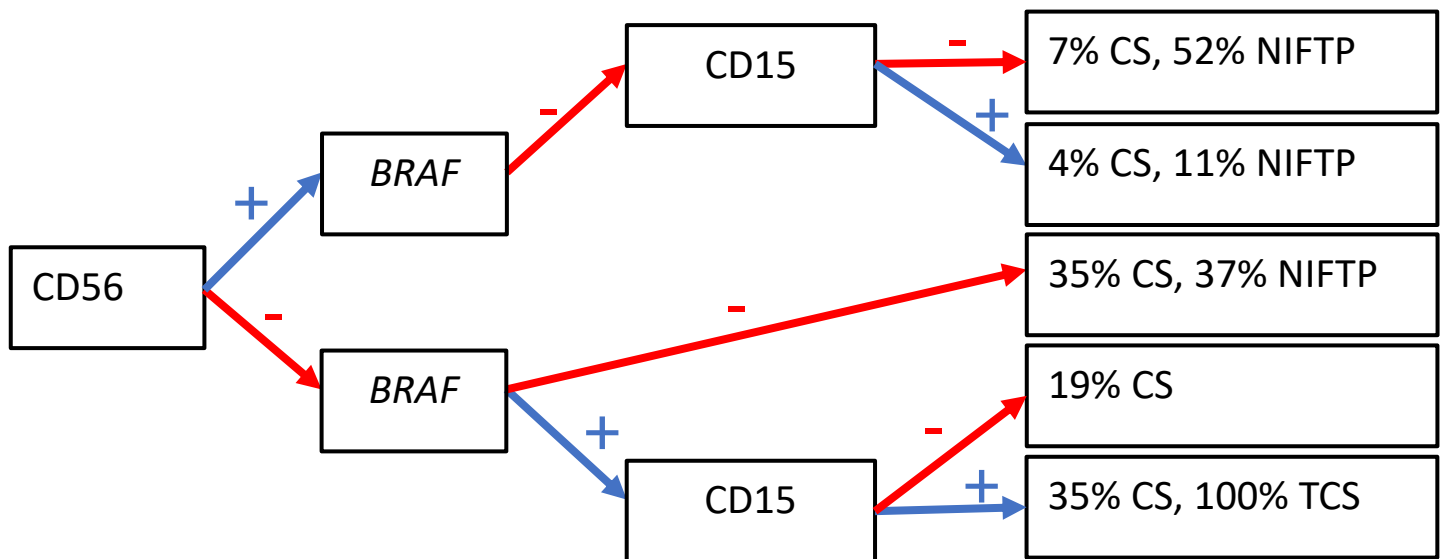
Assessment of CD15. A: Negative staining, granulocytes act as internal control. B: Negative staining, pattern of isolated positive cells, with complete membrane staining. C: Positive staining, focal pattern where a cluster of multiple consecutive cells shows membrane staining. D: Positive staining, diffuse pattern where all or almost all tumour cells show membrane staining.

Figure 2



A: Case of a heterogeneous tumour. The upper part (*) morphologically is TCS, and lower part (+) is CS. B: TCA. C: TCA, positive for BRAF. D: TCA, positive for CD15. E: CS area. F: CS area, negative for BRAF. G: CS area, negative for CD15.

Figure 3



Schematic representation of the algorithm to classify the groups. Percentages correspond to the total of cases of each diagnosis (CS, TCS and NIFTP) of our series.