

Immunohistochemical Expression of HBME-1, E-cadherin, and CD56 in the Differential Diagnosis of Thyroid Nodules

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Summary. Background and Objective. Distinction between benign and malignant thyroid tumors is essential for proper clinical management. The aim of this study was to evaluate the diagnostic potential of a set of 3 molecular markers in the differential diagnosis of thyroid tumors.

Material and Methods. Immunohistochemistry for HBME-1, E-cadherin (E-CAD), and CD56 was carried out in 36 follicular adenomas, 77 colloid goiters, 36 papillary thyroid carcinomas, and 14 follicular carcinomas. Sixty-eight thyroid fine needle aspiration (FNA) cases confirmed by subsequent surgical resection specimens were selected. Immunocytochemistry for HBME-1, E-CAD, and CD56 was performed in these cases, including 25 papillary thyroid carcinomas, 1 follicular carcinoma, 22 follicular adenomas, and 20 colloid goiters.

Results. PTC was characterized by a decreased expression of E-CAD and CD56 contrary to the surrounding benign thyroid tissues. There was no HBME-1 expression in benign thyroid tissues, but it was high in papillary thyroid carcinomas and weak in follicular adenomas. The expression of E-CAD and CD56 was significantly higher in follicular adenomas than in the surrounding thyroid tissues. Analyzing the FNA material, HBME-1 expression was documented in 96% of papillary thyroid carcinomas, but there was no expression in the benign lesions. E-CAD and CD56 expression was significantly weakened in papillary thyroid carcinomas, but enhanced in follicular adenomas.

Conclusions. HBME-1 was found only in malignant lesions and can be considered the most sensitive, specific single marker in papillary thyroid carcinomas. CD56 and E-CAD can assist in the decision-making on the benign and malignant nature of the nodule. Immunocytochemistry is of value as an ancillary test to enhance the diagnostic accuracy of thyroid FNA samples.

Introduction

Thyroid nodules are a very frequent finding, and their prevalence steadily increases with age. Nodular thyroid disease refers to the presence of a solid nodule, a multinodular gland, or one or more cystic lesions. It is estimated that 5%–7% of adults have clinically detectable nodules in the thyroid, and with the emergence of modern ultrasonographic (US) techniques detecting thyroid nodules of a few millimeters, the frequency of nodularity is estimated to be 16%–67% in unselected subjects (1, 2).

Most of the discovered nodules are benign; however, there were approximately 44 000 estimated new cases of thyroid cancer and 1700 estimated deaths in the United States in 2010 (3). The annual incidence increased from 3.6 per 100 000 in 1973 to 8.7 per 100 000 in 2002 (2.4-fold increase), and this trend appears to be continuing. Mostly it is caused by an increase in the incidence of papillary thyroid carcinoma (PTC), which increased from 2.7 to 7.7 per

100 000 (2.9-fold increase) (4). More than 80% of the malignancies present in palpable thyroid nodules are PTC followed by follicular cancer (FC) and significantly less frequent anaplastic carcinomas. Thyroid cancer accounts for about 1%–2% of all malignancies and 90% of all neuroendocrine tumors (5).

The primary goal in the evaluation of the thyroid nodules is to distinguish those nodules that require surgical intervention from those that can be safely observed. Usually medical concerns according to the thyroid nodules revolve around 3 questions: 1) the presence of thyroid dysfunction; 2) the presence of malignancy; and 3) the likelihood of a progressive increase in size of the nodule eventually leading to symptoms (6).

Fine needle aspiration (FNA) has become the ultimate test in conjunction with clinical findings to select those patients who will benefit from surgery the most. FNA is now considered the most accurate, cost-effective, and simplest screening method for the fast diagnosis of thyroid nodules, with the accuracy being around 95% (7). It would be wrong to assume that the most precise quality of care is ob-

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tained by evaluating all thyroid nodules with FNA, apart from their size and their clinical presence (multinodular thyroid, single nodule, age of the patient, radiation history, etc.), since the probability for the presence of cytologically “suspicious” lesions is 20%. Considering this, most of the patients will eventually undergo a thyroidectomy to exclude the presence of cancer, which is present in 10%–20% of all microfollicular lesions. Hence, once the decision is made to do FNA biopsy of the nodule, the patient has an a priori probability of 10%–20% for a thyroidectomy, which is unnecessary in 80%–90% of the cases (6).

The differential diagnosis of thyroid nodules could be difficult due to the overlapping morphological features; therefore, many attempts have been described to find the additional criteria to distinguish thyroid pathologies in surgical material and in less explored FNA cytological specimens. A growing number of molecular or immunohistochemical (IHC) markers are being identified and tested with a considerable variability in the outcomes of these studies.

In this study, we evaluated the usefulness of applying the panel of 3 IHC markers – HBME-1, E-cadherin (E-CAD) and CD56 – on the histological samples of various thyroid lesions as well as on thyroid FNA samples to determine their diagnostic accuracy in the differential diagnosis of thyroid nodules.

Material and Methods

During the first stage of the study, 163 thyroidectomy specimens were selected consecutively from the files of the Institute of Pathology, Pauls Stradins Clinical University hospital, Riga, Latvia, between 2006 and 2011. After the histological verification of diagnosis, the study group consisted of 50 malignant and 113 benign thyroid lesions including 36 PTCs and 14 FCs as well as 36 FAs and 77 colloid goiters (CGs).

The inclusion criteria were as follows: differentiated thyroid cancer originating from follicular epithelial cells including all types of papillary thyroid cancer and follicular cancer, except a Hürthle cell variant; unequivocal morphological findings; enough archival paraffin-embedded tissue material for analysis; and the period from operation to immunohistochemical investigation not exceeding 8 years.

During the second stage of the study, 68 thyroid FNA cases confirmed by subsequent surgical resection specimens during the period of 2010–2011 were selected from the Institute of Pathology, Pauls Stradins Clinical University Hospital, Riga, Latvia. The study group consisted of 26 malignant and 42 benign thyroid lesions including 25 PTC and 1 FC as well as 22 FA and 20 cases of CG.

The inclusion criteria in the second stage of the study were as follows: available FNA smear as well as operation material for the same case according to the abovementioned inclusion criteria and the period of less than one year from FNA biopsy of the thyroid to operation and final morphology.

The institutional Ethics Committee approved the study. Written consent was obtained from the patients before the FNA procedure.

The tissue processing and the general histological report were performed as described previously by Ozolins et al. (8). During the screening, the archival diagnoses were verified by the examination of these slides by an independent pathologist. The diagnostics, typing, and grading of thyroid pathology were performed according to the World Health Organization Classification of Tumors (9) by an independent reviewer experienced in thyroid pathology. Only cases with an unequivocal histological diagnosis were included in the study group.

For immunophenotypic studies, formalin-fixed and paraffin-embedded tissue specimens were cut in 3- μ m-thick sections on electrostatically charged Histobond glass slides and incubated at 60°C for 1 hour to ensure the tissue adhesion to the slides. Deparaffining and rehydration were carried out by routine treatment in xylene for 4 \times 5 minutes and graded ethanol for 2 \times 3 minutes (99.9%), 4 \times 3 minutes (96%), and 5 min (70%). Endogenous peroxidase activity was blocked by 0.5% hydrogen peroxide in methanol for 10 minutes. All chemicals were produced by Sigma-Aldrich (Steinheim, Germany).

After rinsing in the TBS buffer (pH 7.6, Tris-buffered saline, THAM-HCl 50 mmol/L, NaCl 150 mmol/L) for 5 minutes, the slides were subjected to heat-induced antigen retrieval (HIER) treatment in a domestic microwave oven for 3 \times 5 minutes at maximum power in basic buffer (TEG, pH 9.0, 10 mmol/L Tris base, 0.5 mmol/L EGTA). After the HIER, the slides were allowed to cool at room temperature for 20 minutes in the HIER buffer.

The slides were encircled with a Dako pen (Dako, Glostrup, Denmark) and transferred to magnetic immunostaining trays (CellPath plc, Newtown, UK). After the rinse with TBS buffer for 5 minutes, the incubation with primary antibodies was carried out at room temperature for 60 minutes. The following monoclonal antibodies were used: HBME-1 (clone HBME-1; 1:50; Dako, Glostrup, Denmark), E-CAD (clone NCH-38; 1:50 Dako, Glostrup, Denmark), and CD56 (clone 123C3; 1:100; Dako, Glostrup, Denmark).

Unbound primary antibodies later were removed by repeated rinses with TBS buffer for 2 \times 5 minutes. A commercially available polymeric EnVision+ System, bound with horseradish peroxidase (Dako), was used for visualization. The slides were

incubated in a humid chamber for 30 minutes with EnVision+ with following rinses in TBS for 2×5 minutes. The color developed after incubation with 3,3-diaminobenzidine (Dako) for 10 minutes. The slides then were rinsed in water and counterstained with hematoxylin for 3 minutes. After color development in tap water for 5 minutes, the slides were cover-slipped using an aqueous mounting medium Faramount (Dako). The slides of positive and negative control were included in each run.

The membranous expression of HBME-1, E-CAD, and CD56 was scored semiquantitatively by the intensity of staining and percentage of positive cells. The staining intensity was estimated as negative (0), weakly positive (1), moderately positive (2), or positive (3). To evaluate the expression of the considered markers in the entire analyzable tissue, the expression intensity was computed as the multiplication of the percentage of positive cells by staining intensity. The lesion was considered positive for a marker when the expression intensity was at least 1.5.

Fine Needle Aspiration and Immunocytochemistry. US followed by FNA was performed by one experienced radiologist using a GE Voluson E8 ultrasound machine and a 11L-D linear transducer at the Institute of Diagnostic Radiology, Pauls Stradins Clinical University Hospital, Riga, Latvia.

In case of a single thyroid nodule, only nodules greater than 1 cm were further evaluated by FNA. In a multinodular gland, either the dominant nodule or the nodule with the most suspicious US findings was evaluated. The following US characteristics associated with a higher risk of malignancy were considered: marked hypoechoogenicity compared with normal thyroid parenchyma, microcalcifications, irregular or microlobulated margins, increased intranodular vascularity, and a elongated shape characterized by higher height exceeding width.

During the FNA procedure, patients were placed in the supine position; the puncture site was prepared under sterile conditions and draped. A US probe was covered under sterile conditions and disinfected with a Cutasept F solution. Lidocaine, a local anesthetic (1.0 mL, 20 mg/mL), was used. A

21-gauge needle was attached to a 20-mL syringe. Under the real-time visualization, the needle tip was introduced into suspicious nodules. To generate an appropriate negative pressure, the sucker of syringe was pulled out for 5–10 mL. A minimum of 2 passes was employed. The placement of the needle was documented by taking pictures. The aspirated material was placed, smeared on Histobond adhesive glass slides, and air-dried.

For immunophenotypic studies, the cell smears were air-dried and fixed in 96% ethanol for 10 minutes. Further immunophenotypic testing was done as described previously.

The statistical evaluation of the data was carried out using the Statistical Package for Social Sciences (SPSS® version 17.0) and Microsoft® Excel for Mac 2011 programs. In the present study, descriptive statistics was used as well as 95% confidence interval for a single proportion and a mean as described by Altman et al. (10). The calculations of confidence intervals were made by the Confidence Interval Analysis (CIA) software. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated as described by Altman et al. (10).

Results

The results of immunohistochemical staining in benign and malignant thyroid lesions are summarized in Table 1. The expression of HBME-1 was absent in benign thyroid lesions including FA, CG, and pericancerous tissue, but was notably high in PTC (Fig. 1) with a mean intensity of 2.80 (95% CI, 2.68–2.91) and FC (Fig. 2) with a mean intensity of 0.90 (95% CI, 0.09–1.70). To distinguish PTC from the tissue surrounding PTC, the sensitivity, specificity, PPV, and NPV for HBME-1 was 1.00. To differentiate FA from PTC, the sensitivity, specificity, PPV, and NPV were 1.00, 0.96, 0.96, and 1.00, respectively. HBME-1 had an extremely high value in the differential diagnostics of PTC showing a high ability to discriminate between PTC and benign tissues.

The expression of E-CAD was significantly higher in FA than in the surrounding thyroid tis-

Table 1. Intensity of Immunohistochemical Marker Expression in Thyroid Lesions

Target	HBME-1		E-CAD		CD56	
	Mean (SD)	95% CI	Mean (SD)	95% CI	Mean (SD)	95% CI
FA	0.09 (0.37)	0–0.22	2.20 (0.82)	1.92–2.48	2.20 (0.92)	1.88–2.51
TtS FA	0.001 (0.003)	0–0.002	0.63 (0.71)	0.39–0.87	0.95 (0.77)	0.69–1.21
PTC	2.80 (0.33)	2.68–2.91	0.55 (0.61)	0.34–0.75	0.20 (0.19)	0.13–0.26
TtS PTC	0.006 (0.03)	0–0.016	1.60 (0.85)	1.30–1.90	1.02 (0.47)	0.86–1.18
CG	0	0	1.39 (0.69)	1.23–1.54	0.85 (0.63)	0.70–1.00
FC	0.90 (1.40)	0.09–1.70	2.10 (0.80)	1.64–2.60	2.30 (1.00)	1.72–2.90

FA, follicular adenoma; TtS FA, thyroid tissue surrounding FA; PTC, papillary thyroid carcinoma; TtS PTC, thyroid tissue surrounding PTC; CG, colloid goiter; FC, follicular cancer; CI, confidence interval.

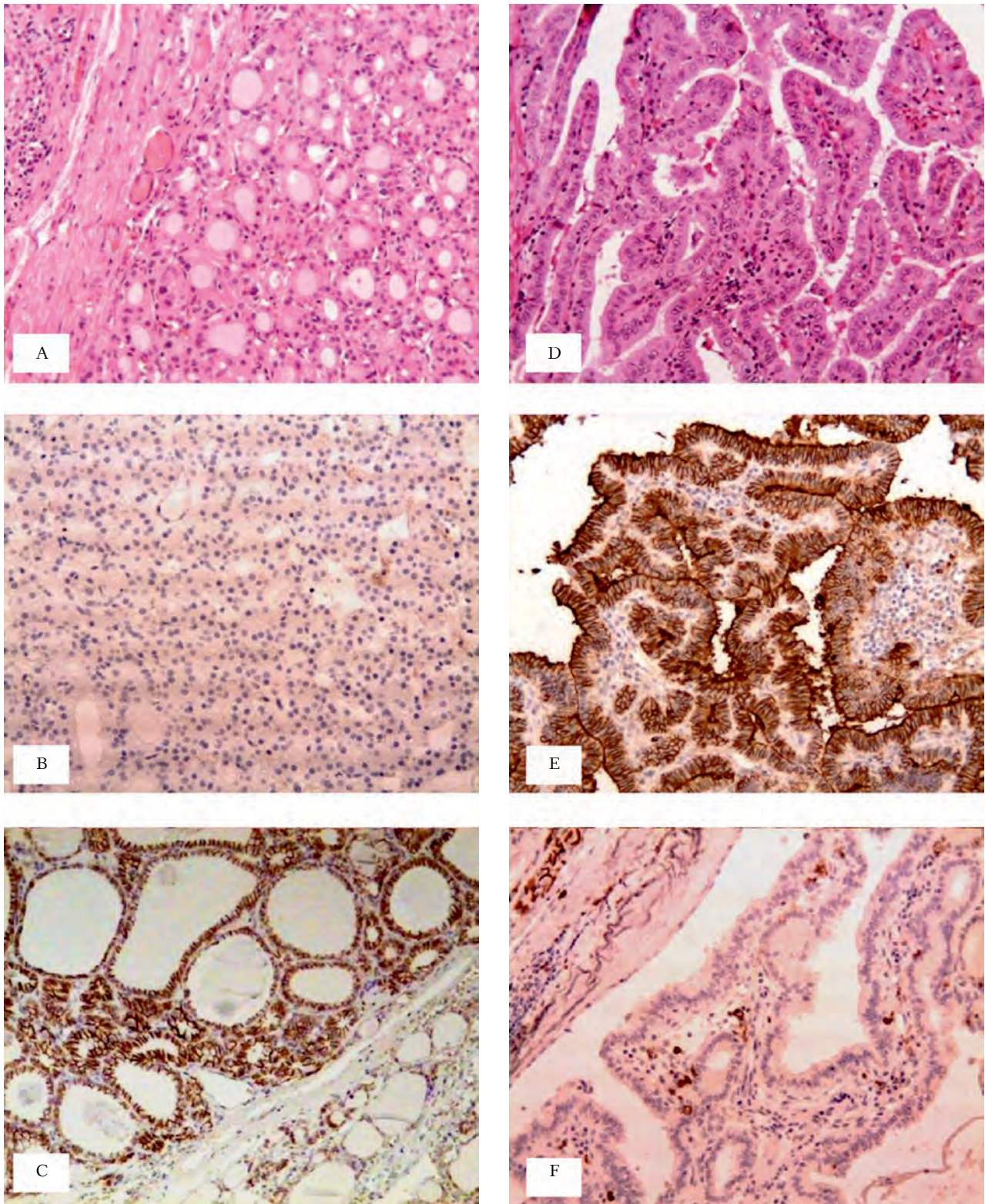


Fig. 1. Morphologic structure and immunophenotype of follicular adenoma (A–C) in contrast to papillary thyroid carcinoma (D–F) A, tissue structure of follicular adenoma. Note the presence of capsule (hematoxylin-eosin [HE], original magnification [OM] $\times 100$); B, lack of HBME-1 in follicular adenoma (immunoperoxidase [IP], anti-HBME-1, OM $\times 100$); C, intense expression of CD56 in follicular adenoma (IP, anti-CD56, OM $\times 100$); D, tissue structure of papillary thyroid carcinoma. Note the characteristic architecture and nuclear structure (HE, OM $\times 100$); E, intense expression of HBME-1 in papillary thyroid cancer (IP, anti-HBME-1, OM $\times 100$); F, lack of CD56 expression in papillary thyroid carcinoma (IP, anti-CD56, OM $\times 100$).

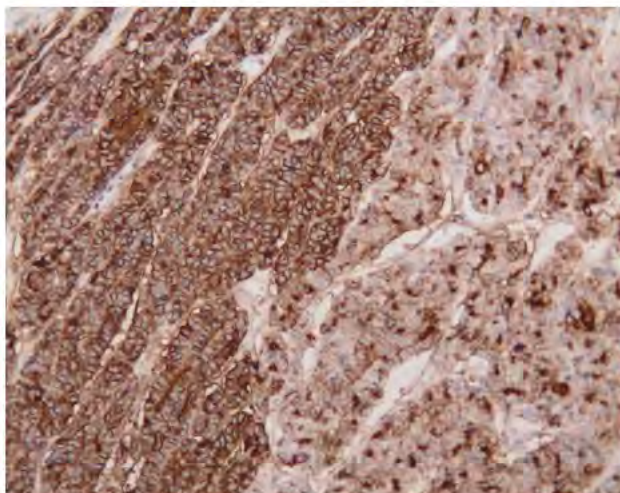


Fig. 2. Intense heterogeneous expression of HBME-1 in follicular cancer (immunoperoxidase, anti-HBME-1, original magnification $\times 100$)

sue. FC also showed a high expression of E-CAD. In contrast, PTC showed an extremely low expression of E-CAD (0.55; 95% CI, 0.34–0.75). The sensitivity, specificity, PPV, and NPV for E-CAD to distinguish FA from the tissue surrounding FA were 0.89, 0.87, 0.88, and 0.87, respectively. The sensitivity, specificity, PPV, and NPV for E-CAD to differenti-

ate FA from PTC were 0.88, 0.88, 0.91, and 0.85, respectively.

The expression of CD56 as well as E-CAD was significantly higher in FA than in the surrounding thyroid tissue (Fig. 1C). PTC showed an extremely low expression of CD56 compared with the surrounding tissue (Fig. 1F). FC similarly to FA showed a very high expression of CD56. The sensitivity, specificity, PPV, and NPV for CD56 to distinguish FA from the tissue surrounding FA were 0.82, 0.87, 0.88, and 0.87, respectively. For differentiating FA from PTC, the sensitivity and specificity for CD56 were 0.82 and 1.00, but the PPV and the NPV were 1.00 and 0.81, respectively.

Unfortunately, there was no possibility to systematically analyze the expression of those immunohistochemical markers in thyroid tissue surrounding FC due to its widespread invasion.

The results of immunocytochemical staining in benign and malignant thyroid lesions are summarized in Table 2. Regarding FA ($n=22$), E-CAD (Fig. 3A) was expressed in 72.7% (95% CI, 50.0%–88.0%) and CD56 (Fig. 3B) was expressed in 54.5% (95% CI, 32.6%–75.0%) of cases. The expression of HBME-1 was documented in 96% (95% CI, 77.0%–99.0%) of PTC cases, but there was no expression in FA, CG, and the only case of FC. The expres-

Table 2. Immunocytochemical Marker Expression in Thyroid Lesions

Marker	FA (n=22)		CG (n=20)		PTC (n=25)		FC (n=1)	
	Expression n (%)	95% CI	Expression n (%)	95% CI	Expression n (%)	95% CI	Expression n (%)	95% CI
HBME-1	0 (0)	0–18.5	0 (0)	0–20.0	24 (96)	77.0–99.0	0 (0)	0–95.0
E-CAD	16 (72.7)	50.0–88.0	2 (10)	1.75–33.1	2 (8)	1.40–27.5	0 (0)	0–95.0
CD56	12 (54.5)	32.6–75.0	1 (5)	0.26–27.0	1 (4)	0.21–22.3	0 (0)	0–95.0

FA, follicular adenoma; CG, colloid goiter; PTC, papillary thyroid carcinoma; FC, follicular cancer; CI, confidence interval.

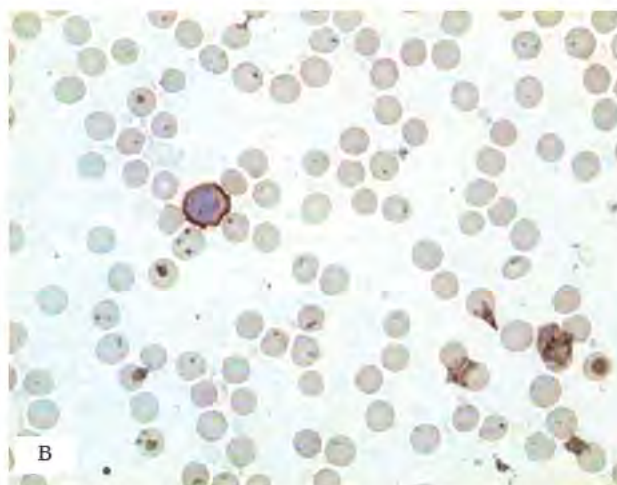
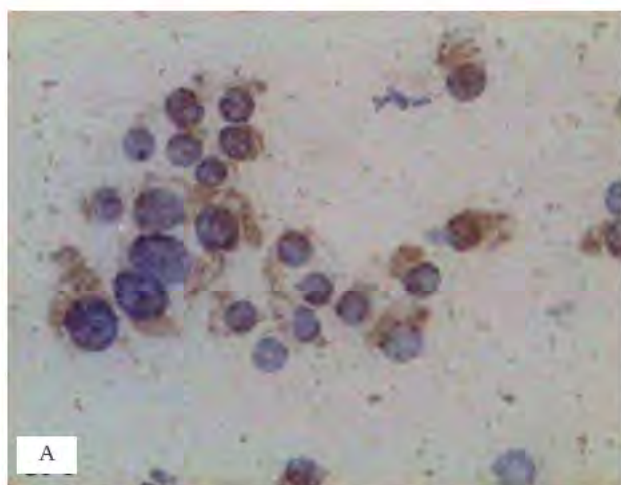


Fig. 3. Selected immunocytochemical findings in FNA smears

A, cytoplasmic expression of E-CAD in a group of thyroid epithelial cells (immunoperoxidase, anti-E-CAD, magnification $\times 400$); B, intense membranous expression of CD56 in a single epithelial cell despite the abundance of red blood cells in the clearly suboptimal smear (immunoperoxidase, anti-CD56, magnification $\times 400$).

sion of E-CAD and CD56 was observed in 8% (95% CI, 1.40%–27.5%) and 4% (95% CI, 0.21%–22.3%) of PTC cases, respectively. Regarding CG (n=20), 10% and 5% of cases were positive for E-CAD and CD56, respectively.

Discussion

Thyroid nodules are common lesions in clinical practice, but only a minority is malignant or suspicious tumors that require surgery. As early as in 1982, it was stated that FNA is the most sensitive and specific test for the diagnosis of thyroid nodules. In fact, the present consensus is that thyroid FNA biopsy is the procedure of choice for evaluation of nodules therefore cytologic interpretation can play a very important role in further clinical management of the patient. There are well-known limitations in the role of thyroid FNA, most importantly its inability to differentiate benign from malignant follicular neoplasms, since this diagnosis rests on the histologic identification of capsular and/or vascular invasion (11, 12). Immunohistochemistry was introduced to the practice of pathology in the early 1970s; however, in a thyroid pathology, originally its use is restricted. New techniques have been introduced to the thyroid FNA procedure to enhance its diagnostic value and improve the accuracy. Despite these improvements, many authors have concluded that even in “the right hands,” the rate of inadequate smears is rarely lower than 10% and there remain difficulties in the cytologic diagnosis of follicular-derived lesions. For this reason, the characterization of follicular thyroid nodules is widely considered as the “gray zone of FNA cytology.” Much attention has shifted to the identification of molecular or IHC markers that can help distinguish adenomatous colloid nodules or a follicular adenoma from a follicular carcinoma on the one hand and PTC from a follicular neoplasm on the other hand (13).

The diagnosis of PTC in FNA specimens is usually unsophisticated when classic cytologic features are present. However, in clinical practice, often it is difficult to make an unequivocal cytologic diagnosis of PTC. The diagnostic difficulties are related to the observation that some of typical cytologic features of PTC (nuclear grooves, giant cells, psammoma bodies, and papillary fragments) can also be observed in nonneoplastic lesions and follicular neoplasms of the thyroid (14).

During the last several years, a large number of IHC markers have been tested in histologic samples and, to a lesser extent, in FNA samples with variable success rates. The evaluation of IHC markers was mostly performed in surgically resected thyroid specimens. However, similar studies were also done on FNA cytologic specimens using cellblock prepara-

tions (15, 16). In general, the studies have shown similar results of markers expression comparing surgical specimens and FNA cellblock sections.

According to the published studies, many IHC markers have been investigated for the evaluation of thyroid nodules. However, only few markers have emerged as potentially useful for differentiating benign from malignant thyroid nodules. Considering this information, we chose to test these most promising markers (HBME-1, CD56, and E-CAD) on histological samples of benign and malignant thyroid lesions. Within the next step of our study, we tested the possibility to use these markers for the evaluation of FNA cytology material of the thyroid.

Anti-HBME-1, first described in 1992 by Battifora et al., is a monoclonal antibody directed against an antigen of the microvillous surface of mesothelioma cells (17). Most studies using HBME-1 for the diagnosis of thyroid tumors have been performed in tissue samples, and only few studies using FNA products have been carried out in cellblocks or thin layers (18, 19). Our findings showed that PTCs had a high-level immunoreexpression of HBME-1 and FC had a weak expression in the tissue samples. A high level of HBME-1 immunoreexpression was also documented in the FNA samples. Unfortunately, there was only one case of FC in the FNA study group. No HBME-1 immunoreexpression was observed in benign lesions. This agrees with the study by Nasr et al. (20), who reported HBME-1 to be the most sensitive and specific marker, staining 96% of PTCs, whereas normal thyroid tissues were consistently negative for HBME-1. A study by Saleh et al. (21) showed that the sensitivity and specificity of immunoreexpression for HBME-1 to distinguish benign from malignant lesions was one of the highest among all the markers tested (88.9% versus 72.7%, respectively). Several recent investigators have reported a high expression of HBME-1 in PTCs and some FCs, but a low or negative expression in FAs (20, 21).

E-CAD is a transmembrane glycoprotein, which is present in most epithelial cells and appears to play an important role in epithelial integrity, cell adhesion and differentiation, and the maintenance of cell polarity and tissue architecture. Its impairment correlates with the development and progression of human carcinomas. The expression of E-CAD in the normal thyroid was first described by Eidelman et al. (22). Since then, it is known that a decrease in the E-CAD expression is associated with thyroid neoplasms (23). In our study, PTCs demonstrated a significant reduction in the E-CAD expression when compared with FAs or the thyroid tissue surrounding PTCs. In contrast, FCs showed no significant differences in the E-CAD expression compared

with FAs. As mentioned before, in the second stage of the study, there was only one case of FC to analyze. The low and heterogenous expression of E-CAD in PTCs confirms the results of Soares et al. (24) and Mitselou et al. (25), who observed a reduction of E-CAD expression in PTC compared to normal thyroid tissue. Contrary to the abovementioned studies as well as to our study, Smyth et al. demonstrated a significant reduction in the E-CAD expression in FCs compared with FAs, but no significant decrease in expression in case of PTCs (26).

CD56 is a neural cell adhesion molecule that is present on follicular epithelial cells of the normal thyroid (27). Our results showed a positive immunorexpression of CD56 in thyroid lesions and tumors except for PTCs where it was remarkably decreased. These findings are very similar to the results reported by Scarpino et al. showing a low or no expression of CD56 in PTCs using immunohistochemistry (28). Using the immunocytochemical staining of CD56, our results showed its expression mainly in FA. El Demellawy et al. showed CD56 as an extremely useful marker in the distinction between PTCs and follicular lesions or neoplasms. According to their results, a diffuse expression of CD56 was

present in normal and neoplastic follicular epithelium, but was absent in PTCs (29, 30).

Conclusions

Both in the histological evaluation of formalin-fixed, paraffin-embedded tissues and in the cytological examination of FNA, an intense positive reaction of HBME-1 was observed only in malignant lesions. CD56 and E-CAD can assist in the decision-making about the benign and malignant nature of aspirated material. The panel consisting of 3 immunohistochemical markers – HBME-1, E-CAD, and CD56 – can aid both FNA diagnostics and evaluation of surgically removed thyroid tissue and can be recommended as an adjunct to morphological criteria.

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Statement of Conflicts of Interest

The authors state no conflict of interest.

References

- Gharib H, Papini E. Thyroid nodules: clinical importance, assessment, and treatment. *Endocrinol Metab Clin North Am* 2007;36(3):707-35.
- Tan GH, Gharib H. Thyroid incidentalomas: management approaches to nonpalpable nodules discovered incidentally on thyroid imaging. *Ann Intern Med* 1997;126(3):226-31.
- Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. *CA Cancer J Clin* 2010;60(5):277-300.
- Davies L, Welch HG. Increasing incidence of thyroid cancer in the United States, 1973–2002. *JAMA* 2006;295(18):2164-7.
- Hundahl SA, Fleming ID, Fremgen AM, Menck HR. A National Cancer Data Base report on 53,856 cases of thyroid carcinoma treated in the U.S., 1985–1995. *Cancer* 1998;83(12):2638-48.
- Procopiou M, Meier AC. Evaluation of thyroid nodules. In: Oertli D, Udelsman R, editors. *Surgery of the thyroid and parathyroid glands*. New York: Springer; 2007. p. 45–57.
- Wu HH, Jones JN, Osman J. Fine-needle aspiration cytology of the thyroid: ten years experience in a community teaching hospital. *Diagn Cytopathol* 2006;34(2):93-6.
- Ozolins A, Narbutis Z, Strumfa I, Volanska G, Gardovskis J. Diagnostic utility of immunohistochemical panel in various thyroid pathologies. *Langenbecks Arch Surg* 2010;395(7):885-91.
- DeLellis RA. Pathology and genetics of thyroid carcinoma. *J Surg Oncol* 2006;94(8):662-9.
- Altman D, David M, Trevor B, Martin G. *Statistics with confidence – confidence intervals and statistical guidelines*. Bristol: BMJ Books; 2000.
- Shih SR, Shun CT, Su DH, Hsiao YL, Chang TC. Follicular variant of papillary thyroid carcinoma: diagnostic limitations of fine needle aspiration cytology. *Acta Cytol* 2005;49(4):383-6.
- Wu HH, Jones JN, Grzybicki DM, Elsheikh TM. Sensitive cytologic criteria for the identification of follicular variant of papillary thyroid carcinoma in fine-needle aspiration biopsy. *Diagn Cytopathol* 2003;29(5):262-6.
- Filie AC, Asa SL, Geisinger KR, Logani S, Merino M, Nikiforov YE, et al. Utilization of ancillary studies in thyroid fine needle aspirates: a synopsis of the National Cancer Institute Thyroid Fine Needle Aspiration State of the Science Conference. *Diagn Cytopathol* 2008;36(6):438-41.
- Baloch ZW, LiVolsi VA, Asa SL, Rosai J, Merino MJ, Randolph G, et al. Diagnostic terminology and morphologic criteria for cytologic diagnosis of thyroid lesions: a synopsis of the National Cancer Institute Thyroid Fine-Needle Aspiration State of the Science Conference. *Diagn Cytopathol* 2008;36(6):425-37.
- Fadda G, Rossi ED, Mule A, Miraglia A, Vecchio FM, Capelli A. Diagnostic efficacy of immunocytochemistry on fine needle aspiration biopsies processed by thin-layer cytology. *Acta Cytol* 2006;50(2):129-35.
- Rossi ED, Raffaelli M, Minimo C, Mule A, Lombardi CP, Vecchio FM, et al. Immunocytochemical evaluation of thyroid neoplasms on thin-layer smears from fine-needle aspiration biopsies. *Cancer* 2005;105(2):87-95.
- Sack MJ, Astengo-Osuna C, Lin BT, Battifora H, LiVolsi VA. HBME-1 immunostaining in thyroid fine-needle aspirations: a useful marker in the diagnosis of carcinoma. *Mod Pathol* 1997;10(7):668-74.
- de Micco C, Savchenko V, Giorgi R, Sebag F, Henry JF. Utility of malignancy markers in fine-needle aspiration cytology of thyroid nodules: comparison of Hectol Battifora mesothelial antigen-1, thyroid peroxidase and dipeptidyl aminopeptidase IV. *Br J Cancer* 2008;98(4):818-23.
- Schmitt AC, Cohen C, Siddiqui MT. Paired box gene 8, HBME-1, and cytokeratin 19 expression in preoperative fine-needle aspiration of papillary thyroid carcinoma: diagnostic utility. *Cancer Cytopathol* 2010;118(4):196-202.

20. Nasr MR, Mukhopadhyay S, Zhang S, Katzenstein AL. Immunohistochemical markers in diagnosis of papillary thyroid carcinoma: utility of HBME1 combined with CK19 immunostaining. *Mod Pathol* 2006;19(12):1631-7.
21. Saleh HA, Feng J, Tabassum F, Al-Zohaili O, Husain M, Giorgadze T. Differential expression of galectin-3, CK19, HBME1, and Ret oncoprotein in the diagnosis of thyroid neoplasms by fine needle aspiration biopsy. *Cytojournal* 2009;6:18.
22. Eidelman S, Damsky CH, Wheelock MJ, Damjanov I. Expression of the cell-cell adhesion glycoprotein cell-CAM 120/80 in normal human tissues and tumors. *Am J Pathol* 1989;135(1):101-10.
23. von Wasielewski R, Rhein A, Werner M, Scheumann GF, Dralle H, Potter E, et al. Immunohistochemical detection of E-cadherin in differentiated thyroid carcinomas correlates with clinical outcome. *Cancer Res* 1997;57(12):2501-7.
24. Soares P, Berx G, van Roy F, Sobrinho-Simoes M. E-cadherin gene alterations are rare events in thyroid tumors. *Int J Cancer* 1997;70(1):32-8.
25. Mitselou A, Ioachim E, Peschos D, Charalabopoulos K, Michael M, Agnantis NJ, et al. E-cadherin adhesion molecule and syndecan-1 expression in various thyroid pathologies. *Exp Oncol* 2007;29(1):54-60.
26. Smyth P, Sheils O, Finn S, Martin C, O'Leary J, Sweeney EC. Real-time quantitative analysis of E-cadherin expression in ret/PTC-1-activated thyroid neoplasms. *Int J Surg Pathol* 2001;9(4):265-72.
27. Zeromski J, Bagnasco M, Paolieri F, Dworacki G. Expression of CD56 (NKH-1) differentiation antigen in human thyroid epithelium. *Clin Exp Immunol* 1992;89(3):474-8.
28. 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.
29. El Demellawy D, Nasr A, Alowami S. Application of CD56, P63 and CK19 immunohistochemistry in the diagnosis of papillary carcinoma of the thyroid. *Diagn Pathol* 2008;3:5.
30. El Demellawy D, Nasr AL, Babay S, Alowami S. Diagnostic utility of CD56 immunohistochemistry in papillary carcinoma of the thyroid. *Pathol Res Pract* 2009;205(5):303-9.

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