Diagnostic value of HBME-1, CD56, Galectin-3 and Cytokeratin-19 in papillary thyroid carcinomas and thyroid tumors of uncertain malignant potential

ADELA NECHIFOR-BOILĂ¹, RAMONA CĂTANĂ¹, ANDRADA LOGHIN¹, TATIANA GEORGIANA RADU², ANGELA BORDA¹

¹Department of Histology, University of Medicine and Pharmacy of Tirgu Mures, Romania
²PhD student in Histology, University of Medicine and Pharmacy of Craiova, Romania

Abstract

Aim: We aimed to evaluate four immunohistochemical markers (HBME-1, Galectin-3, Cytokeratin-19 and CD56) used alone or in panels in a series of papillary thyroid carcinoma (PTC) and thyroid tumors of uncertain malignant potential (TT-UMP) cases. Materials and Methods: We performed an immunohistochemical analysis on a tissue micro-array of 11 PTCs [six classic (CPTC), five follicular variant (FVPTC)] and 31 TTs-UMP. A control group of 11 benign thyroid lesions/tumors was also included. Results: CD56, whose expression is reduced or absent in thyroid carcinomas, was the most sensitive marker (81.8%), showing a “malignant” profile in 5/6 CPTCs and 4/5 FVPTCs. It was followed by HBME-1 (63.6% sensitivity), Cytokeratin-19 and Galectin-3 were the least sensitive antibodies (45.6%), but the most specific ones (100%). Three panels consisting of CD56 and/or Cytokeratin-19/Galectin-3 and HBME-1 and/or CD56 reached the highest sensitivity (90.9%) and the highest negative predicting value (87.5 and 83.3, respectively). In TTs-UMP, Cytokeratin-19, Galectin-3, HBME-1 and CD56 stained negatively in most of the cases (90.3%, 83.9%, 87.1% and 61%, respectively) and no statistically significant differences compared to the benign thyroid lesions’ immunoprofile could be observed. Conclusions: New panels of antibodies, consisting of CD56 and/or Cytokeratin-19/Galectin-3 and CD56 and/or HBME-1 that were found to be highly sensitive for PTC in our study, are reported. Applying these panels to TTs-UMP seems also useful. Our results showed that these tumors have an immunoprofile similar to the benign thyroid lesions, suggesting that they are most likely to have a benign rather than a malignant biological behavior.

Keywords: thyroid cancer, papillary, thyroid tumors of uncertain malignant potential, immunohistochemistry, HBME-1 antigen, CD56 antigen.

Introduction

Thyroid nodules are extremely common in the general population and are usually discovered during routine medical care. It is estimated that up to 7% of the general population develops clinically palpable thyroid nodules [1]. However, the widely spread of new and more sophisticated diagnostic techniques, such as ultrasonography and fine-needle aspiration biopsy has led to the discovery of an infra-clinical reservoir of thyroid nodules in 20 to 76% of the general population [2]. Most thyroid nodules are benign, while thyroid cancer represents only 5–24%. However, increase in thyroid cancer incidence has been reported along the time [3–5]. Papillary thyroid carcinoma (PTC) is by far the most common type of thyroid malignancy (85%) and it is characterized by a distinctive set of nuclear features [6].

In the great majority of cases, the pathological diagnosis of surgically removed thyroid nodules is possible by morphological examination in routine Hematoxylin–Eosin (HE) stained sections. However, there are cases in which pathological criteria do not allow the differentiation between benign and malignant follicular-patterned thyroid tumors/lesions, making the distinction between these two groups quite subtle and challenging [7, 8]. Consequently, some tumors are required to be included in the category of thyroid tumors of uncertain malignant potential (TTs-UMP). TT-UMP is a newly described, controversial entity, referring to a borderline lesion that shows questionable, incomplete PTC-nuclear features and/or capsular and vascular invasion [9]. Large intra- and inter-observer variability among expert pathologists in the diagnosis of these lesions is well documented in the literature [10–12].

All of these diagnostic dilemmas have important consequences for the management and prognosis of these patients. Several studies have shown that immunohistochemistry may provide additional support in the evaluation and diagnosis of thyroid tumors/lesions. Hector Battifora Mesothelial Cell-1 (HBME-1), Galectin-3 (Gal-3) and Cytokeratin-19 (CK19) have been the most frequently used antibodies in thyroid pathology, although a wide range of sensibility and specificity values of these markers has been reported by different studies along the time [13–20]. CD56 is a newly reported, “promising” marker in thyroid pathology [20–25], but, to present date, literature data is few and inconsistent.

In this study, we aimed to determine the diagnostic value of HBME-1, Gal-3, CK19 and CD56, used alone and in different combinations, in a series of PTC and TT-UMP cases.
Materials and Methods

Selection of cases

A total of 55 cases were selected from the Department of Pathology, Emergency County Hospital of Tîrgu Mureș, Romania database between January 2006 and December 2012. These cases consisted of PTCs (six CPTCs and five FVPTCs) and TTs-UMP (33 cases), measuring more than 1 cm in the largest diameter and whose diagnosis were in accordance to the 2004 WHO criteria [6]. A control group of 11 benign thyroid lesions/tumors (follicular adenomas, nodular goiter and chronic autoimmune thyroiditis) was also included in the study.

HE-stained slides from all of the cases included in the study were reviewed by two pathologists (ANB and RC) to confirm the diagnosis and to classify the lesions into one of the study categories. Follicular adenoma was defined as a solitary, encapsulated, follicular-patterned thyroid tumor, showing no evidence of nuclear atypia and/or capsular and vascular invasion. The microscopic diagnosis of PTC was based exclusively on nuclear features: enlargement, overlapping, irregularity of the nuclear contours, grooves, clearing or a ground glass appearance and nuclear pseudo-inclusions. CPTCs revealed a characteristic papillary architecture, pure or admixed with a variable proportion of follicles. The tumors defined as FVPTC were composed of small to medium sized, irregularly shaped follicles, with characteristic PTC nuclear features in most of the cells lining these follicles and virtually no papillary structures. TTs-UMP were encapsulated tumors showing incompletely developed PTC-type nuclear changes and/or questionable capsular or vascular penetration.

Tissue microarray (TMA)

Five TMA blocks were constructed from archival, formalin-fixed, paraffin-embedded tissues (donor blocks) with the help of a tissue microarrayer (TMA Builder Kit, Histopathology Ltd., Hungary) (Figure 1). The TMAs were constructed by arraying 55 tissue cores of 3 mm diameter each into five low-density TMA blocks, representing 11 benign thyroid lesions (one TMA block), 11 PTCs (one TMA block) and 33 TTs-UMP (three TMA blocks).

Immunohistochemical technique

The immunohistochemical technique was performed on 4-µm thick sections of the TMA blocks using the labeled Streptavidin–Biotin peroxidase complex system from a commercially available kit (DAKO LSAB2 System-HRP Kit). Heat induced antigen retrieval was carried out for each antibody at 98°C using specific buffers. Sections were then incubated with the primary antibodies for 30 to 60 minutes at room temperature, depending on the antibody. Positive controls were used for each antibody (Table 1).

Table 1 – Characteristics of the antibodies used in the study

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone</th>
<th>Host species</th>
<th>Antigen retrieval (buffer)</th>
<th>Dilution</th>
<th>Incubation time</th>
<th>Positive control</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBME-1</td>
<td>HBME-1</td>
<td>Mouse (monoclonal)</td>
<td>WB (EDTA)</td>
<td>1:50</td>
<td>60 minutes</td>
<td>CPTC</td>
<td>Dako, North America Inc., USA</td>
</tr>
<tr>
<td>Gal-3</td>
<td>9C4</td>
<td>Mouse (monoclonal)</td>
<td>WB (citrate, pH 6)</td>
<td>1:50</td>
<td>60 minutes</td>
<td>CPTC</td>
<td>Novocastra, Newcastle, UK</td>
</tr>
<tr>
<td>CK19</td>
<td>PCK108</td>
<td>Mouse (monoclonal)</td>
<td>WB (citrate, pH 6)</td>
<td>1:80</td>
<td>30 minutes</td>
<td>CPTC</td>
<td>Dako, Denmark</td>
</tr>
<tr>
<td>CD56</td>
<td>123C3</td>
<td>Mouse (monoclonal)</td>
<td>WB (Target Retrieval Solution)</td>
<td>1:20</td>
<td>30 minutes</td>
<td>NTT</td>
<td>Dako, Denmark</td>
</tr>
</tbody>
</table>

WB: Water bath; CPTC: Classic/conventional papillary thyroid carcinoma; NTT: Normal thyroid tissue.

Immunohistochemical evaluation

The following staining patterns were considered positive: HBME-1 membrane staining along the lateral and abluminal surfaces ± cytoplasmic and cytoplasmic and/or membrane expression of CK19 and Gal-3. A positive immunoreactivity was considered when at least 10% of the tumor cells stained positively for these antibodies. The intensity of staining was further evaluated using a scoring system, as following: “1”, “2”, “3” or “4” when 10–25%, 26–50%, 51–75% or 76–100% of the tumor cells showed positive expression, respectively.

Expression of CD56 was evaluated based on the membrane staining. Since CD56 is a marker whose expression is reduced or absent in thyroid carcinoma [26, 27], a “malignant” profile (or positive result) was considered when the tumor was entirely or almost entirely negative for this antibody (less than 10% of tumor cells stained positively).

The immunostaining results for all of the cases were assessed by three pathologists (ANB, AB and RC), and a consensus on the controversial cases was reached with the aid of a double-headed microscope.

The study was approved by the Ethical Committee of the University of Medicine and Pharmacy of Tîrgu Mureș.
Statistical analysis

Descriptive statistics were used to summarize the study data.

Sensitivity (true positive/true positive + false negative), specificity (true negative/false positive + false negative), positive predictive values (true positive/true positive + false positive) and negative predictive values (true negative/true negative + false negative) of each marker and their combinations were assessed both in PTC and FVPTC cases. When assessing the most valid panels of antibodies, a series of “positivity” rules were defined, ranging from considering a panel positive if at least one of the antibodies present in the panel was positive (or) to only considering a panel positive if at least two antibodies were positive (and).

As for TTs-UMP, the rates of positive results for each antibody in relation to the current reference examination (HE staining) were calculated, considering a minimal 10% positive threshold.

EpInfo Software version 3.5.3 (CDC, Atlanta, USA) was used for all statistical analysis. Association between categorical variables was evaluated by using the Fisher’s exact test or the chi-square test as appropriate and categorical variables was evaluated by using the Fisher’s exact test or the chi-square test as appropriate.

Results

Clinico-pathological characteristics

Our series consisted of 11 PTC cases, that included CPTCs (n=6) and FVPTCs (n=5), and 11 benign follicular-pattern thyroid lesions/tumors (five follicular adenomas, three autoimmune thyroiditis and three nodular goiter) (Table 2). All but one of the PTC cases were females, with a mean age of 46.7 and 35.6 years for CPTC and FVPTC cases, respectively. Half of the CPTC cases were associated with extrathyroidal extension ± lymph node metastasis, whereas none of the FVPTC cases was associated with these two conditions.

Thirty-three cases of TTs-UMP were also evaluated (Table 2). More than half of these cases were females (22 cases, 67%), and the patients’ mean age was 50.2 years. All of the tumors were encapsulated, follicular neoplasms, showing nuclear atypia that did not meet the criteria for PTC and/or incomplete capsular or vascular invasion.

Table 2 – Clinico-pathological data for the thyroid tumor cases included in the study

<table>
<thead>
<tr>
<th>F/M</th>
<th>Mean age (years)</th>
<th>Multifocality</th>
<th>Extrathyroidal extension</th>
<th>Lymph node metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>0/5</td>
<td>44</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>5/6</td>
<td>46.7</td>
<td>2/6</td>
<td>1/5</td>
<td>0/6</td>
</tr>
<tr>
<td>5/0</td>
<td>35.6</td>
<td>1/5</td>
<td>0/5</td>
<td>0/33</td>
</tr>
<tr>
<td>2/6</td>
<td>50.2</td>
<td>0/5</td>
<td>0/5</td>
<td>0/33</td>
</tr>
</tbody>
</table>

F/M: Female/male ratio; CPTC: Classic/conventional papillary thyroid carcinoma; FVPTC: Follicular variant of papillary thyroid carcinoma; TT-UMP: Thyroid tumor of uncertain malignant potential.

Table 3 – Immunohistochemical results using single antibodies in benign lesions, PTCs and TTs-UMP

<table>
<thead>
<tr>
<th>Benign lesions</th>
<th>PTC</th>
<th>TT-UMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK19</td>
<td>0/5</td>
<td>0/3</td>
</tr>
<tr>
<td>Gal-3</td>
<td>0/5</td>
<td>0/3</td>
</tr>
<tr>
<td>HMBE-1</td>
<td>2/5</td>
<td>1/3</td>
</tr>
<tr>
<td>CD56 (positive staining = negative result)</td>
<td>3/5</td>
<td>3/3</td>
</tr>
</tbody>
</table>

PTC: Papillary thyroid carcinoma; CPTC: Classic/conventional papillary thyroid carcinoma; FVPTC: Follicular variant of papillary thyroid carcinoma; TT-UMP: Thyroid tumor of uncertain malignant potential.

Table 4 – Sensitivity, specificity, PPV and NPV of the four markers, alone and in various combinations, in PTCs versus benign thyroid lesions

<table>
<thead>
<tr>
<th>Single marker</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK19</td>
<td>45.6%</td>
<td>100%</td>
<td>100</td>
<td>64.7</td>
</tr>
<tr>
<td>Gal-3</td>
<td>45.6%</td>
<td>90%</td>
<td>90</td>
<td>64.7</td>
</tr>
<tr>
<td>HMBE-1</td>
<td>63.6%</td>
<td>72.7%</td>
<td>70.0</td>
<td>66.7</td>
</tr>
<tr>
<td>CD56</td>
<td>81.8%</td>
<td>63.6%</td>
<td>69.2</td>
<td>77.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Double markers</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD56 and/or HMBE-1</td>
<td>90.9%</td>
<td>63.6%</td>
<td>62.5</td>
<td>83.3</td>
</tr>
<tr>
<td>CD56 and/or CK19/Gal-3</td>
<td>90.9%</td>
<td>63.6%</td>
<td>71.4</td>
<td>87.5</td>
</tr>
</tbody>
</table>

CK19: Cytokeratin-19; Gal-3: Galectin-3; PPV: Positive predictive value; NPV: Negative predicting value. For CD56, a marker whose expression is reduced or absent in thyroid carcinoma, a "malignant" profile or a positive result was considered when the tumor was entirely or almost entirely negative for this antibody (less than 10% of the tumor cells stained positively), while a "benign", negative result was considered when more than 10% of the tumor cells stained positively.
Table 5 – Sensitivity, specificity, PPV and NPV of HBME-1 and CD56, alone and in various combinations, in follicular variant of papillary thyroid carcinoma cases versus benign thyroid lesions/tumors

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD56*</td>
<td>83.3%</td>
<td>63.6%</td>
<td>55.6</td>
<td>63.6</td>
</tr>
<tr>
<td>HBME-1</td>
<td>66.6%</td>
<td>72.7%</td>
<td>57.1</td>
<td>80.0</td>
</tr>
<tr>
<td>CD56 and/or HBME-1</td>
<td>100%</td>
<td>45.5%</td>
<td>50.0</td>
<td>100</td>
</tr>
<tr>
<td>CD56 and/or CK19/Gal-3</td>
<td>83.3%</td>
<td>66.6%</td>
<td>60.0</td>
<td>100</td>
</tr>
<tr>
<td>CD56 and HBME-1*</td>
<td>60%</td>
<td>90%</td>
<td>60.0</td>
<td>100</td>
</tr>
</tbody>
</table>

PPV: Positive predictive value; NPV: Negative predicting value. *For CD56, a marker whose expression is reduced or absent in thyroid carcinoma, a “malignant” profile or a positive result was considered when the tumor was entirely or almost entirely negative for this antibody (less than 10% of the tumor cells stained positively). **When various combinations of markers were tested, Gal-3/CK19 formula was used because similar results were obtained in the panels containing either CK19 or Gal-3.

Table 6 – Expression of CK19, Gal-3, HBME-1 and CD56 in benign thyroid lesions/tumors versus thyroid tumors of uncertain malignant potential cases

<table>
<thead>
<tr>
<th></th>
<th>Benign lesions</th>
<th>TT-UMP</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK19</td>
<td>0/11 (0%)</td>
<td>3/31 (9.7%)</td>
<td>0.74</td>
</tr>
<tr>
<td>Gal-3</td>
<td>0/11 (0%)</td>
<td>5/31 (16.1%)</td>
<td>0.38</td>
</tr>
<tr>
<td>HBME-1</td>
<td>3/11 (27.3%)</td>
<td>4/31 (12.9%)</td>
<td>0.49</td>
</tr>
<tr>
<td>CD56 (positive staining = negative result)**</td>
<td>7/11 (63.6%)</td>
<td>19/31(61.3%)</td>
<td>0.80</td>
</tr>
</tbody>
</table>

TT-UMP: Thyroid tumor of uncertain malignant potential. *For CD56, a marker whose expression is reduced or absent in thyroid carcinoma, a “malignant” profile or a positive result was considered when the tumor was entirely or almost entirely negative for this antibody (less than 10% of the tumor cells stained positively), while a “benign”, negative result was considered when more than 10% of the tumor cell stained positively. **The p-value was calculated by comparing the percentage of positive benign thyroid lesions/tumors versus positive TTs-UMP.

Benign thyroid lesions

None of the benign thyroid cases (thyroid follicular adenomas, chronic autoimmune thyroiditis or nodular goiter) stained positively for CK19 and Gal-3, making these two antibodies 100% specific for differentiating benign thyroid lesions/tumors from PTC cases (Table 4). On the other hand, HBME-1 was positive in three and one thyroid follicular adenoma and chronic autoimmune thyroiditis cases, respectively, resulting thus in a lower specificity for HBME-1 (72.7%). CD56, a marker whose expression is reduced or absent in thyroid carcinoma, revealed a positive, benign immunostaining in 7/11 (63.6%) benign thyroid lesions/tumors (Table 3).

Papillary thyroid carcinoma

CD56 was the most sensitive marker in our study, with 5/6 CPTCs and 4/5 FVPTCs revealing a malignant profile for this antibody, it’s sensitivity in identifying PTC cases reaching up to 81.1% (Table 4). It was followed by HBME-1, with a 63.6 sensitivity (4/6 CPTCs and 3/5 FVPTCs positive cases). When assessing the staining intensity in HBME-1 positive PTC cases, the predominant score was 3, with strong, characteristic membrane staining, with apical reinforcement in more than 50% of the tumor cells (Figures 2B and 3B). CK19 and Gal-3 stained positively in 5/6 CPTCs, while no FVPTC case was immunoreactive for CK19 or Gal-3 (Table 4). The immunoreactivity for these markers was predominantly cytoplasmic and a 4 grade intensity score was assigned for most of the positive cases (positive staining in more than 75% of the tumor cells) (Figure 2, C and D).

Figure 2 – Classic/conventional papillary thyroid carcinomas revealed a characteristic papillary architecture and typical PTC nuclear features (A – HE staining, ×200). Strong and diffuse membrane (+ cytoplasmic) HBME-1 positivity (B – HBME-1, ×200), strong and diffuse cytoplasmic CK19 (C – CK19, ×100) and Gal-3 (D – Gal-3, ×200) positivity are presented.
These markers were evaluated not only alone, but also in various panels (Table 4). As single antibody, CD56 was the most sensitive marker (81.1%), but it also had the lowest specificity among the four markers (63.6%). CK19 and Gal-3, on the other hand, were the most specific (100%), but also the least sensitive (45.5%) ones. In various combinations, the most interesting results were obtained for the panels consisting of CD56 and/or CK19/Gal-3 and HBME-1 and/or CD56, with the highest sensitivities (90.9%) and negative predicting values (87.5 and 83.3, respectively). When looking at specificity, the association of HBME-1 with CK19/Gal-3 reached the highest value (72.7%). The addition of a third marker to the immunopanel (the association of HBME-1, CD56 and Gal-3/CK19) not only did not improve the sensitivity, but it lowered the specificity (45.5%). We used Gal-3/CK19 formula because similar results were obtained in the panels containing either CK19 or Gal-3.

Regarding FVPTC cases, as a single marker, CD56 was the most sensitive one (83.3%), followed by HBME-1 (66.6%) (Table 5). However, CD56 was less specific (63.6%), as compared to HBME-1 (72.7%). When various combinations of markers were tested, the panel consisting of CD56 and/or HBME-1 reached 100% sensitivity. The most frequent positive association of markers was represented also by CD56 and HBME-1, with 60% sensitivity and 90% specificity.

Thyroid tumors of uncertain malignant potential

Only 31 TTs-UMP cases could be included in the final immunohistochemical evaluation. CD56 revealed a “malignant” profile (absent expression in more than 90% of the tumor cells) in 12/31 (38.7%) cases and a negative or “benign” profile (>10% positive tumor cells) in 19/31 (61.3%) cases (Figure 4C). Gal-3, HBME-1 (Figure 4D) and CK19 were positive in 5/31 (16.1%), 4/31 (12.9%) and 3/31 (9.7%) TTs-UMP cases, respectively. However, when TTs-UMP immunohistochemical profile was compared to the benign thyroid lesions’ profile, no statistically significant differences were noted between these two groups (Table 6).

Discussion

In this study, using TMA technology multiple markers (HBME-1, CK19, Gal-3 and CD56), alone and in various combinations were evaluated in a series of benign thyroid lesions/tumors and PTCs, aiming to determine their diagnostic value (in terms of sensitivity, specificity, positive and negative predicting value). The results obtained from this first series of tumors were then applied to assess the expression and the possible diagnostic role of these markers in 31 challenging TTs-UMP cases.

In our study, CD56 was the most sensitive marker, staining 81.8% of PTC cases, but also the least specific one (63.6%). CD56 or NCAM (Neural cell adhesion molecules, Leu19) is a membrane glycoprotein with important roles in cell-to-cell adhesion [28]. Loss of CD56 expression was correlated with metastatic potential and a poor prognostic in some malignant tumors [29, 30]. Previous studies have demonstrated that CD56 is present in the normal follicular cells of the thyroid gland and also in benign thyroid lesions (thyroid follicular adenomas, chronic autoimmune thyroiditis, nodular goiter or Basedow disease), but its expression is low or absent when malignant transformation occurs [26, 27, 31, 32]. Recently, El Demellawy et al. has suggested that CD56 is of great value in selecting PTC from other follicular cell-derived thyroid lesions/tumors, with a 100% sensitivity and a 100% specificity [21, 22]. In a previous, recent study on a large series of PTCs (204 cases), we have also found CD56 to be a highly sensitive marker of PTC. CD56 showed a “malignant” profile (no expression) in the majority of cases (84.8%) that was associated with positive membrane staining in the corresponding normal thyroid tissues [20].

HBME-1 was the second most sensitive antibody in our study (63.6%). HBME-1 was originally described as a marker of normal and malignant mesothelial cells because it recognizes a currently unknown antigen that is abundant on the surface of these cells [33]. Miettinen and Kärkkäinen (1996) [34] later reported that PTCs and follicular carcinomas exhibit strong and diffuse immunoreactivity for this antibody, whereas normal thyroid and nodular goiter do not stain or show only weak and focal staining. Recent and past studies have shown that most of CPTCs express HBME-1 and to a lesser extent the FVPTCs [14–19, 20, 35]. We obtained similar results to those reported by Cheung et al. [15], who found HBME-1 expression in 38/54 (70%) CPTCs and 38/70...
(45%) FVPTCs. Regarding its specificity, HBME-1 is almost always negative in the normal thyroid tissue, but literature data describes a positivity ranging from 10 to 56% and from 0 to 20% in thyroid follicular adenomas and nodular goiter, respectively [17, 18, 35–37]. In our study, HBME-1 revealed 72.7% specificity, with three thyroid follicular adenomas and one chronic autoimmune thyroiditis cases staining positively.

Figure 4 – A case of thyroid tumor of uncertain malignant potential showing incompletely developed PTC-type nuclear changes (A – HE staining, ×100 and B – HE staining, ×200). This case revealed a negative, “benign” profile for CD56, with positive membrane expression in the majority of the tumor cells (C – CD56, ×200) and stained positively for HBME-1 (D – HBME-1, ×200).

In addition to HBME-1 and CD56, Gal-3 and CK19 were also evaluated as potential markers of malignancy in PTC. Although not very sensitive (45.5%), these two markers, due to their high specificity (100%), were of great value when used in various panels, associated with much more sensitive markers (CD56 or HBME-1).

Galectin-3 is a beta-galactosyl-binding lectin that is normally expressed in macrophages, mast cells, Langerhans cells and various malignant cells, including thyroid cells [38]. It has been suggested that Galectin-3 could also play a role in the malignant transformation of thyroid cells and many studies have shown that PTC cases, and especially CPTC, are characterized by strong, intense Gal-3 expression [13, 17, 18, 37, 39].

CK19 is a low molecular weight cytokeratin, present in a wide range of normal and neoplastic tissues. Previous studies have shown that CK19 has a diffuse, strong expression in PTC (CPTC in particular), but it is also focally expressed in the normal thyroid epithelium, Hashimoto’s thyroiditis and follicular adenomas [14, 15, 17, 39, 40].

In our study, none of the benign thyroid lesions and none of the FVPTCs stained positively for Gal-3 or CK19, but most of the CPTC cases (4/6) revealed a strong, intense Gal-3 and CK19 expression in more than 75% of the tumor cells (staining intensity score 4).

The value of combined markers use was also tested. The panels consisting of CD56 and/or CK19/Gal-3 and CD56 and/or HBME-1 reached the highest sensitivity (90.9%) in identifying PTC cases, while the most specific combination of markers was represented by the association of HBME-1 with CK19/Gal-3 (72.7%). The addition of a third marker to the immunopanel not only did not improve the sensitivity, but it lowered the specificity.

However, the diagnostic problems encountered in PTC cases are not usually related to CPTC, but are much more often related to the FVPTC. Our results revealed 100% sensitivity for the panel consisting of CD56 and/or HBME-1 in differentiating FVPTC cases from other benign thyroid lesions/tumors. Further on, when more strict rules of positivity were imposed (that both HBME-1 and CD56 be positive), the association of these two markers was highly specific (90%).

We also evaluated the possible diagnostic role of these markers and their combinations in TTs-UMP cases. In these tumors, the use of immunohistochemistry could be even more useful as morphological criteria do not allow a precise diagnosis. Our results showed that CK19, Gal-3...
and HBME-1 stained negatively in most of the cases (90.3%, 83.9% and 87.1% respectively). Regarding CD56, more than half of the TTs-UMP cases (61.3%) revealed a benign, positive membrane CD56 expression in more than 10% of the tumor cells. Moreover, no statistically significant differences could be observed between TTs-UMP immunohistochemical profile and that of the benign thyroid lesions/tumors. Considering these results and since all the antibodies that we have studied are known as markers of thyroid malignancies, it seems most likely that TTs-UMP to be rather linked to a benign biological behavior.

Literature data is few and controversial regarding the expression and possible diagnostic role of various immunohistochemical markers in TTs-UMP cases [12, 19, 41]. The study performed by Hofman et al. [12] on a series of 31 TTs-UMP did not found immunohistochemical and molecular genetics profiling that could be useful in detecting a low- or high-risk population of patients among the different TT-UMP subgroups. On the other hand, Scognamiglio et al. [19] concluded that a panel consisting of HBME-1, Gal-3, CK19 and CITED1 can be helpful in a proportion of FL/QPTC (encapsulated follicular lesions with questionable features of FTC) cases, with its limitation, likely reflecting the biologic ambiguity of these lesions.

Conclusions

CD56 was the most sensitive marker in our study, followed by HBME-1. Although, CK19 and Gal-3 were not very sensitive, these two markers, due to their high specificity, proved to be extremely valuable when used in various panels. The panels consisting of CD56 and/or CK19/Gal-3 and CD56 and/or HBME-1 reached the highest sensitivity (90.9%), while the association of HBME-1 with CK19 or Gal-3 represented the most specific combination of markers. Our results regarding TTs-UMP revealed an immunohistochemical profile similar to the benign thyroid lesions, suggesting that TTs-UMP are probably much more likely to have a benign rather than a malignant biological behavior. However, this is only a hypothesis and only long-term follow-up studies can determine the true biologic nature of these borderline lesions. Until then, morphological diagnosis remains the ‘gold standard’ and should always be privileged.

Acknowledgments

We would like to thank Dorina Ciurca and Valeria Mesaroș for their very precious technical help.

References


Corresponding author
Andrada Loghin, Lecturer, MD, PhD, Department of Histology, University of Medicine and Pharmacy of Tîrgu Mureș, 38 Gheorghe Marinescu Street, 540139 Tîrgu Mureș, Romania; Phone +40744–772 031, e-mail: andradaloghin@yahoo.com

Received: April 8, 2013
Accepted: February 10, 2014