Histopathology 2005, **47**, 391–401. DOI: 10.1111/j.1365-2559.2005.02221.x

Usefulness of HBME-1, cytokeratin 19 and galectin-3 immunostaining in the diagnosis of thyroid malignancy

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Date of submission 13 December 2004
Accepted for publication 11 March 2005

de Matos P S, Ferreira A P, de Oliveira Facuri F, Assumpção L V M, Metze K & Ward L S
(2005) *Histopathology* **47**, 391–401

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Aims: To investigate the usefulness of immunohistochemical expression and immunolocalization of a panel of thyroid malignancy markers including HBME-1, cytokeratin (CK) 19 and galectin-3.
Methods and results: We evaluated 170 thyroid lesions including 148 neoplastic lesions [84 papillary carcinomas (PC), 38 follicular carcinomas (FC), 18 follicular adenomas, one hyalinizing trabecular tumour, five medullary carcinomas, two anaplastic carcinomas] and 22 non-neoplastic lesions (12 adenomatous nodules and 10 Hashimoto's thyroiditis). HBME-1, galectin-3 and CK19 were expressed in 94%, 72.6%, 72.6% of PCs and in 63%, 21%, 21% of FCs. The three markers were mostly negative in all normal tissues. Although the most helpful marker in terms of sensitivity and specificity for the follicular variant of PC and for FC diagnosis was HBME-1, when we consider the differentiation between cases of follicular variant of papillary carcinoma (FVPC) and FC or adenoma, in terms of percentage of positive cells, galectin-3 and CK19 were more relevant.
Conclusions: HBME-1 is the most sensitive marker for thyroid malignancy but the three markers may be useful in specific cases. This panel of markers is useful to differentiate the follicular patterned lesions, with special reference to the FVPC.

Keywords: diagnosis, FVPC, prognosis, sensitivity, specificity

Abbreviations: FC, follicular carcinoma; FVPC, follicular variant of papillary carcinoma; PC, papillary carcinoma

Introduction

The differential diagnosis between some thyroid lesions is often difficult to determine, even with permanent sections. In particular, the so called 'follicular-patterned' thyroid lesions, a group that includes follicular adenomas, follicular carcinomas (FC), the follicular variant of papillary carcinomas (FVPC), Hürthle cell carcinomas, Hürthle cell adenomas and adenomatous nodules, may pose a diagnostic challenge even to the most experienced pathologists.[1,2] A precise pathological diagnosis is essential to clinical and surgical planning. Patients submitted to surgery with a previous fine-needle biopsy diagnostic of a follicular patterned lesion may need a complete resection and even neck exploration if they present capsular and/or vascular invasion, while benign lesions may be treated with much less invasive procedures. It is not unusual to have to resubmit patients to surgery in order to complete the resection of a lesion mistaken as benign at fine-needle biopsy or at frozen section. Furthermore, the different follow-up approaches to benign and malignant tumours, and even between papillary and follicular carcinomas, may endanger the outcome of misdiagnosed patients. Thus, new methods that can, simply and accurately, distinguish benign from malignant tumours are greatly desired.

Several markers of malignancy have been investigated but they all present some advantages and some limitations. Among the most promising, galectin-3, HBME-1 (Hector Battifora Mesothelial cell) and

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cytokeratin (CK) 19 have been most frequently used in thyroid pathology. Galectin-3 has been reported to be a very sensitive and reliable diagnostic marker for preoperative identification of thyroid carcinomas with high sensitivity and specificity in tissue specimens, cytological cell blocks and in fresh cytological samples.[3] This marker was initially suggested as an indicator of malignancy and a potential tool in the differential diagnosis of the follicular-patterned lesions of the thyroid.[3–5] Unfortunately, many further studies, either by immunohistochemistry or reverse transcriptase-polymerase chain reaction (RT-PCR), were not able to prove galectin-3 capable of distinguishing follicular-patterned lesions since it was demonstrated to be expressed also in normal thyroid tissues and in benign thyroid lesions.[4–10] HBME-1 has also been reported to be useful in the diagnosis of malignant tumours of follicular epithelial derivation.[11–13] However, HBME-1 expression seems to decrease with Hürthle cell and apocrine-like changes.[14] A third marker, CK19, may be useful in the diagnosis of papillary carcinoma (PC), where it has been shown to have strong diffuse cytoplasmic reactivity.[15–17] Other markers, such as CAV1, CAV2, GDF10, GPC3 and CHRDL, were found to be down-regulated in most FC and have been proposed as helpful in distinguishing follicular adenoma and FC.[18,19] However, further analysis is still needed to evaluate their potential clinical use.

The present study was designed to evaluate the utility of a combination of markers including galectin-3, HBME-1 and CK19 in the routine histological differentiation of the various thyroid malignancies.

Materials and methods

TISSUE SPECIMENS

Ten percent formalin-fixed, paraffin-embedded blocks routinely prepared from surgical specimens of 170 cases of thyroid tumours were selected for this study. All cases included normal thyroid tissue adjacent to tumour. One hundred and forty-eight thyroid lesions were diagnosed as neoplastic lesions and consisted of 18 follicular adenomas; one hyalinizing trabecular tumour; 38 FC (13 widely invasive and 25 minimally invasive carcinomas); 84 PC (39 of the classic variant, 25 follicular variants, 10 tall cell variants, one columnar cell, one oxiphylic variant and eight papillary microcarcinoma); five medullary carcinomas and two anaplastic (undifferentiated) carcinomas. Lesions smaller than 10 mm were classified as papillary microcarcinomas ($n = 8$). Four of the FC were classified as oxyphilic cell variants, one of them considered widely invasive and the other three minimally invasive. Another 22 thyroid lesions were considered non-neoplastic and included 12 adenomatous nodules and 10 cases of Hashimoto's thyroiditis. Four out of the 18 follicular adenomas were further classified as oxyphilic variants according to diagnostic criteria based on the World Health Organization Histological Classification, 2nd edition, and Livolsi's classification of thyroid pathology.[20,21]

IMMUNOHISTOCHEMISTRY

Tissue sections 5 μm thick were deparaffinized in xylene and dehydrated in a graded ethanol series. Endogenous peroxidase activity and non-specific binding were blocked with 0.3% hydrogen peroxide in methanol for 15 min. After rinsing in phosphate-buffered saline at pH 7.2 (PBS), 10% bovine serum (Wako, Osaka, Japan) was applied for 20 min to block non-specific reactions. Sections were then incubated with the primary antibody overnight at 4°C. After rinsing in PBS, they were treated with peroxidase-labelled anti-rabbit immunoglobulin (Nichirei, Tokyo, Japan) for 30 min. The peroxidase reaction was visualized by incubating the sections with 0.02% 3,3′-diaminobenzidine tetrahydrochloride in 0.05 M Tris buffer with 0.01% hydrogen peroxide (Nichirei). The sections were counterstained with haematoxylin. Immunostaining for CK19 and HBME-1 was performed using monoclonal antibodies (Dako, Carpinteria, CA, USA) diluted 1 : 50 and 1 : 100, respectively; for galectin-3 staining we used a monoclonal antibody provided by Novocastra (Newscastle upon Tyne, UK) diluted 1 : 200. Positive control sections were prepared of prostate carcinoma for CK19, uterine adenomatoid tumour for HBME-1 and a lymph node for galectin-3. Negative controls were obtained by replacing the primary antibody with Tris-buffered saline.

IMMUNOHISTOCHEMICAL EVALUATION

The cells were regarded as positive for these proteins when immunoreactivity was clearly observed in their nuclei and/or cytoplasm. Staining of the follicular colloid in the absence of staining of the follicular epithelium and/or cytoplasm was considered non-specific and negative. Evaluation included the proportion of reactive cells within tumours as well as the staining intensity and its distribution pattern. The proportion score described the estimated fraction of positively stained cells (0, no visible reaction; 1+, < 5%; 2+, 5–25%; 3+, > 25–75%; 4+, > 75% of tumour cells stained). The intensity score represented the estimated staining intensity (0, no staining; 1+, weak; 2+,

moderate; 3+, strong reaction intensity). In addition, we recorded results based on the distribution of the immunoreactive cells classified as: diffuse when most of the cells were stained by the marker; focal, segmental when some portions of the tissue presented clusters of stained cells while other portions did not; and focal, cellular when we observed that the marker was restricted to a few scattered cells.

STATISTICAL ANALYSES

Statistical analysis was conducted using SAS (Statistical Analysis System, version 8.1; SAS Institute Inc., Cary, NC, USA, 1999–2000). Linear discriminant analysis using a step-wise selection process of the relevant variables was performed using the SPSS 10.0 program (SPSS Inc., Chicago, IL, USA). The stability of the system was evaluated using the leave one out cross-validation (LOOCV) (jackknife procedure). Associations were assessed using 2×2 or $2 \times n$ contingency table analysis and χ^2 or Fisher's (F) exact tests were used where appropriate. *P*-values < 0.05 were regarded as statistically significant.

Results

IMMUNOHISTOCHEMICAL CK19 EXPRESSION IN THYROID LESIONS

The staining pattern was predominantly pancytoplasmic. Normal thyroid tissues were negative or presented scattered, weakly stained follicular cells. Most of the benign lesions were negative or weakly stained in a few cells as exemplified in Figure 1C,D. Also, most adenomatous nodules were negative or presented only slight positivity, with the exception of two cases that presen-

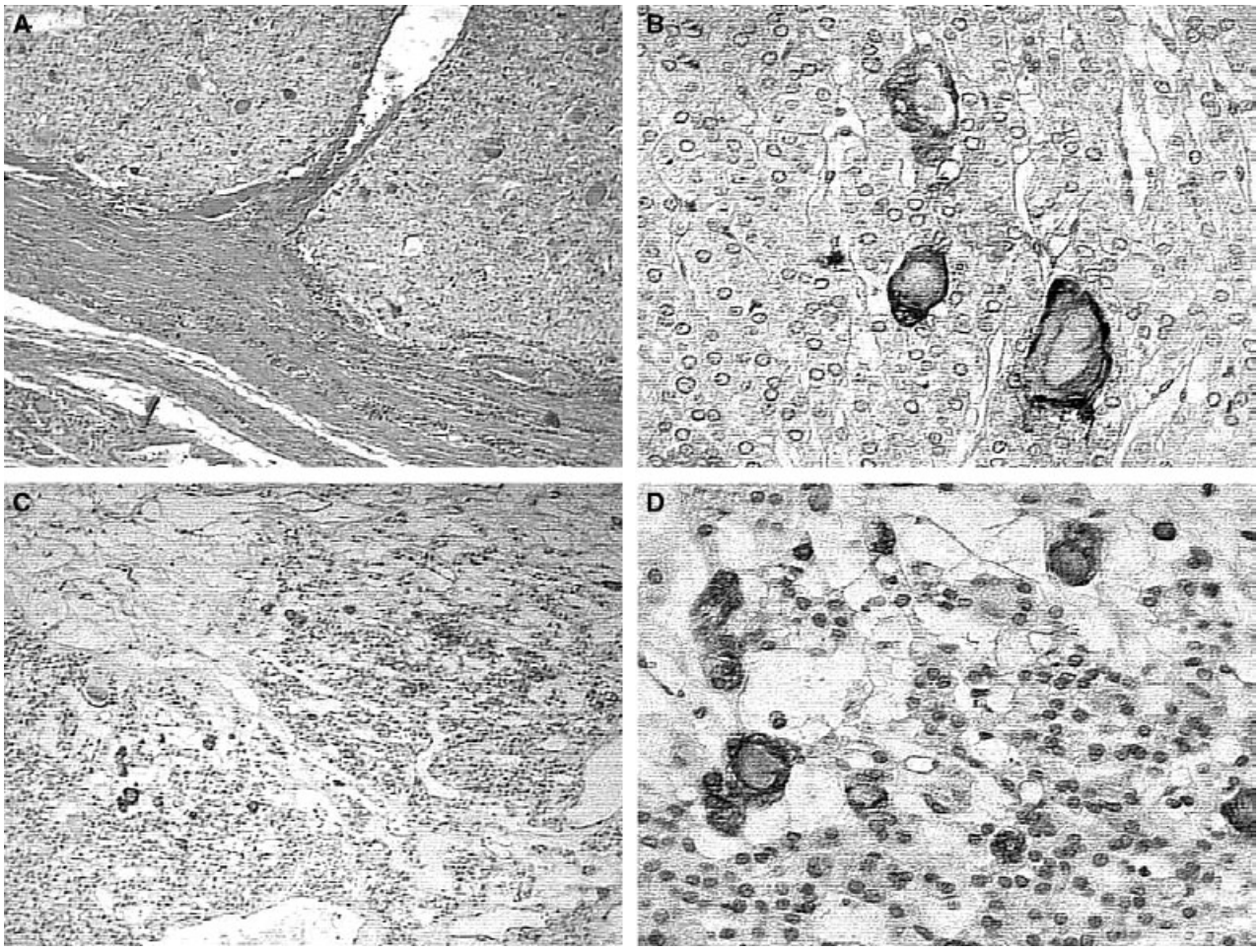

Figure 1. Follicular adenoma. **A,** H&E stain showing a complete encapsulated nodule as a follicular patterned lesion, without any capsular or vascular invasion. **B,** Most of the lesion is negative for HBME-1, with just a few scattered cells showing immunoreactivity. **C,** Focal cellular positivity for CK19. **D,** A few cells showing cytoplasmic positivity for CK19.

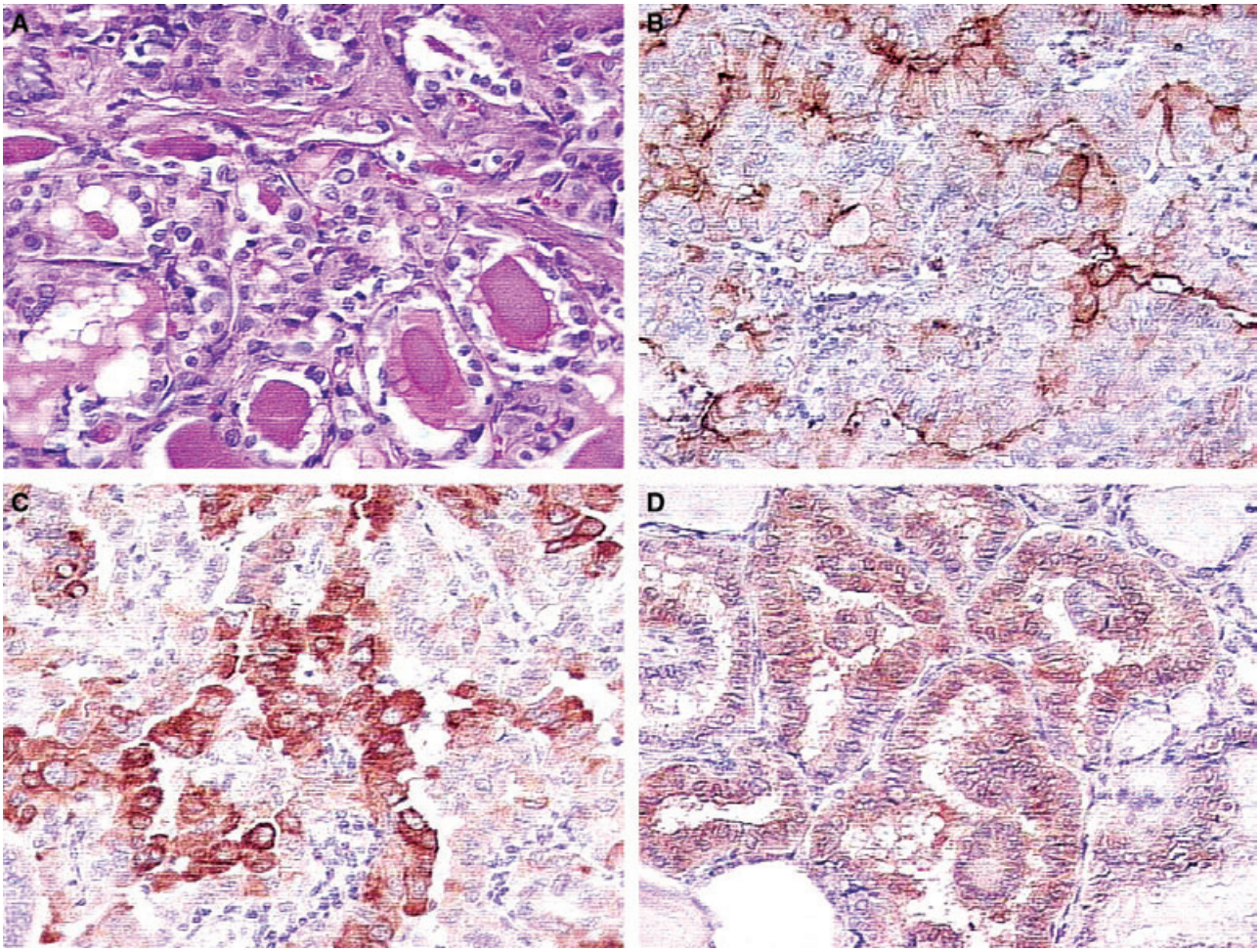

Figure 2. Follicular variant of papillary carcinoma. **A,** H&E stain showing proliferating follicles formed by follicular cells with characteristic nuclear features of papillary carcinoma. **B,** Diffuse and strong immunoreactivity for HBME-1 in the cytoplasm and cytoplasmic membrane of the neoplastic cells. **C,** Diffuse and strong reactivity for CK19. **D,** Diffuse predominantly cytoplasmic positivity for galectin-3.

ted focal staining; one of the cases is exemplified in Figure 5A,B. It also showed a papillary microcarcinoma or microtumour (Figure 5A,C), as an incidental finding, that was positive for CK19 and HBME-1 (Figure 5D). Most PC cases were strongly immunopositive for CK19, as show in Figures 2C and 6C and Table 1.

In contrast to PC, that presented a high percentage of positive cases (72.6%) strongly and diffusely stained in 65.6% of the cases, FCs were positive in only 21% of the cases and the distribution of the markers was always focal, as exemplified in Figure 4C. Most of the FCs (79%) were negative for this marker, as exemplified in Figure 3C. This difference in CK19 staining intensity allowed us to distinguish the FVPC from other follicular-patterned lesions with a sensitivity of 52%, specificity of 13.7%, a positive predictive power of 34.2% and negative predictive power of 25%. When we considered the diffuse pattern of staining of the FVPC (Figure 2A), exemplified in Figure 2C, we were able to ascertain the diagnosis of 77% of the cases (Table 4). The staining features of CK19 also helped avoiding confusion between hyperplasia and the papillary areas of FVPC cases. Among PC, the microcarcinomas, all 10 cases of tall cell variant and the oxyphilic cell cases were positive. The classic form PCs (Figure 6A,C) were positive in 84.6% of the cases while the FVPC (Figure 2A,C) was positive in 52% of the cases. The columnar cell variant case was negative for CK19.

IMMUNOHISTOCHEMICAL HBME-1 EXPRESSION IN THYROID LESIONS

Table 2 represents the percentage of cases that expressed HBME-1 and the corresponding staining pattern.

Table 1. Immunohistochemical expression of CK19 in neoplastic and non-neoplastic thyroid lesions

Diagnosis	No. of cases	CK19+ (%)	Positivity pattern, predominant/secondary (%)
Carcinomas			
PC	84	61 (72.6)	D (65.6)/F++ (24.6)
FC	38	8 (21)	F++ (50)/F+ (37.5)
MC	5	1 (20)	F+ (100)
AC	2	0 (0)	–
Normal	170	0 (0)	–
FA	18	6 (33.3)	F+ (66.6)/F++ (33.4)
AN	12	2 (16.7)	F+ (100)
HT	10	6[60]	F+ (100)

PC, Papillary carcinoma; FC, follicular carcinoma; MC, medullary carcinoma; AC, anaplastic carcinoma; FA, follicular adenoma; AN, adenomatous nodule; HT, Hashimoto's thyroiditis; D, diffuse reactivity; F++, focal segmental reactivity; F+, focal cellular reactivity.

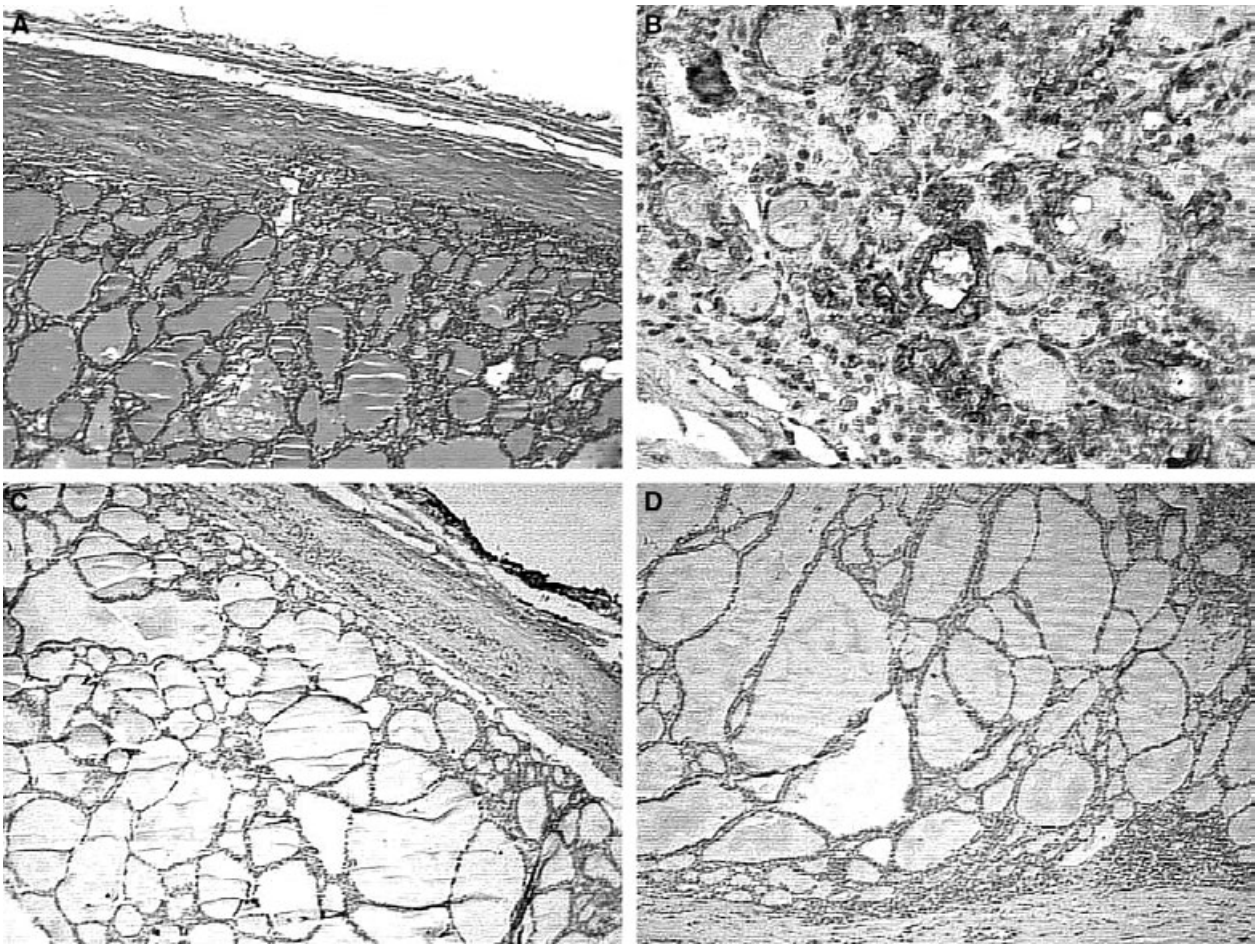

Figure 3. Minimally invasive follicular carcinoma. **A,** H&E stain. Follicular patterned lesion showing capsular invasion in the upper field. **B,** Diffuse positivity for HBME-1, predominantly cytoplasmic. **C,** Total negativity of the lesion for CK19. **D,** Total negativity of the lesion for galectin-3.

Table 2. Immunohistochemical expression of HBME-1 in neoplastic and non-neoplastic thyroid lesions

Diagnosis	No. of cases	HBME-1+ (%)	Positivity pattern, predominant/secondary (%)
Carcinomas			
PC	84	79 (94)	D (91.1)/F++ (6.3)
FC	38	24 (63)	D (50)/F++ (33)
MC	5	0 (0)	–
AC	2	0 (0)	–
Normal	170	0 (0)	–
FA	18	10 (55.6)	F++ (66.7)/F+ (22.2)
AN	12	4 (33.3)	F+ (75)/F++ (25)
HT	10	9 (90)	F+ (100)

PC, Papillary carcinoma; FC, follicular carcinoma; MC, medullary carcinoma; AC, anaplastic carcinoma; FA, follicular adenoma; AN, adenomatous nodule; HT, Hashimoto's thyroiditis; D, diffuse reactivity; F++, focal segmental reactivity; F+, focal cellular reactivity.

Characteristically, immunoreactivity was mainly in the cell membranes and cytoplasm, as exemplified in Figures 1B, 2B, 3B, 4B, 5D and 6B. Normal tissues were all negative. Likewise, the medullary and the undifferentiated carcinomas were HBME-1–. Most cases of Hashimoto's thyroiditis were positive but less than 5% of the cells were immunoreactive. Also, most adenomatous nodules were negative or presented slight positivity with the exception of four cases that presented a focal staining pattern. One of these cases showed a papillary microtumour, as an incidental finding (Figure 5C,D), that was also positive for CK19.

HBME-1 immunoreacted with a significant proportion of PC (94%), including both classical (97.4%) (Figure 6B) and FVPC (84%) (Figure 2B). Both types presented a predominantly diffuse pattern of staining (91% and 90.4%, respectively, for classic PC and FVPC). Table 4 shows a comparative analysis of HBME-1, CK19 and galectin-3 rates of expression and staining patterns. It is evident that HBME-1 was the best among the three markers evaluated in the diagnosis of follicular-patterned lesions. Unfortunately, HBME-1 staining was not significantly different between classic PC (97.4%) and FVPC (84%). All PC variants were positive for this marker. Concerning the differential diagnosis between follicular adenoma (Figure 1A) and FC (Figures 3A and 4A), both showed similar proportions of staining (55.6% and 63%, respectively), but the staining pattern was predominantly focal in the follicular adenoma (66.7% of the cases) (Figure 1B), while it was diffuse (50%) or focal segmental (33%) in most of the FC (Figures 3B and 4B). HBME-1 had a similar positivity in minimally (60%) and widely (69.2%) invasive FC. Although nine out of 10 cases of Hashimoto's thyroiditis were positive for HBME-1, immunoreactivity was restricted to scattered follicular cells and macrophages.

IMMUNOHISTOCHEMICAL GALECTIN-3 EXPRESSION IN THYROID LESIONS

Immunoreactivity was seen predominantly in the cytoplasm but additional nuclear staining was observed in many clusters of cells (Figures 2D, 4D and 6D). Adjacent normal thyroid tissue was consistently negative, although a few sparse follicular cells and macrophages were also stained with this marker. Likewise for CK19, the elevated percentage of positive PC (72.6%) presenting a diffuse pattern (73.8%) (Figures 2D and 6D) contrasted with a lower percentage of positive FCs (21%) and its focal segmental (50%) or focal cellular (50%) (Figure 4D) distribution (Table 4). Most of the FCs (79%) were negative for this marker, as exemplified in Figure 3D. Galectin-3 was expressed less in benign thyroid lesions and its expression was always focal, and restricted to a few cells (Table 3). All the medullary, the undifferentiated carcinomas and the hyalinizing trabecular tumour were negative.

Galectin-3 stained 82% of the classic PC and 52% of the FVPC cases, all tall cell and 75% of the oxyphilic PC variants. However, the columnar cell variant was negative. Among the follicular-patterned lesions, galectin-3 stained just 20% and 23% of the minimally and widely invasive FC, respectively; and two out of the four

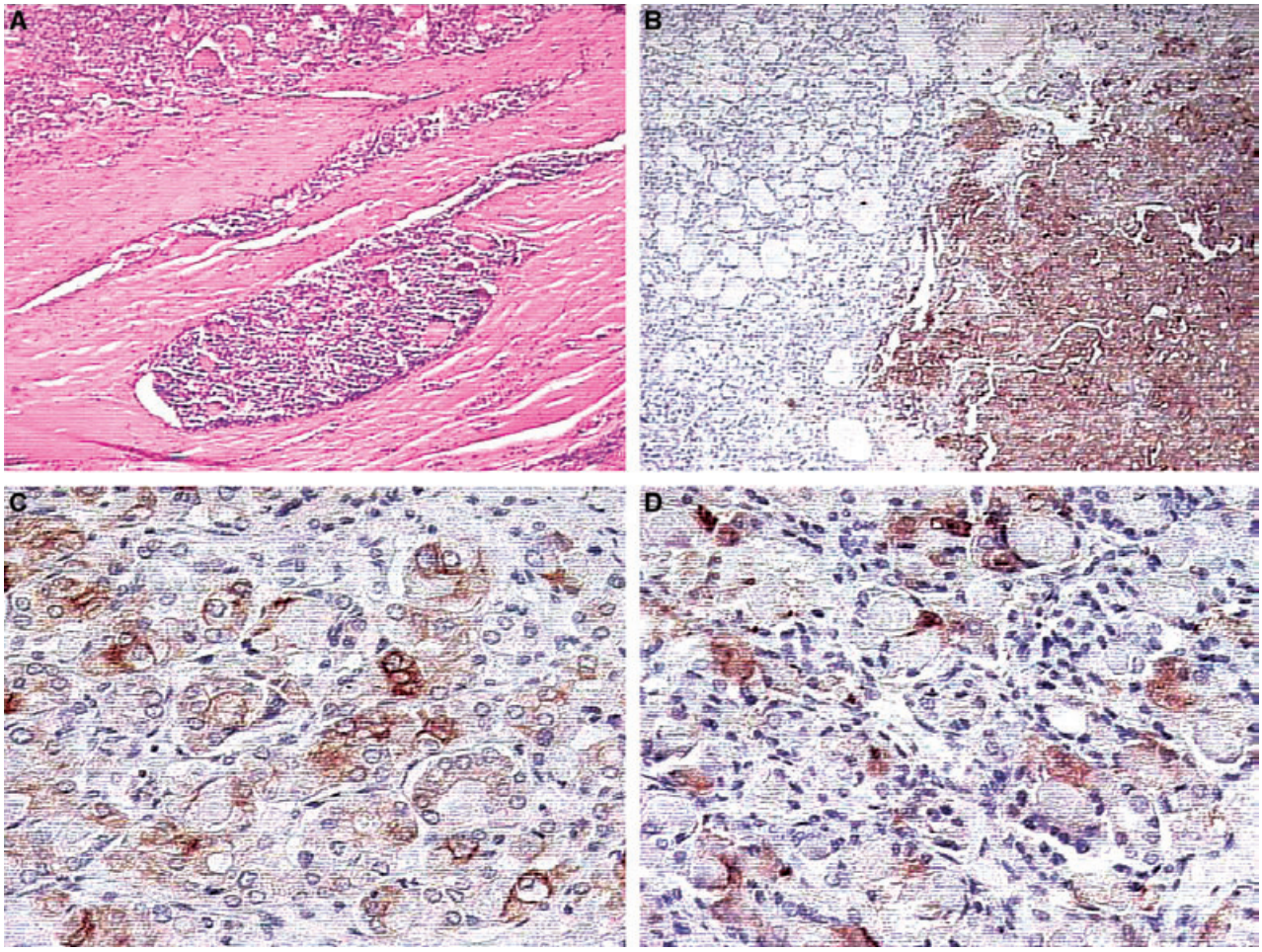

Figure 4. Frankly invasive follicular carcinoma. **A,** H&E stain. A follicular carcinoma showing multiple vascular invasion. **B,** Focal segmental positivity for HBME-1. **C,** Most of the lesion is negative with focal cells positive for CK19. **D,** Scattered cells positive for galectin-3 in a focal cellular pattern. Note the focal cytoplasmic and nuclear staining pattern for galectin-3.

oxyphilic FC variants, one minimally and the other widely invasive. These immunohistochemical features helped to distinguish FVPC (Figure 2D) that presented a diffuse pattern in 77% of the cases from FC (Figure 4D) (50% focal cellular and 50% focal segmental), the follicular adenoma and adenomatous nodule cases that were always just focally stained (Table 4).

CK19 and galectin-3 had a similar 21% sensitivity, 36.8% specificity, 25% positive predictive and 31.8% negative predictive power regarding the diagnosis of FC, contrasting with 63.1% sensitivity, 78.9% specificity, 75% positive and 68% negative predictive power for HBME-1. The panel of markers did not identify three out of 84 PCs (3.5%) but 27 PCs were negative for one or another of these markers. Likewise, HBME-1 was more efficient in the diagnosis of the FVPC, with 84% sensitivity, 48% specificity, 61.7% positive and 75% negative predictive power, while both CK19 and galectin-3 presented a sensitivity of 52%, specificity of 13.7%, positive predictive power of 34.2% and negative predictive power of 25%. However, the pattern of immunoreactivity differed among the three markers, helping to characterize follicular-patterned lesions since follicular adenomas and adenomatous nodules presented mostly weak and focal immunoreactivity, while the FVPC was, generally, diffusely stained.

Twenty-seven out of the 84 PC (32%) were negative for one or another of the three markers employed, but only three cases, all them FVPC, were negative for all three markers combined. On the other hand, the use of the three markers combined allowed the diagnosis of 81 out of the 84 PCs (96.5%) but only 24 out of the 38 FCs (63.1%). Concerning the differential diagnosis of follicular-patterned lesions, the combined use of

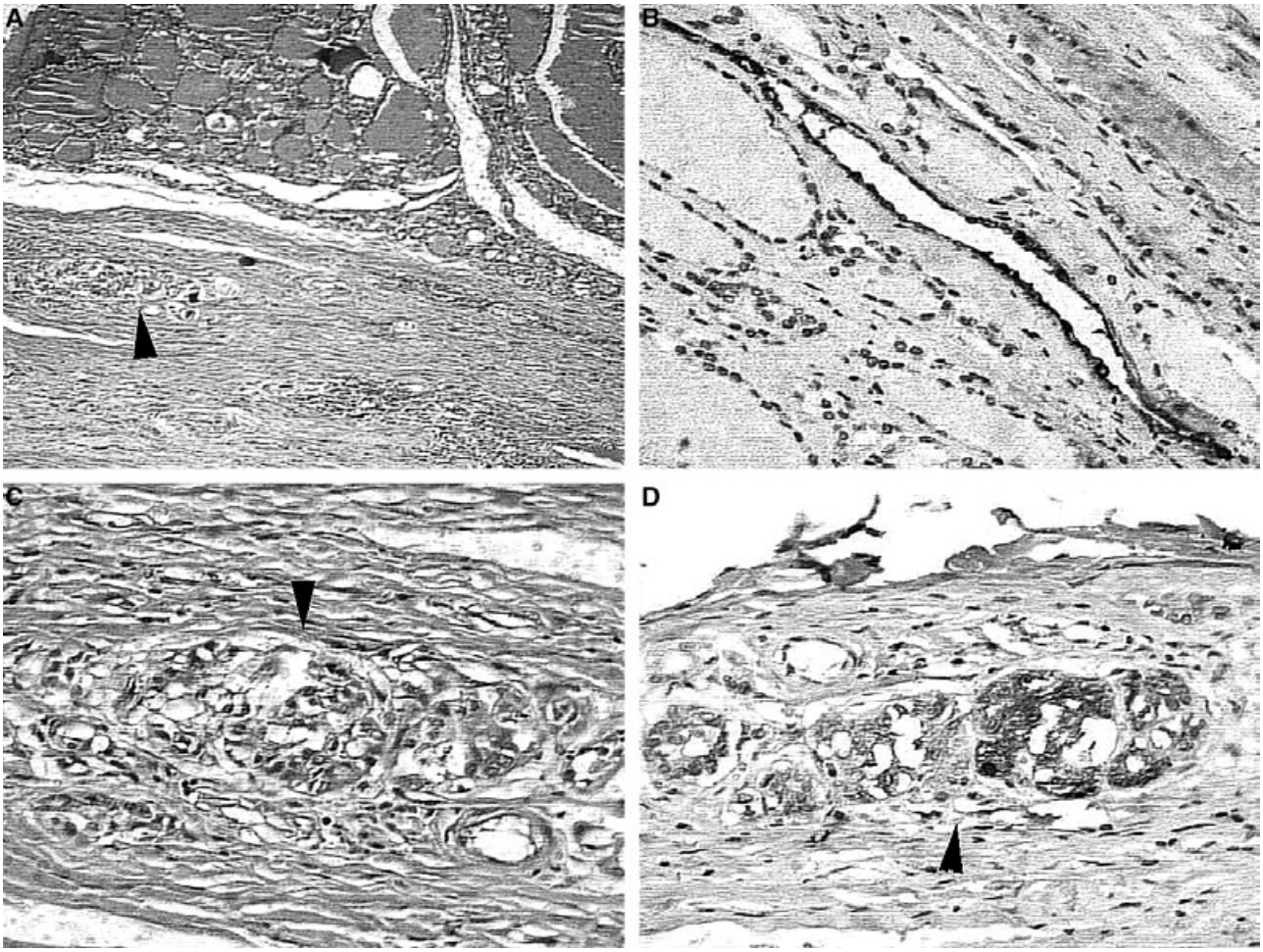

Figure 5. Adenomatous nodule. **A,** H&E stain showing a partially encapsulated nodule with a follicular patterned lesion and a papillary microcarcinoma or microtumour at the periphery (arrow). **B,** Focal cellular immunoreactivity for CK19. Note that the only site of positivity is an atrophic follicle in the middle of the hyperplastic nodule. **C,** Papillary microcarcinoma or microtumour (arrow) (H&E). **D,** Papillary microcarcinoma positive for HBME-1.

immunoreactivity of the three markers in combination with their corresponding staining patterns helped to identify 84% of the FVPC. Follicular adenomas were negative for the three markers in 38.8% of the cases and the remaining 61.2% cases showed focal positivity that helped to differentiate these lesions from encapsulated FVPC.

Linear analysis with step-wise selection process of relevant variables showed that FVPC could be correctly differentiated from other follicular lesions (follicular carcinomas, adenomas or adenomatous nodules) in 80.8%, 79.5% and 71.2% of the cases using galectin-3, CK19 and HBME-1, respectively. The combination of galectin-3 and CK19 achieved a correct classification in 84.9% of the cases (83.6% after jackknife procedure). Also, no matter how positive the lesion was for HBME-1, the presence of more than 20% of positive cells for CK19 or galectin-3 indicated a probable FVPC.

Discussion

Proposed molecular markers of PC include HBME-1, specific cytokeratins such as CK19, galectin-3 and RET, among others. Although at the molecular genetic level PC is characterized by rearrangements of the *RET* gene, the rearrangement is found in only 35% of PC in the general population and RET immunoreactivity appears to be less sensitive and specific for PC than CK19.[22,23] On the other hand, CK19, HBME-1 and galectin-3 have been demonstrated to be highly sensitive and specific in the diagnosis of PC by most

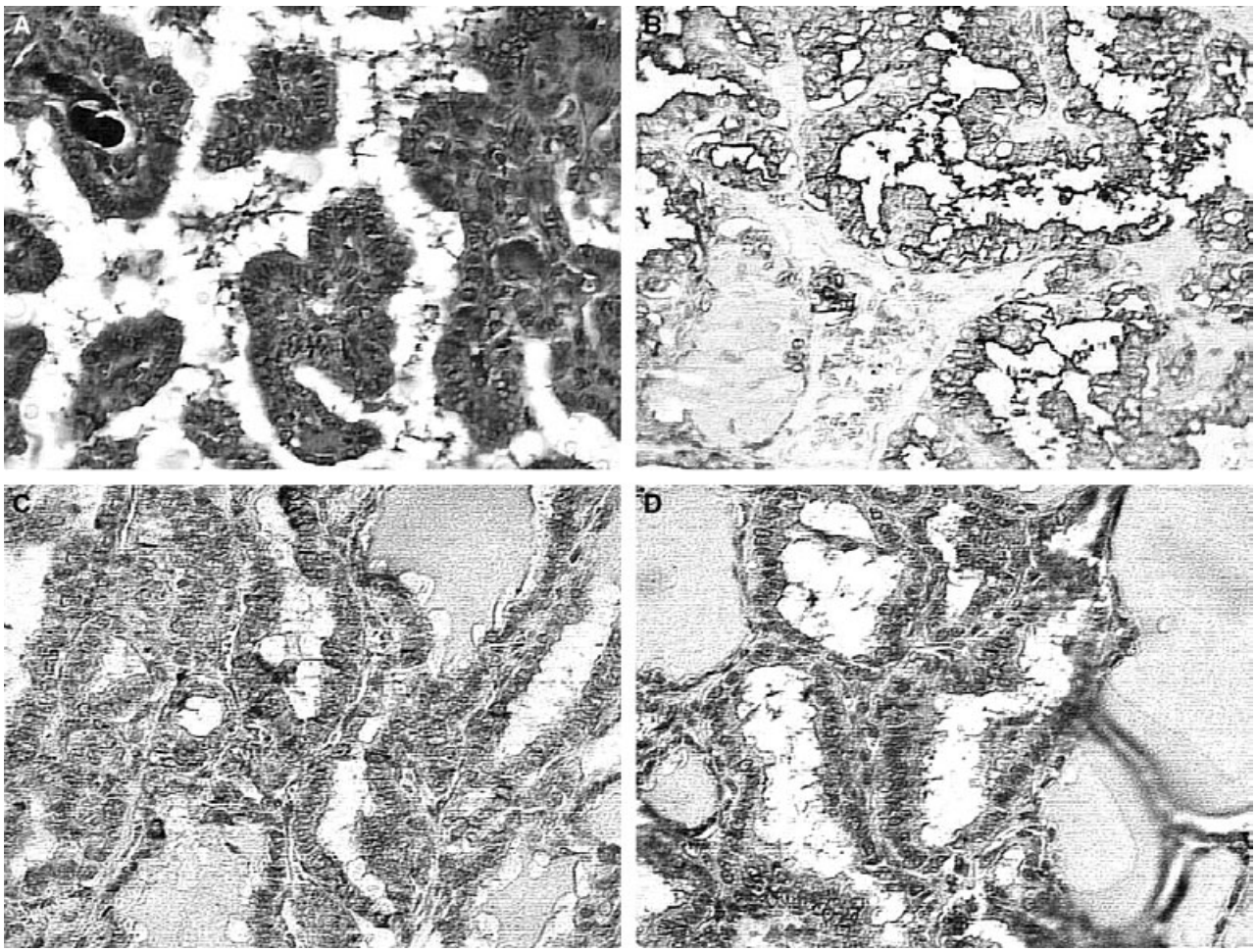

Figure 6. Classic variant of papillary carcinoma. **A,** H&E stain showing the characteristic papillary pattern and nuclear features of papillary carcinoma. **B,** Strong and diffuse immunoreactivity for HBME-1. **C,** Diffuse moderate immunoreactivity for CK19. **D,** Diffuse immunoreactivity for galectin-3. Note predominant cytoplasmic stain and scattered nuclear positivity.

reports.[3–17,24,25] Indeed, confirming Casey *et al.*'s data, we were able to ascertain the diagnosis of 96.4% of the PCs and most of the hyperplasia and other diagnostically difficult cases we evaluated with these three markers.[26]

Unfortunately, in contrast to many reports concerning the differential diagnosis of follicular-patterned lesions, we found none of these markers sufficient to confer a sensitive and specific diagnosis.[3–5,13,15,27] In particular, solitary encapsulated follicular-patterned nodules may be challenging especially when presenting scattered nuclear atypia. Some FVPC cases may look like follicular adenoma, when the nuclear features are not clear enough and a false diagnosis may mislead the clinicians to underestimate malignant thyroid pathology. Most of all, FC lesions are hard to distinguish and we have confirmed other reports on the limited utility of CK19 and galectin-3 concerning follicular-patterned lesions.[17,28,29] The best marker, in terms of sensitivity and specificity, was HBME-1 that stained 63.1% of the FCs but also 55% of the follicular adenomas and 33.3% of the adenomatous nodules. Also, galectin-3 showed reduced expression in benign thyroid lesions which was always focal, restricted to a few cells (Table 3). Unfortunately, half of the positive FCs also presented a focal staining pattern.

An interesting result was the strong and diffuse positivity for HBME-1 observed in our case of hyalinizing trabecular tumour in contrast to negative CK19 and galectin-3 immunoreactivity. The classification and biological behaviour of this particular type of tumour has been causing considerable interest recently. This tumour has been regarded as a variant of PC, since *RET* rearrangements have been demonstrated by

Table 3. Immunohistochemical expression of galactin-3 in neoplastic and non-neoplastic thyroid lesions

Diagnosis	No. of cases	Galactin-3+ (%)	Positivity pattern, predominant/secondary (%)
Carcinomas			
PC	84	61 (72.6)	D (73.8)/F+ (16.4)
FC	38	8 (21)	F++, 50/F+ (50)
MC	5	0 (0)	–
AC	2	0 (0)	–
Normal	170	0 (0)	–
FA	18	2 (11)	F+ (100)
AN	12	1 (8.3)	F+ (100)
HT	10	7 (70)	F+ (100)

PC, Papillary carcinoma; FC, follicular carcinoma; MC, medullary carcinoma; AC, anaplastic carcinoma; FA, follicular adenoma; AN, adenomatous nodule; HT, Hashimoto's thyroiditis; D, diffuse reactivity; F++, focal segmental reactivity; F+, focal cellular reactivity.

Table 4. Immunohistochemical positivity for HBME-1, CK19 and galactin-3 in thyroid follicular lesions

		Positivity (%)			Positivity pattern (%) predominant		
Diagnosis	No. of cases	HBME-1	CK19	Galactin-3	HBME-1	CK19	Galactin-3
FVPC	25	21 (84)	13 (52)	13 (52)	D (90.4)	D (77)	D (77)
FC	38	24 (63)	8 (21)	8 (21)	D (50)	F++ (50	F++ (50)
FA	18	10 (55.6)	6 (33)	2 (11)	F++ (66.7)	F++ (66.6)	F+ (100)
AN	12	4 (33.3)	2 (16.7)	1 (8.4)	F+ (75)	F+ (100)	F+ (100)

FVPC, Follicular variant of papillary carcinoma; FC, follicular carcinoma; FA, follicular adenoma; AN, adenomatous nodule; D, diffuse reactivity; F++, focal segmental reactivity; F+, focal cellular reactivity.

RT-PCR and immunohistochemistry in many cases.[30] Gaffney *et al*. found 60% of the 58 hyalinizing trabecular tumours he examined to be negative or weakly (1+) stained and 40% strongly stained by galectin-3.[31] The positive expression of simple epithelial-type cytokeratins, including CK19, was found in all 11 cases studied by Fonseca *et al*., but only in the malignant tumours studied by Hirokawa.[27,32]

The immunoreactive distribution of the markers may be helpful in differential diagnosis. Although focal HBME-1, CK19 and galectin-3 staining may be found in benign lesions, diffuse positivity is characteristic of malignancy. For example, we have confirmed the focal and pale staining for CK19 in multinodular goitres or adenomatous nodules with papillary formations but diffuse and intense positivity in the cells of almost all cases of PC.[33]

In conclusion, our series documents the advantage of combining CK19, HBME-1 and galectin-3 expression and the distribution of immunoreactivity to differentiate the various histological types of thyroid malignancy.

Acknowledgements

We are indebted to the Laboratory of Experimental Pathology, FCM/UNICAMP and we are specially thankful to Mrs M. Matsura, who did all the immunohistochemical reactions. We are also indebted to the statistical team of the Faculty of Medical Sciences, FCM/UNICAMP for useful discussion and important suggestions. This work was supported by grants from FAPESP 03/02309-7 and 02/03827-9 and from CNPQ 470784/2003-2.

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