Cytokeratin 19 Immunoreactivity in the Diagnosis of Papillary Thyroid Carcinoma

A Note of Caution

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Abstract

To evaluate the expression of cytokeratin (CK) 19, we stained sections obtained from formalin-fixed, paraffin tissue blocks of 35 thyroid tumors (follicular adenoma [FA], 20; papillary thyroid carcinoma [PTC], 10 follicular variant [FV] and 5 usual type) and scored the extent of staining as follows: 1+ (<5% positively stained cells), 2+ (5%-25% positively stained cells), 3+ (25%-75% positively stained cells), and 4+ (>75% positively stained cells). All 15 PTCs (including 10 FV-PTCs) were CK19 positive: 14 were 4+ and 1 (FV-PTC) was 2+. All 20 FAs also were CK19 positive: 15 were 1+, 1 was 2+, 4 were 3+, and none was 4+. In the FAs that were scored 1+, reactivity usually was confined to follicular cells lining cystically dilated atrophic follicles that lacked the typical nuclear features of PTC. The remaining FAs showed more diffuse reactivity, which was, however, less intense than that observed in the PTCs. Thus, immunoreactivity for CK19 is not specific for PTC, although we acknowledge that the extent and intensity of staining are considerably greater in this tumor than in FA. There were no significant differences in staining for CK19 between nonneoplastic follicles adjacent to PTCs and those adjacent to FAs.

Papillary thyroid carcinoma (PTC) constitutes about 80% of all thyroid malignant neoplasms.[1] Among the subtypes of papillary carcinoma, the follicular variant (FV-PTC) is the most common.[2-4] This lesion is characterized by an exclusive or almost exclusive follicular growth pattern and a set of nuclear features identical to those of the usual type of papillary carcinoma (UT-PTC). Some cases of FV-PTC are surrounded by a fibrous capsule, which may or may not be invaded by tumor.

A common diagnostic dilemma arises when an encapsulated nodule with a follicular pattern of growth exhibits some but not all of the features of PTC, such as clear nuclei with grooves or darkly staining colloid. In such instances, distinguishing FA from encapsulated FV-PTC becomes difficult. Cytokeratin (CK) 19 has been proposed as an immunohistochemical marker to distinguish PTC (including FV-PTC) from other benign and malignant follicular lesions.[5-9] It also has been reported that, among nonneoplastic follicles, CK19 staining is limited to those adjacent to FV-PTC[6] and those located within or near areas with chronic inflammation.[10] The purpose of the present study was to compare the reactivity of CK19 in PTC (including the FV-PTC) with that observed in FA. We also sought to examine the pattern of CK19 reactivity in the nonneoplastic thyroid tissue adjacent to both tumor types.

Materials and Methods

We retrieved 20 cases of FA, 10 cases of FV-PTC, and 5 cases of UT-PTC from the files of the Department of Pathology, New York Presbyterian Hospital–Weill Cornell

Table 1
Patient and Tumor Characteristics

Diagnosis	Male/Female	Mean Age (Range), y	Mean Tumor Size (Range), cm
Follicular adenoma (n = 20)	7/13	49 (32-76)	3.4 (1.0-7.0)
Papillary thyroid carcinoma			
Follicular variant (n = 10)	1/9	47 (32-65)	2.4 (1.2-5.2)
Usual type (n = 5)	3/2	46 (33-78)	1.7 (0.7-3.0)

Table 2
Percentage of Thyroid Tumors Staining Positive for Cytokeratin 19

	Papillary Thyroid Carcinoma		
Score (% of Positivity)	Usual Type (n = 5)	Follicular Variant (n = 10)	Follicular Adenoma (n = 20)
1+ (<5%)	0	0	15 (75)
2+ (5%-25%)	0	1 (10)	1 (5)
3+ (25%-75%)	0	0	4 (20)
4+ (>75%)	5 (100)	9 (90)	0

Medical Center, New York, NY. The original slides were reviewed by all authors, and diagnostic consensus was achieved in all cases. The diagnosis of UT-PTC was made when the tumor had a predominant papillary pattern and the nuclei showed clearing, grooves, and pseudoinclusions, as described in the third series of the Armed Forces Institute of Pathology fascicle.[4] The diagnosis of FV-PTC was made when the tumor had an exclusive follicular growth pattern and the nuclei showed the typical, aforementioned features of PTC. Follicular adenomas (FAs) were diagnosed when an encapsulated tumor had an exclusive follicular pattern of growth and lacked the nuclear features of PTC.

Immunohistochemical staining was performed on 1 representative formalin-fixed, paraffin-embedded tissue section from each case using an automated immunostainer (Techmate 500, Ventana Medical Systems, Tucson, AZ). A monoclonal antibody to CK19 (BA17, DAKO, Carpinteria, CA) at a dilution of 1:200 was used for the study. Immunostaining was performed using the ChemMate ABC peroxidase secondary detection system (Ventana Medical Systems). Antigen retrieval was performed by heating the slides in a microwave for 20 minutes in a 10-mmol/L concentration of citrate buffer, pH 6.0. The peroxidase reaction was developed using diaminobenzidine substrate chromogen provided in the secondary detection system. Appropriate positive and negative controls were prepared.

The following semiquantitative method was used to score the immunoreactivity of the tumor and nonneoplastic adjacent thyroid: 1+, fewer than 5% positively stained cells; 2+, 5% to 25% positively stained cells; 3+, 25% to 75% positively stained cells; and 4+, more than 75% positively stained cells.

Results

Pathologic Findings

Cases of UT-PTC included both partially encapsulated and nonencapsulated tumors. All 10 cases of FV-PTC were encapsulated and showed an exclusive follicular growth pattern. In 9 cases, the follicles were of different sizes with a predominance of microfollicles, while 1 case showed a predominant macrofollicular growth pattern. The nuclear features of PTC, ie, nuclear enlargement and clearing, nuclear grooves, and pseudoinclusions, were present diffusely in more than 95% of the tumor cells in 7 cases and were less extensive in 3 cases in which they were present in 75% to 95% of the tumor cells. Patchy areas of oncocytic change with characteristic nuclear changes of PTC were present in 2 cases.

Most FAs showed a mixture of small and large follicles. In some FAs, cystically dilated follicles and peripherally located follicles were lined by cells with enlarged nuclei occasionally having visible nucleoli but lacking the characteristic nuclear features of PTC. Ten of 20 FAs showed focal regressive changes in the form of hemorrhage, cystic degeneration, fibrosis, or calcification. Data on patient and tumor characteristics are summarized in **Table 1**.

Immunohistochemical Findings

The cellular localization of immunoreactivity with CK19 was similar in all positive tissues examined, ie, cytoplasmic with frequent enhancement adjacent to the cell membrane. The distribution and extent of CK19 immunoreactivity in UT-PTCs, FV-PTCs, and FAs are summarized in **Table 2**. All cases of UT-PTC and all but

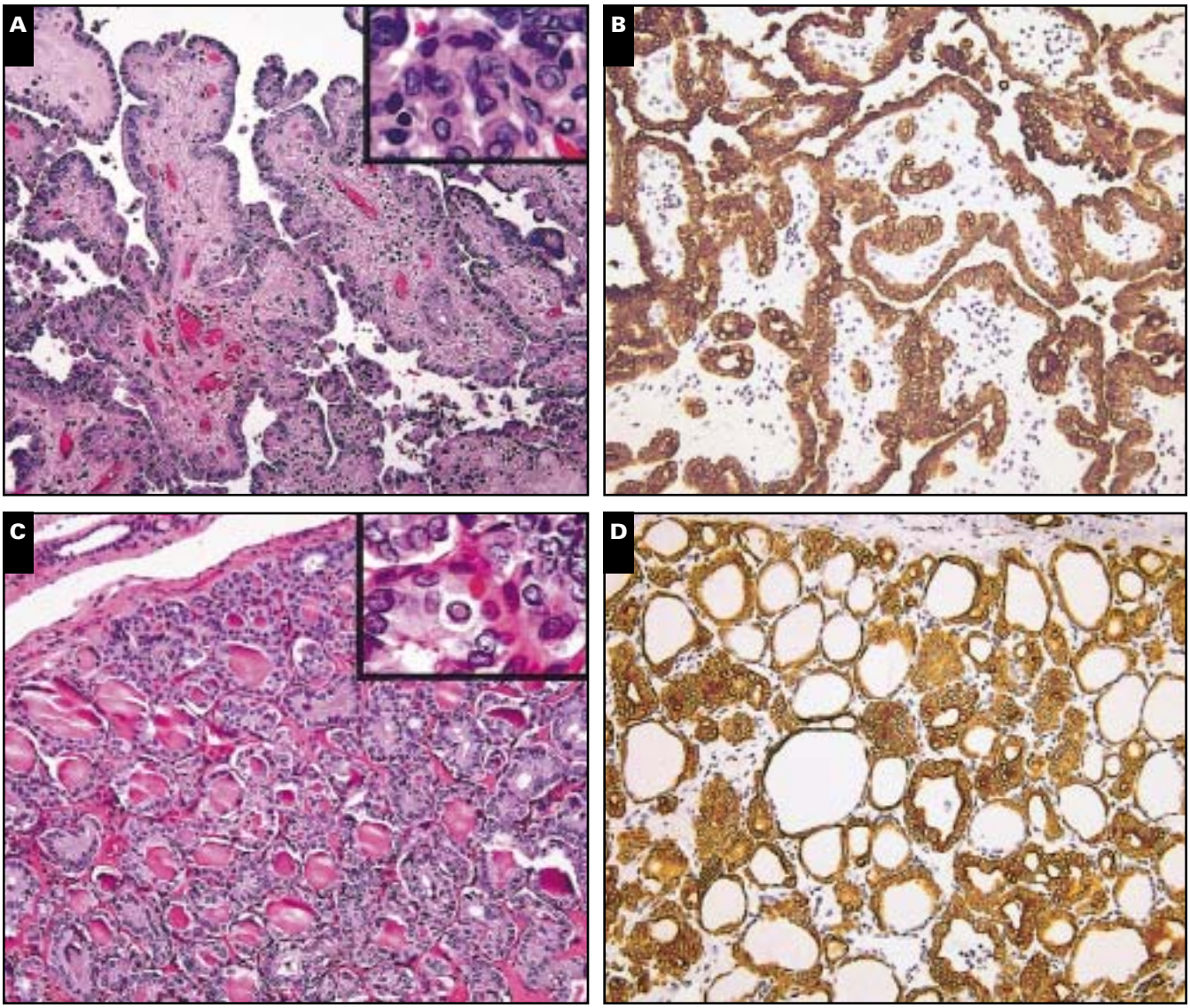

Image 1 **A**, Papillary thyroid carcinoma of the usual type (UT-PTC) showing typical nuclear features (H&E, ×100). Inset, High-power view of tumor cells (H&E, ×400). **B**, UT-PTC shows strong and diffuse cytoplasmic staining of tumor cells lining the papillae (cytokeratin [CK] 19, ×100). **C**, Encapsulated follicular variant of papillary carcinoma (FV-PTC) (H&E, ×100). Inset, High-power view of follicular cells with nuclear features of PTC (H&E, ×400). **D**, FV-PTC shows strong and diffuse cytoplasmic staining (CK19, ×100).

1 case of FV-PTC showed strong diffuse reactivity for CK19 **Image 1**. In general, the CK19 immunoreactivity was stronger in the cells with nuclear features of PTC, while staining in cells lacking nuclear changes of PTC varied from completely negative to moderately positive. The exception was represented by a single case of FV-PTC that scored as 2+, with the staining concentrated in the subcapsular areas. This tumor exhibited typical nuclear changes of PTC throughout the tumor on the routinely stained sections **Image 2**. CK19 expression was variable in FAs. Of the cases, 75% (15/20) showed scattered positive cells that accounted for less than 5% reactivity. In the remaining cases, the staining was more widespread (2+ in 1 case and 3+ in 4 cases). In all of these cases, CK19 staining was patchy and often restricted to follicular cells located at the periphery of the nodule or those lining cystic follicles. The intensity of staining was moderately strong **Image 3**.

Nonneoplastic thyroid tissue was available for evaluation in 32 of 35 cases. The pattern of immunostaining for CK19 is summarized in **Table 3**. The extent and intensity of staining of nonneoplastic normal thyroid (whether affected by lymphocytic thyroiditis or not) were similar in cases of PTC and FA **Image 4**.

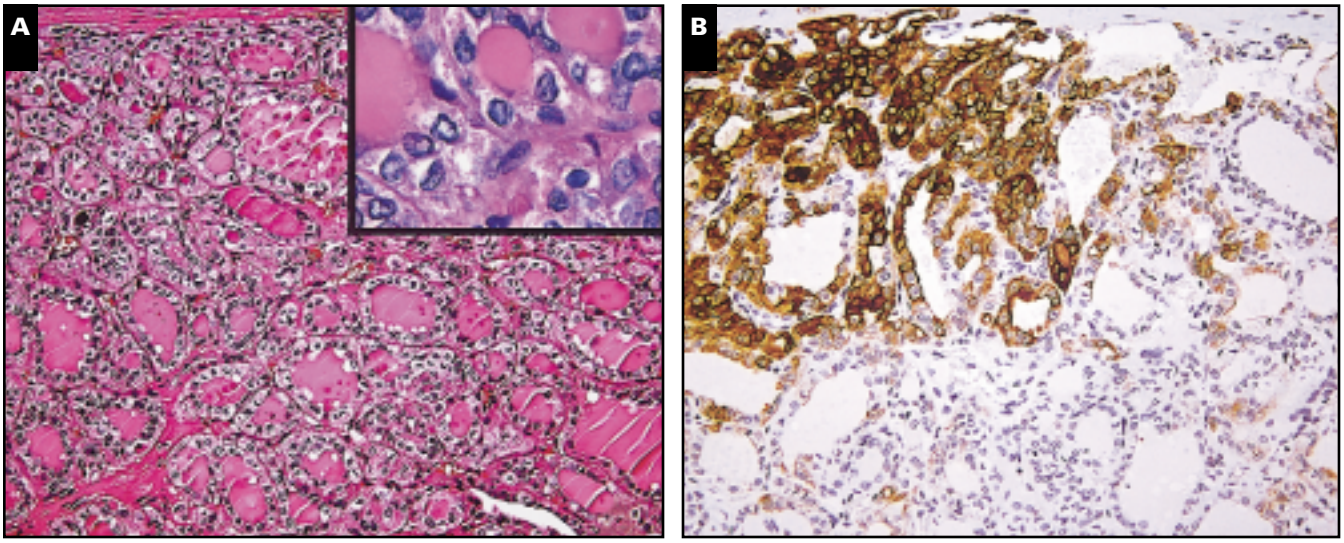

Image 2 **A**, Follicular variant of papillary thyroid carcinoma (H&E, ×100). Inset, High-power view of follicular cells with nuclear features of papillary thyroid carcinoma (H&E, ×400). **B**, Strong cytoplasmic staining limited to the peripheral follicles (cytokeratin 19, ×100).

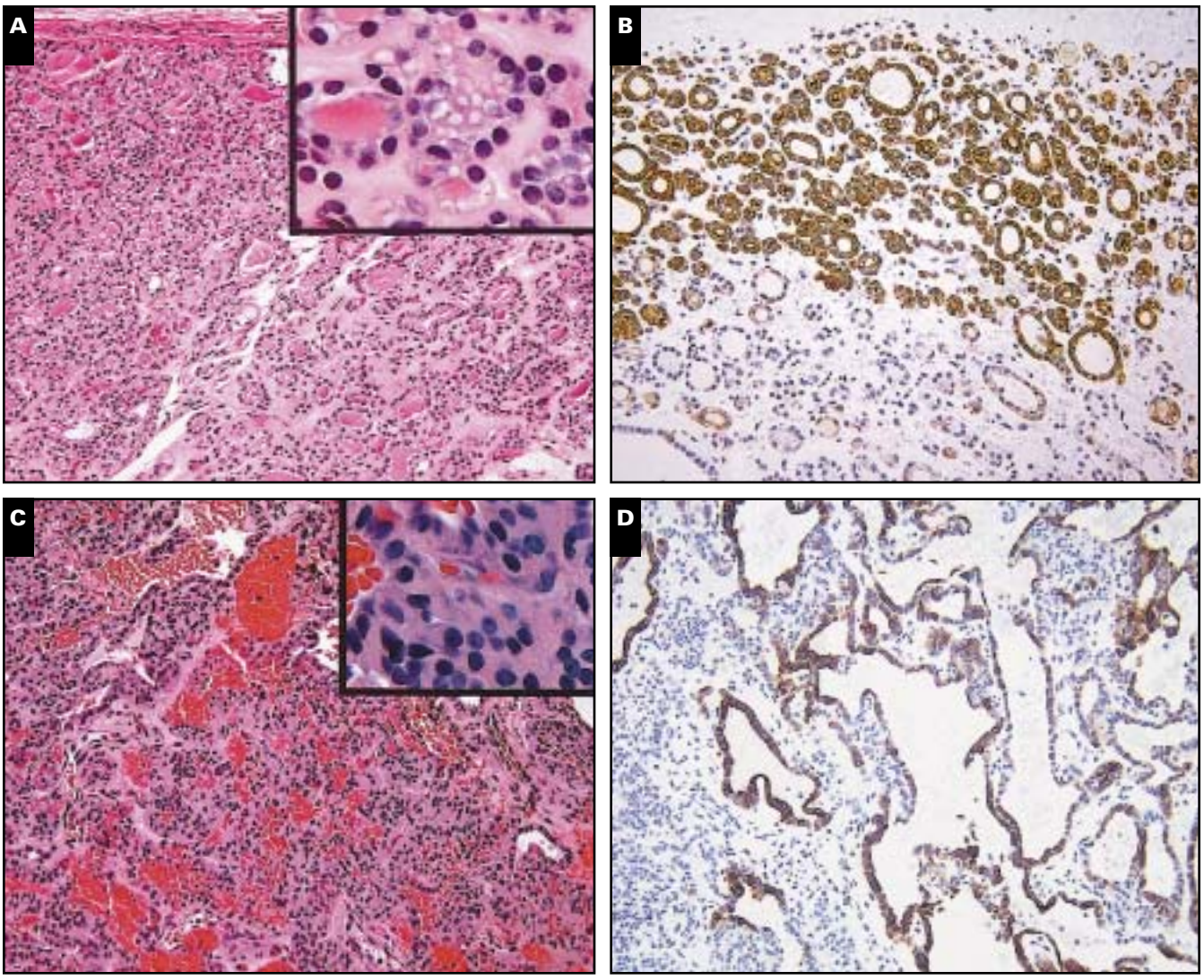

Image 3 **A**, Encapsulated follicular adenoma (FA) (H&E, ×100). Inset, High-power view of the follicles (H&E, ×400). **B**, This FA exhibits patchy, moderately strong cytoplasmic staining of follicular cells (cytokeratin [CK] 19, ×100). **C**, FA with scattered cystic follicles (H&E, ×100). Inset, High-power view of the follicles (H&E, ×400). **D**, Strong cytoplasmic staining limited to follicular cells lining the cystic follicles of the FA (CK19, ×100).

Table 3
Percentage of Nonneoplastic Thyroid Adjacent to Tumors Staining Positive for Cytokeratin 19

	Papillary Thyroid Carcinoma		
Score (% of Positivity)	Usual Type (n = 4)	Follicular Variant (n = 10)	Follicular Adenoma (n = 18)
1+ (<5%)	1 (25)	3 (30)	2 (11)
2+ (5%-25%)	0	2 (20)	1 (6)
3+ (25%-75%)	1 (25)	2 (20)	8 (44)
4+ (>75%)	2 (50)	3 (30)	7 (39)

Discussion

Immunohistochemical studies using various antibodies to cytokeratins have been performed by several authors in an attempt to distinguish papillary from follicular tumors of the thyroid and to differentiate the former from nonneoplastic lesions on the basis of the differential expression of this marker in normal and neoplastic follicular cells.[11-15] Although some studies have concluded that these antibodies are useful in the distinction, others have shown equivocal results.

Broad-spectrum cytokeratins are present in normal and hyperplastic follicular cells, in the follicular cells in chronic thyroiditis, and in all tumor types. A study using antibodies to high-molecular-weight keratins (plantar callus) reported reactivity in some follicular cells in 8% (1/12) of normal thyroids, 14% (1/7) of follicular carcinomas, 44% (8/18) of hyperplastic nodules, 100% (12/12) of thyroiditis cases, and 100% (12/12) of PTCs.[11] However, these results were not confirmed by 2 subsequent studies.[12,14] Liberman and Weidner[15] studied the distribution of high-molecular-weight cytokeratin using the 34betaE12 antibody. They found reactivity in 91% (41/45) of PTCs (including FV-PTCs) and in 20% (10/51) of follicular neoplasms (adenomas and carcinomas). In general, the staining pattern in PTC was strong and patchy, whereas follicular neoplasms stained weakly.[15]

Studies have shown that normal thyroid strongly expresses the simple epithelial cytokeratins, CK7 and CK18, and, to a lesser extent, CK8 and CK19, but not stratified epithelium-type cytokeratins such as CK5/6 and CK13.[9] The latter also have been found lacking in follicular carcinoma. Instead, PTCs expressed CK5/6 and CK13 in 66% (27/41) and 34% (14/41) of cases, respectively.[9] Immunoreactivity for CK7, CK8, CK18, and CK19 is present in both PTCs and follicular carcinomas, but the extent and intensity of CK19 staining has been said to be greater in the former. Miettinen et al[5] observed diffuse CK19 reactivity in all PTCs and in approximately 50% (14/28) of follicular carcinomas. They also found focal expression of CK5/6 in PTCs.[5] Schelfhout and coworkers[8] reported uniform reactivity for CK19 in 100% (12/12) of PTCs. Kragsterman et al[10] concluded that CK19 is of limited value as a marker for the diagnosis of

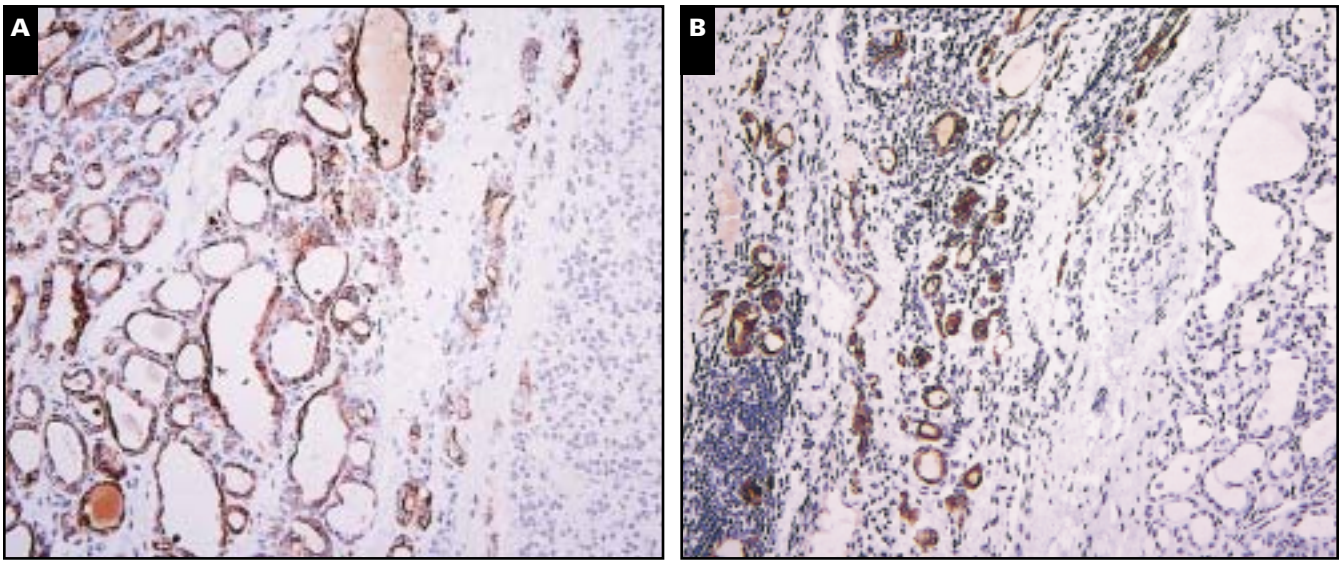

Image 4 **A**, Normal thyroid follicular cells adjacent to follicular adenoma (FA) showing strong cytoplasmic staining (cytokeratin [CK] 19, ×100). **B**, Lack of staining in FA and strong cytoplasmic staining in follicular cells in lymphocytic thyroiditis adjacent to the tumor (CK19, ×100).

Table 4
Summary of Main Published Results of Cytokeratin 19 Expression in Papillary Thyroid Carcinoma (PTC) and Follicular Adenoma (FA)

	Reference and Antibody Clone*							
	Kragsterman et al,[10] BA17		Miettinen et al,[5] RCK108		Schelfhout et al,[8] LP_2K		Baloch et al,[6] b170	
Score (% Positive)	PTC (n = 35)	FA (n = 2)	PTC (n = 137)	FA (n = 54)	FV-PTC (n = 12)	FA (n = 10)	FV-PTC (n = 26)	FA (n = 4)
<1%	1	0	0	22	0	0	5	3
1%-10%	2	0	4	19	0	9	1	1
11%-50%	4	2	3	5	0	1	3	0
>50%	28	0	130	8	12	0	17	0

FV-PTC, follicular variant of papillary carcinoma.
* BA17, DAKO, Glostrup, Denmark; RCK108, DAKO, Carpinteria, CA; LP_2K, Amersham, Buckinghamshire, England; b170, Novocastra, Newcastle upon Tyne, England.

thyroid tumors, but acknowledged that the presence of this marker should raise the suspicion of PTC. In a recent study, Cheung et al[16] reported diffuse CK19 staining in 80% (43/54) of UT-PTCs and 57% (48/84) of FV-PTCs. They also observed that 17% (6/35) of FAs showed focal staining with CK19 and 3% (1/35) showed diffuse CK19 staining.[16] Baloch and coworkers[6] studied UT-PTCs and FV-PTCs with a wide spectrum of cytokeratins, including CK19. In that study, all cases of PTC (including FV-PTC) were positive for CK19. The FV-PTC showed consistently strong immunoreactivity in areas having the typical nuclear features of PTC, whereas other areas showed moderate to strong staining in only one half of the cases. Nonneoplastic thyroid parenchyma immediately adjacent to the FV-PTC stained with CK19, whereas normal thyroid adjacent to the UT-PTC was negative.[6] The authors suggested that some unknown factors secreted by FV-PTC might have altered cytokeratin expression of the follicular epithelium adjacent to the tumors.[6] In our study, there were no significant differences in the staining pattern of nonneoplastic thyroid adjacent to FAs or UT-PTCs compared with FV-PTCs.

The extent of reactivity with CK19 reported in some of the previous studies on PTCs and FAs is summarized in **Table 4**. The reasons for the discrepancies in the immunoreactivity for CK19 and other cytokeratins in papillary and follicular tumors reported by different authors are not clear. Possible explanations include variations in technical methods, the antibody clone used, time of fixation, and observer bias in the interpretation of the results. The significance of focal expression of CK19 in some FAs is unknown. Further studies will be necessary to show whether these tumors have a different clinical behavior or molecular profile.

In agreement with previous reports,[5-10] we found strong and diffuse staining of CK19 in almost all cases of PTC, regardless of the subtypes. However, CK19 immunoreactivity also was present in 100% of FAs (20/20). Of the cases, 75% (15/20) showed only 1+ reactivity, which was confined largely to flattened cells lining cystic follicles, an observation also made by Schelfhout et al.[8] However, in 25% of the FAs, CK19 immunoreactivity was more extensive (2+ in 1, 3+ in 4). These findings indicate that the presence of CK19 immunoreactivity, even if moderately strong, cannot be used by itself to establish a diagnosis of PTC. The fact remains that the presence of strong and diffuse CK19 staining in a thyroid tumor with a follicular growth pattern should raise the suspicion of FV-PTC, and the lesion should be examined carefully for the presence of the nuclear and other features of PTC. Occasionally, the CK19 staining of PTC (including FV-PTC) can be patchy rather than diffuse, as demonstrated by our study and by others (Table 4). Therefore, less than diffuse staining for CK19 does not rule out a diagnosis of FV-PTC if the diffuse nuclear changes typical of this type are present. Finally, staining of peritumoral thyroid follicles for CK19 is not restricted to cases of FV-PTC.

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