HBME-1 and CK19 Are Highly Discriminatory in the Cytological Diagnosis of Papillary Thyroid Carcinoma

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The cytologic diagnosis of papillary thyroid carcinoma is straightforward in most instances. However, there are some mimics including goitrous nodules and Hurthle cell neoplasms. Many studies have shown the combination of HBME-1 and CK19 expression to be useful in reaching a correct histologic diagnosis on tissue sections. We aim to assess the value of these markers in the setting of cell blocks prepared from needle aspiration specimens. We performed immunohistochemical staining of HBME-1 and CK19 on cell block material from 22 thyroid nodules that also had follow-up histology. Both CK19 and HBME-1 were strongly positive in all nine cases of papillary thyroid carcinoma, the latter showing distinct luminal accentuation. In the non-papillary carcinomas, none showed positivity for both HBME-1 and CK19. Two of six Hurthle cell neoplasms were positive for CK19, however all were negative for HBME-1. One of nine goitrous nodules was strongly positive for HBME-1 with luminal/membranous staining, but this were negative for CK19.

The sensitivity, specificity and positive predictive value of HBME-1 in distinguishing between papillary thyroid carcinoma and goitrous nodules/Hurthle cell neoplasms were found to be 100%, 92.9% and 0.9, respectively; and that of HBME-1 and CK19 combination was 100%, 100% and 1.

We thus conclude that the combination of positive HBME-1 (luminal/membranous) and CK19 (cytoplasmic) staining on cell blocks of thyroid cytologic specimens is highly discriminatory in the diagnostic workup for papillary thyroid carcinoma. Diagn. Cytopathol. 2008;36:550–556. © 2008 Wiley-Liss, Inc.

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Amongst the various subtypes of thyroid malignancies, papillary thyroid carcinoma (PTC) is the most prevalent. An accurate diagnosis of PTC is therefore important in determining the clinical management of the patient. Not infrequently, the pathologist’s first encounter with the thyroid nodule is in the form of a fine-needle aspiration cytology (FNAC) specimen. FNAC is an invaluable tool in the pre-surgical diagnosis of PTCs. In most instances, a confident diagnosis can be made on FNAC preparations. The technique is simple, cheap and safe. Disadvantages, however, include the problem of sampling as well as the low specificity of many cytologic features. Typical cytologic features of PTC such as nuclear grooves and pseudoinclusions or their mimics may be found in some cases of goitrous nodules (GNs) and Hurthle cell nodules (HCNs). Additionally, in both cytology and histology, the follicular and macrofollicular variants of PTC may pose particular difficulties because of morphologic overlap with non-neoplastic and other neoplastic conditions. Hence, it is not surprising that many studies have been conducted to evaluate the usefulness of immunohistochemical staining in the differential diagnostic workup of these nodules.1–13 Immunostains which have been studied include HBME-1, CK19, Galectin-3, CD15, CD57, CITED-1 and others. While a large proportion of these address immunostaining of surgical resection specimens, a few have investigated the reliability of immunostaining on cytologic specimens, whether on cell block material or thin layer cytology preparations.8–13

Among the various reports of immunohistochemical panels, HBME-1 and CK19 have been found to be two of the more consistently reliable markers in the distinc-
tion of papillary from non-papillary neoplasms and non neoplastic lesions according to studies done on histological material. There are no reports of the value of the combination of CK19 and HBME-1 in cell block material.

Therefore we aim to assess the value of these markers in differentiating PTC from GNs and HCNs in cell blocks (CBs) prepared from cytologic specimens, using two readily available antibodies, HBME-1 and CK19 which have been shown to be of good use in surgical specimens.

**Materials and Methods**

**Training Set**

Prior to the study on CB material, a training set of full histologic sections of 102 thyroid cases from the archives of the Department of Pathology, National University of Singapore was reviewed. These cases included 46 benign lesions (10 hyperplastic nodules (HPNs) and six cases of Graves’ disease (GD); 11 follicular adenomas (FAs); 16 Hurthle cell adenomas (HAs), two hyperplastic nodules with Hurthle cell change and one case of Hashimoto thyroiditis) and 56 malignant lesions (seven follicular carcinomas (FCs), four Hurthle cell carcinomas (HCs) and 45 PTCs [including 32 conventional PTCs, 12 follicular variant PTCs and one cribriform morular variant PTC]). Two 4-μm sections were cut from each case and subjected to immunohistochemical staining with HBME-1 and CK19 in the manner described below. The results were interpreted and agreed upon by three pathologists (GSL, MPK and MEN) and were scored according to extent of staining (score 1: <1% of cells; score 2: 2–50% of cells; score 3: >50% of cells) and intensity (score from 0 to 3+). Positive staining was regarded as reactivity in at least 50% of the cells at an intensity of at least 2+. The results of this training set were used to ensure the validity of the immunostaining results in the test set of CBs.

**Fig. C-1.** PTC (case 11). A: Well formed papillary structure lined by cells with classical nuclear features, haematoxylin and eosin, ×200; B: HBME-1 staining showing strong cytoplasmic and membranous staining with luminal accentuation, ×200; C: CK19 staining showing strong cytoplasmic and membranous reactivity, ×200.
Test Set (Cytology Cases)

A total of 22 cases of thyroid nodules were subject to FNAC, all of which had accompanying CB material. Follow-up histology was available for all 22 cases. Briefly, the CBs were prepared by placing the contents of the needle hub into a tissue cassette and adding a drop of Bouin’s solution to the tissue for the formation of a pellet. The pellet was processed in a similar fashion to a small biopsy in the usual manner, with paraffin wax impregnation and embedding. Two 4-μm sections were cut from each case, in addition to the original haematoxylin and eosin stained sections.

Immunohistochemical staining for HBME-1 (Dako, Glostrup, Denmark, dilution 1:100) and CK19 (Novocastra, Newcastle, United Kingdom, dilution 1:400) was performed on the cell block for each case, using a standard streptavidin-biotin-peroxidase method, with appropriate positive and negative controls.

Parameters assessed included the percentage of cells staining positive, staining intensity (score 0–3+) and the pattern of staining (cytoplasmic, cell membrane, luminal). Positive staining was defined as reactivity in >25% of cells at an intensity of at least 2+.

The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and the diagnostic accuracy were calculated for each stain separately and for the combination of both stains, using histologic diagnosis as the gold standard of these two immunostains, comparing this to the gold standard of the histologic diagnosis.

Results

Training Set (Histology)

The detailed and complete results of this cohort, currently being written up, were presented at the Scientific Session of the International Academy of Pathology-Asia Pacific Division, in May 2007 in Singapore. Briefly, among the benign lesions, only three of 46 cases stained significantly for HBME-1 (2FAs and 1 HPN); none of these three showed luminal accentuation. None of the Hurthle cell lesions stained for HBME-1. A higher proportion (10 of 46) of the benign lesions stained with CK19 (4/11 FAs, 3/16 HPNs, 2/6 HAs and one case of Hashimoto thyroiditis). Among the malignant cases, 45/56 were positive for HBME-1, including 3/7 FCs, 42/45 PTCs. Of the PTCs, 34/42 showed luminal accentuation of membrane staining. All four cases of HC were negative for this marker. CK19 reacted with 49/56 malignant cases, including 2/7 FCs and 44/45 PTCs and 1/4 HCs.

The overall sensitivity and specificity of combined positivity of HBME-1 and CK19 in the differentiation of PTC from benign follicular lesions was 100 and 93.3%, respectively.

Test Set (Cytology)

Papillary thyroid carcinomas. Both CK19 and HBME-1 were positive in all nine cases of PTCs (100%). HBME-1 was positive in >40% cells in all cases, showing at least moderate staining intensity (2+). The staining pattern was distinctly membranous (n = 8/9, 89%), with luminal accentuation in a majority of the cases (n = 7/9, 78%) (Fig. C-1B).

CK19 showed diffuse, strong cytoplasmic or membranous (intensity 3+) staining in all nine cases (Fig. C-1C).

Goitrous nodules and Hurthle cell nodules. Two of six HCs (33%) were strongly positive for CK19 but all six cases were negative for HBME-1 (Fig. C-2).

One of nine GNs (14%) was strongly positive for HBME-1 with luminal/membranous staining, but this case was negative for CK19. None of the nine GNs stained positively for CK19. None of the GNs or HCNs showed positivity for both HBME-1 and CK19.

The results of immunostaining are presented in Table I. The sensitivity, specificity and PPV of HBME-1 and CK19 in distinguishing between PTC and GN/HCN are presented in Table II. Notably, when both stains are combined and interpreted in concert, the sensitivity, specificity and PPV are 100%, 100% and 1, respectively.

Discussion

The reported accuracy of FNAC in the diagnosis of thyroid lesions ranges from 70 to 97%, with false-negative rates ranging from 1 to 6%.14 This is largely dependent on the experience of the aspirator and the cytopathologist.

The use of immunohistochemical markers have been proven on many histological studies to aid in distinguishing PTCs from its mimics.1–7 Likewise, the use of such markers on cytologic material prepared from FNAC of thyroid lesions has been previously studied by a few groups and found to be helpful in distinguishing morphologically equivocal lesions.8–13 While using cytologic material, some authors have used restained, alcohol-fixed or air-dried slides while others have used thin layer preparation specimens, but the majority of investigators have favoured the use of cell blocks.8–13 Nasser et al. attempted immunocytochemical staining for CK19 on a variety of specimen types, including methanol fixed thin layer preparation slides, alcohol-fixed smears and air-dried smears, the latter two of which were destained in isopropyl alcohol before immunostaining. They found that the best results were obtained using methanol-fixed thin layer preparations, which showed the strongest immunoreactivity in positive cases. This group did not, however, compare the reproducibility of staining using cell block preparations.
HBME-1 is a monoclonal antibody that binds to an unknown microvillous surface antigen that is present in mesothelial cells, as well as other epithelial cells. Previous studies have found it to be a sensitive marker for PTCs. We find this to be borne out in this study, using cytological material, where the cells consistently exhibited focal staining (<25% extent) for CK19 in one of the cases that was considered to be negative for this marker.

Fig. C-2. GN (case 17), A: Morphology on the cell block, haematoxylin and eosin, ×20; B: Negative staining for HBME-1, ×40; C: Very focal staining (<25% extent) for CK19 in one of the cases that was considered to be negative for this marker, ×200.

Fig. C-3. HC (case 3), A: Islands of plump oncocytic cells on CB, haematoxylin and eosin, ×40; B: Very focal staining for HBME-1, ×200; C: Positive staining for CK19, ×200.
similar patterns of immunoreactivity to those seen in histologic specimens. Interestingly, most cases of PTCs showed cytoplasmic and membranous staining with luminal accentuation. This finding was first noticed in the test set of histologic cases on which the immunostaining was performed, and was particularly obvious in the follicular variants of PTC, although some classical PTCs also demonstrated this cytolocalization. The reactivity of HBME-1 to a protein localized to the cell surface could in part explain the prominent luminal accentuation demonstrated in this study, however, cytoplasmic reactivity was also noted in a fair proportion of cases. The strong luminal or apical accentuation of HBME-1 staining has not been specifically highlighted in other studies, however, in the photomicrographs in the papers by Matos and Cheung, respectively, the staining pattern of HBME-1 on histologic sections showed distinct luminal/surface accentuation.2,3 Similarly, in cytologic specimens, the photomicrographs in the paper by Van Hoeven also showed stronger luminal membranous staining in their cell block sections.11 We believe that this feature is valuable in the critical evaluation of HBME-1 staining, as a high proportion of PTCs demonstrated such a pattern. However, in the present study, there was one GN that was positive for HBME-1 showing luminal accentuation. This cannot be explained at this point in time.

Mai et al. found that HCTs and tumours with H cells or apocrine-like changes showed a negative or reduced reactivity for HBME-1.16 However, all their cases of PTC with H cell change showed at least focal staining (12/12 cases, up to 50% staining). We also observed this phenomenon in our study, where none of the pure H cell lesions showed significant staining for HBME-1 while the single case of oncocytic PTC was strongly positive for this marker, albeit with loss of the luminal accentuation pattern. The specific mechanism of this loss is unexplained, although it was suggested by Mai et al. that the decreased staining could have been due to the absence of surface glycoprotein that reacts with the HBME-1 antibody in Hürthle cells.16

Most studies have shown CK19 to be a relatively sensitive but non-specific marker of thyroid malignancies, as it also stains non-neoplastic lesions such as FAs and even normal thyroid tissue, usually focally.1–7 Cheung et al. found that 20% (15/75) of their benign thyroid lesions (hyperplastic nodules and FAs) were at least focally positive for CK19, although the staining appeared to favour areas of reactive/degenerative change.3 De Matos et al.
found an even higher percentage of benign lesions to be reactive for this marker, namely 6/18 (33.3%) of FAs, 2/12 (16.7%) of adenomatous nodules and 6/10 (60%) of Hashimoto thyroiditis. They found the staining to be focal and weak, in comparison to diffuse staining in the malignant lesions. In our study, the sensitivity and specificity of CK19 for the diagnosis of PTC was 100% and 84.6%, respectively, being positive in all nine PTCs, but also in two cases of HCs (Fig. C-3). Unlike the former two studies, though, CBs of the benign nodules were all negative in the cytology cases. However, this may have been due to the small numbers of cases. The larger cohort in the training set of histologic cases yielded 10/46 (21.7%) of benign cases showing significant CK19 positivity. Although the normal thyroid parenchyma was not quantitatively and specifically assessed, we also noted that in a fair proportion of cases, patchy positivity was discernible in the non-lesional parenchyma.

Most authors advocate the use of not one antibody but a panel of immunomarkers in the differential diagnosis of contentious lesions. We agree with this approach, as we and others have shown a fair degree of overlap in the reactivity of PTCs and non-PTCs to these two markers. Cheung et al. suggested that HBME-1 served as an indicator of thyroid malignancy (including papillary, follicular and insular carcinomas), while CK19 in combination with Ret immunostaining, suggested a differentiation toward PTC. However, we were not able to demonstrate the increased specificity of CK19 for PTCs, but instead found HBME-1 to be more specific, especially in the presence of luminal accentuation. Ret is a protein encoded by the RET proto-oncogene, which has been found to exhibit RET/PTC gene rearrangements in PTCs. Furthermore, others have shown that the prevalence of Ret positivity in PTC on immunohistochemistry is less than universal, with only just over 60% or so cases demonstrating reactivity. Nevertheless, we find that a simple panel of HBME-1 and CK19 is more helpful than the use of a single immunomarker as it improves the specificity, positive and NPV and thus diagnostic accuracy.

The cut-off for defining a positive staining is stricter here than that used in other studies. In histologic studies, it ranges from any degree of positivity at all to at least 10% of cells being positive. In cytologic studies, a cut-off of >10% staining extent has been used to define a positive reaction, while in others, any degree of staining was regarded as positive. None of the cytologic studies used staining intensity to define positivity, unlike the present study. We find that a “stricter” threshold for positivity improves the specificity of these markers while not significantly compromising on the sensitivity. Applying a cut-off value of greater than 25% of cells with at least moderate (2+) staining intensity, we found that both sensitivity and specificity of the combination of HBME-1 and CK19 was 100%. Similarly, the predictive value for the diagnosis of PTC over benign GNs and Hurthle cell neoplasms (HCNs) was 1. Individually, the sensitivity of HBME-1 and CK19 was both 100% but the specificity was 92.3% and 84.6%, respectively. This argues for the need to use a panel of at least two markers to improve the positive and NPVs.

PTC is one of the most commonly encountered thyroid malignancies, and difficulties in differential diagnosis of PTC versus both benign and malignant thyroid lesions do occur in the scope of one’s routine cytopathology practice. We highly recommend that cell blocks be prepared whenever possible and this simple and readily available panel of stains be used to aid in the diagnostic workup, which will afford maximal information for the subsequent clinical management of otherwise equivocal cases.

In conclusion, we find that the combination of positive HBME-1 and CK 19 staining on cell blocks is highly discriminatory in differentiating PTCs from HCNs/GNs. Focal, weak staining is of doubtful diagnostic value. We recommend the use of this panel in cases with features suspicious for but not diagnostic of PTCs. Attention should be paid to both extent, intensity and pattern of staining.

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