Differential Diagnosis between Primary Endocervical and Endometrial Adenocarcinoma using Immunohistochemical Staining of Estrogen Receptor, Vimentin, Carcinoembryonic Antigen and p16

Marut Yanaranop MD*,***,
Sucha Ayuwat MD*, Saranyu Nakrangsee MD**,***

** Department of Obstetrics and Gynecology, Rajavithi Hospital, Bangkok, Thailand*
*** Department of Pathology, Rajavithi Hospital, Bangkok, Thailand*
**** College of Medicine, Rangsit University, Bangkok, Thailand*

Background: *When clinical and histopathological evaluation is not effective in discriminating primary endocervical adenocarcinoma (ECAs) and endometrial adenocarcinoma (EMAs), an immunohistochemistry (IHC) method is regularly used in practice, which involves staining of estrogen receptor (ER), vimentin (Vim), monoclonal carcinoembryonic antigen (mCEA) and p16.*
Objective: *To evaluate the performance of IHC markers, ER, Vim, mCEA and p16, in differentiating between primary ECAs and EMAs and to compare the performances of two-, three- and four-marker panels.*
Material and Method: *Women with cervical or uterine cancers who were diagnosed with mucinous or endometrioid adenocarcinoma or adenocarcinoma of non-otherwise specified, after cervical biopsy, endometrial biopsy or curettage, and who underwent elective surgery at Rajavithi Hospital between January 1, 2011 and June 30, 2012 were retrospectively reviewed. Paraffin-embedded tissue sections from pre-operative specimens were reviewed and stained with ER, Vim, mCEA and p16. Postoperative pathologic slides was reviewed and installed as the reference standard.*
Results: *Of 110 cases, 44 were primary ECAs and 66 were primary EMAs. ER and Vim were significantly expressed in EMAs ($p<0.001$), while mCEA and p16 were significantly expressed in ECAs ($p<0.001$). From multivariable analysis, Vim and p16 were the significant markers for differentiating ECAs and EMAs. A comparison of different combinations showed that panels of Vim/p16, ER/Vim/p16, Vim/mCEA/p16 and ER/Vim/mCEA/p16 achieved the highest overall accuracy of 97.9%.*
Conclusion: *Vim and p16 are the significant IHC markers and a two-marker panel of Vim/p16 is recommended for using in differentiating primary ECAs and EMAs; which a pattern of negative Vim and positive p16 expression favors diagnosis of ECAs while the converse pattern of positive Vim and negative p16 staining points to diagnosis of EMAs.*

Keywords: *Endocervical adenocarcinoma, Endometrial adenocarcinoma, Immuno-histochemistry, Estrogen receptor, Vimentin, Carcinoembryogenic antigen, p16*

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Cervical and endometrial carcinomas are respectively the first and second most common types of gynecologic malignancy worldwide[1]. Endocervical adenocarcinomas (ECAs) and endometrial adenocarcinomas (EMAs) have some degree of histomorphological overlapping, and sometimes it may be difficult to differentiate between the two entities in biopsy or curettage specimens in which tissue fragments are not sufficient to distinguish the two sites of origin. This discrimination has clinical significance since the choice of an appropriate therapeutic plan depends on the site of tumor origin.

ECAs or EMAs can be found in both mucinous and endometrioid histological subtypes. The mucinous subtype is more common in ECAs while the endometrioid subtype is more common in EMAs. In the process of identifying the tumor site origin, pathologists can avail themselves of several histomorphological clues: for example, identification of endometrial stroma or stromal foam cells or

Correspondence to:
Yanaranop M, Department of Obstetrics and Gynecology, Rajavithi Hospital, 2 Phyathai Road, Rajathewi, Bangkok 10400, Thailand.
Phone: +66-2-3548165 ext. 3226, Fax: +66-2-3548084
E-mail: myanaran@hotmail.com

endometrial hyperplasia point to EMA, whereas the finding of intraepithelial neoplasia, either glandular type or squamous type, suggests ECA. Routing histologic examination can often differentiate ECAs versus EMAs of the usual mucinous and endometrioid subtypes, but when clinical and routine histopathological evaluation is not adequate to diagnose whether an ECA or EMA, the immunohistochemistry (IHC) technique is an ancillary method, which is widely recommended in practice. This involves antibody staining of estrogen receptor (ER), vimentin (Vim), monoclonal carcinoembryogenic antigen (mCEA) and p16[2]. Using this panel, EMAs are characterized by an ER positivity, Vim positivity, mCEA negativity, and p16 negativity, whereas ECAs generally exhibit the converse pattern of staining, ER negativity, Vim negativity, mCEA positivity, and p16 positivity[3,4].

The objectives of this study were to evaluate the performances of the commonly used IHC markers ER, Vim, mCEA and p16, in differentiating between primary ECAs and EMAs, and to compare single markers with two-, three- and four-marker panels.

Material and Method

The protocol of this research was approved by Institutional Review Board (IRB) of Rajavithi Hospital (No. 55126). A retrospective review was carried out of the medical records of cervical and uterine cancer patients who had been histologically diagnosed with mucinous or endometrioid adenocarcinoma or adenocarcinoma of non-otherwise specified (NOS) after cervical biopsy, endometrial biopsy or fractional curettage and who had undergone surgical staging at Rajavithi Hospital between January 1, 2011 and June 30, 2012. Clinical data were extracted from the patients' medical records including age at diagnosis, parity, weight, height, underlying diseases, signs and symptoms, surgical procedures and tumor staging. Pathologic slides of pre-operative cervical biopsy, endometrial biopsy and curettage were reviewed by a pathologist (Yanaranop M), and then the selected formalin-fixed, paraffin-embedded tissue blocks were cut as whole sections for IHC staining. These IHC slides were scored semi-quantitatively by two pathologists (Yanaranop M and Nakrangsee S) without detail of the pre-operative pathologic results. Finally, post-operative pathologic slides were reviewed by one pathologist (Yanaranop M), who was also blinded to the pre-operative pathologic and IHC results. Patients were excluded in cases of (1) pathologic diagnosis of adenocarcinoma other than mucinous or endometrioid subtypes or adenocarcinoma, NOS (2) pathologic diagnosis of metastatic adenocarcinoma from other sites, and (3) absence of paraffin-embedded tissue block.

Formalin-fixed, paraffin-embedded tissue sections of 4-5 mm thickness were deparaffinized in xylene, rehydrated through serial dilutions of alcohol, and washed in phosphate buffered saline (pH 7.2). IHC staining was performed using the avidin-biotin complex (ABC) technique by the automated slide immunostainer "BenchMark XT IHC/ISH Slide Staining System" of Roche Diagnostics. The slides were stained with commercially available antibodies of ER, Vim, mCEA and p16 of which the main characteristics are summarized in Table 1. Negative and positive tissue control slides included for every batch of samples were processed and run on the automated slide immunostainer.

In the present study, IHC staining was scored semi-quantitatively according to the German Semi-quantitative Scoring System[5] that consisted of two partitions: the intensity of marker expression and the extent of staining. The final immunoreactive score or expression index (EIsq) equaled the product of the immunostaining intensity (ITIsq) and the area fraction of labeled cells (ALC). The ITIsq was quantified using the following scores: 0 = negative, 1 = weakly positive, 2 = moderately positive, 3 = strongly positive. The ALC was calculated by evaluating the percentage of the

Table 1. Antibodies used in this study

Ag	Clone	Product code	Ab class	Supplier	Dilution	Ag retrieval
ER	Rabbit monoclonal	SP1	IgG	Ventana	No	EDTA
Vim	Mouse monoclonal	V9	IgG1	Dako cytomation	1:100	Citrate
mCEA	Mouse monoclonal	II-7	IgG1	Dako cytomation	1:50	Citrate
p16	Rabbit polyclonal	P14ARF	IgG	GeneTex	1:100	Citrate

Ag = Antigen; Ab = Antibody; ER = Estrogen receptor; Vim = Vimentin; mCEA = Monoclonal carcinoembryonic antigen

positive staining areas in relation to the whole cancer area in tissue sections. A score of 0 was assigned for 0% reactivity, 1 point for 1% to 10% reactivity, 2 points for 11% to 50% reactivity, 3 points for 51% to 80% reactivity and 4 points for 81% to 100% reactivity. The total EIsq yielded a score range of 0 to 12. With regard to patterns of IHC staining, nuclear staining was scored for ER, cytoplasmic staining for Vim and mCEA, and nuclear and cytoplasmic staining for p16 as shown in Fig. 1.

Sample size calculation was based on the formula for one proportion ($N = [Z_{\alpha/2}]^2 P (1-P)/d^2$) using 2-tail alpha equal 0.05 and acceptable error at 0.075. The sensitivity of four markers from two studies by McCluggage et al[6,7] was used for calculation. The estimated ratio of ECAs and EMAs in Rajavithi Hospital, 2008-2012 was 2: 3; therefore, at least 108 subjects were calculated and 120 subjects were required with an expected 10% dropout rate.

Statistical analysis was undertaken using STATA 14 (StataCorp, College Station,TX). The baseline characteristics were described using frequency and percentage for categorical data while mean, standard deviation, median and range were used for continuous data. Comparison of the IHC score was carried out by Mann-Whitney U test. The discrimination ability and optimal cutoff value of the German Semi-quantitative Score were determined by Receiver Operating Characteristic (ROC) curve analysis and Youden index[8] respectively. Sensitivity, specificity, positive and negative predictive values, and overall accuracy were calculated for the performances of each IHC marker and compare with those of two-, three- and four-marker panels using the pathologic diagnosis as the reference standard. Multivariable analysis with the logistic regression method was calculated for the significant markers in differentiating between ECAs and EMAs. A probability value of less than 0.05 was

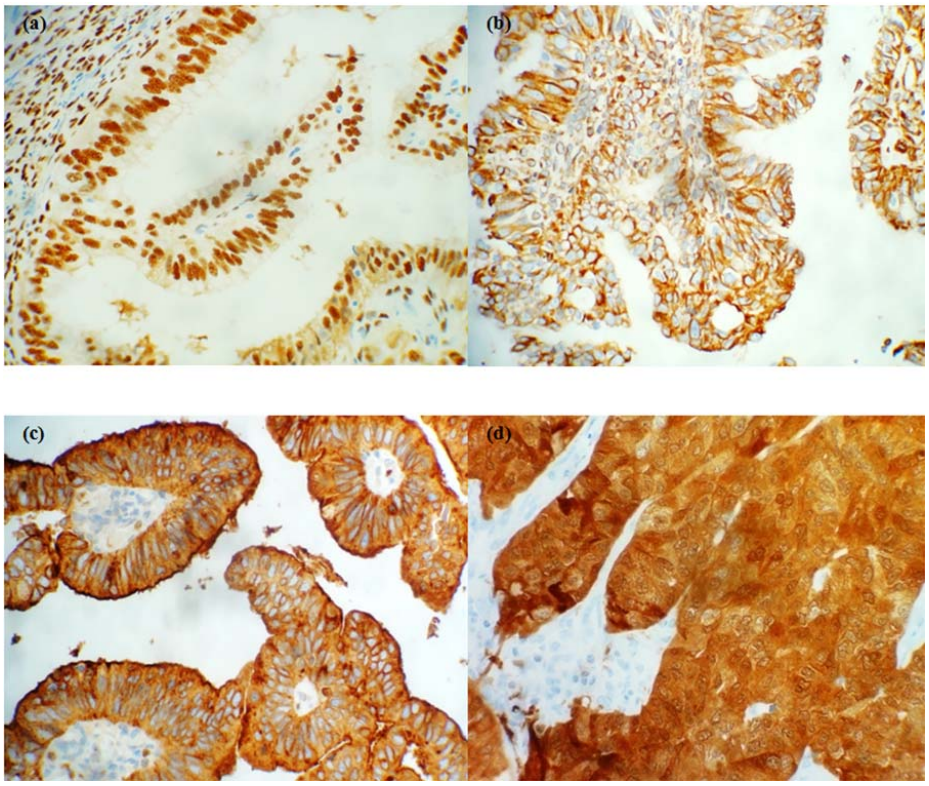


Fig. 1 Immunohistochemical staining patterns of estrogen receptor, vimentin, monoclonal carcinoembryonic antigen and p16. An endometrial adenocarcinoma with (a) a diffuse and strong nuclear expression of estrogen receptor, and (b) a diffuse and strong cytoplasmic expression of vimentin. An endocervical adenocarcinoma with (c) a diffuse cytoplasmic monoclonal carcinoembryonic antigen expression with apical accentuation, and (d) a diffuse, strong, nuclear and cytoplasmic labeling of p16 expression.

considered statistically significant.

Results

Between January 1, 2011 and June 30, 2012, the medical records were reviewed of 124 cervical and uterine cancer patients who had been histologically diagnosed with mucinous or endometrioid adenocarcinoma or adenocarcinoma of NOS, after pre-operative cervical biopsy, endometrial biopsy or curettage, and who had undergone surgical staging at Rajavithi Hospital. After pathologic review of pre-operative specimens, fourteen patients were excluded because of absence of paraffin-embedded tissue blocks. Finally, a total of 110 patients were included and divided into 2 groups of 44 ECAs and 66 EMAs based of post-operative pathologic diagnosis.

The clinico-pathologic characteristics of all patients are summarized in Table 2. The mean age of patients at diagnosis was 54.9 years (standard deviation 9.4 years) and 74.5% of the subjects were postmenopausal. Pre-operative procedures for histopathological diagnosis were cervical biopsy 40.9%, fractional curettage 36.4%, endometrial curettage 20.0% and endometrial biopsy 2.7%. Seven patients (6.4%) had discordance between pre- and postoperative diagnosis of origin of adenocarcinoma. According to the 2009 FIGO staging of cervical and uterine cancer[9], 36 ECA patients (81.8%) were in stage I and 8 patients (18.2%) in stage II, whereas 42 EMA patients (63.6%) were in stage I, 11 patients (16.7%) in stage II, 12 patients (18.2%) in stage III and 1 patient (1.5%) in stage IV.

ER and Vim had significantly higher scores in EMAs (p<0.001, both); on the other hand mCEA and p16 had significantly higher scores in ECAs (p<0.001, both), as shown in Table 3. All markers performed well in area under ROC curve (ROC-AUC) ranging from 89.7% to 93.1% (Fig. 2). Vim and p16 showed the greatest ROC-AUC of 93.1% and 92.6% respectively. Using the Youden index, the optimal cutoff points of IHC score were 4 for ER, Vim and mCEA, while of p16 it was 12. For discrimination of ECAs versus EMAs, expression of ER, Vim and mCEA at least focal and moderately positive staining (EIsq more than or equal 4) was the cutoff threshold but p16 had a different expression. ECAs mostly expressed diffuse and strong pattern (EIsq = 12) for p16 marker while EMAs revealed p16 expression range from negative to patchy and strong pattern (EIsq = 0-9). Multivariable logistic regression analysis found that Vim and p16 were the significant markers for discriminating ECAs and EMCs as exhibited in Table 4.

Table 2. Baseline clinicopathologic characteristics

Variables	Total (n =110)
Age (years), mean (SD)	54.9 (9.4)
Weight (kg), mean (SD)	64.1 (13.1)
Height (cm), mean (SD)	154.3 (6.0)
BMI (kg/m^2), mean (SD)	27.0 (5.7)
Parity, median (range)	2 (0-8)
Underlying diseases (%)	70 (63.6)
Hypertension	59 (53.6)
Diabetes mellitus	23 (20.9)
Breast cancer	3 (2.7)
Other	8 (7.3)
Menopausal status (%)	
Premenopausal women	28 (25.5)
Postmenopausal women	82 (74.5)
Signs and symptoms	
Abnormal vaginal bleeding	80 (72.7)
Abnormal vaginal discharge	11 (10.0)
Pelvic pain	3 (2.7)
Pelvic mass	1 (0.9)
Abnormal cervical cytology	15 (13.6)
Preoperative procedures	
Cervical biopsy	45 (40.9)
Endometrial biopsy	3 (2.7)
Endometrial curettage	22 (20.0)
Fractional curettage	40 (36.4)
Preoperative diagnosis	
ECAs	44 (40.0)
Mucinous type	39 (35.5)
Endometrioid type	2 (1.8)
Adenocarcinoma, NOS	3 (2.7)
EMAs	66 (60.0)
Mucinous type	4 (3.6)
Endometrioid type	60 (54.6)
Adenocarcinoma, NOS	2 (1.8)
Postoperative diagnosis	
ECAs	44 (40.0)
Mucinous type	40 (36.4)
Endometrioid type	4 (3.6)
EMAs	66 (60.0)
Mucinous type	3 (2.7)
Endometrioid type	63 (57.3)
Discordance of pre- and post-operative diagnosis	7 (6.4)

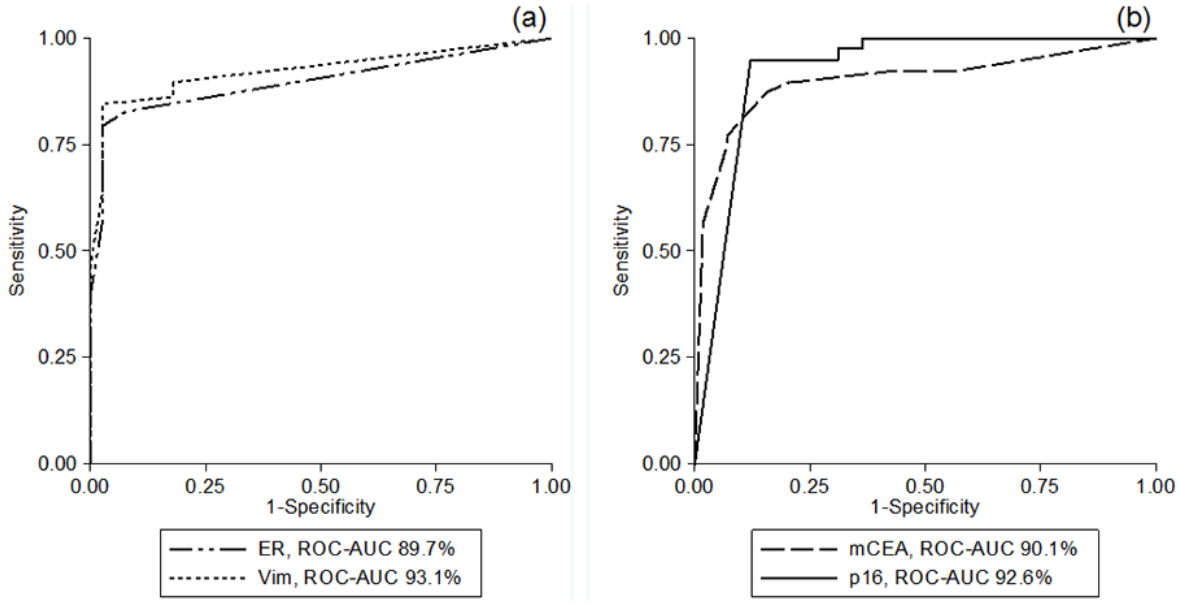
SD = standard deviation; BMI = body mass index; ECAs = cervical adenocarcinomas; EMAs = endometrial adenocarcinomas; NOS = not otherwise specified

Table 5 displayed the comparisons of the test performance of single markers (ER, Vim, mCEA, p16),

Table 3. Summary of discriminatory abilities and optimal cutoff value of each immunohistochemistry markers

Markers	IHC score, median (range)			ROC-AUC (95% CI)	Optimal cutoff
	ECAs (n = 44)	EMAs (n = 66)	*p*-value		
ER	0 (0-9)	9 (0-12)	<0.001*	89.7 (84.1-95.4)	4
Vim	0 (0-12)	12 (0-12)	<0.001*	93.1 (87.8-98.3)	4
mCEA	12 (0-12)	1 (0-12)	<0.001*	90.1 (82.9-97.3)	4
p16	12 (4-12)	3 (0-12)	<0.001*	92.6 (87.8-97.3)	12

IHC = Immunohistochemistry; ECAs = Endocervical adenocarcinomas; EMAs = Endometrial adenocarcinomas; ROC = Receiver Operating Characteristics curves; AUC = Area under curve; CI = Confidence interval; ER = Estrogen receptor; Vim = Vimentin; mCEA = Monoclonal carcinoembryonic antigen
* Significance at $p<0.05$



ROC = Receiver Operating Characteristics curves; AUC = Area under curve; ER = Estrogen receptor; Vim = Vimentin; mCEA = Monoclonal carcinoembryonic antigen

Fig. 2 Receiver Operating Characteristic curve of (a) estrogen receptor and vimentin for diagnosis of primary endometrial adenocarcinomas, and (b) monoclonal carcinoembryonic antigen and p16 for diagnosis of primary endocervical adenocarcinomas.

two-marker panels (ER/mCEA, ER/p16, Vim/mCEA, Vim/p16), three-marker panels (ER/Vim/mCEA, ER/Vim/p16, ER/mCEA/p16, Vim/mCEA/p16) and four-marker panels (ER/Vim/mCEA/p16) in differentiating primary ECAs versus EMAs. Of the single markers, p16 and Vim showed the highest accuracy at 90.7% and 89.7% respectively, while mCEA showed the lowest accuracy of 83.5%. Diagnostic accuracy of IHC marker combinations was evaluated using a logistic regression model, which indicated that four panels of Vim/p16, ER/Vim/p16, Vim/mCEA/p16 and ER/Vim/mCEA/p16 archived the highest overall accuracy of 97.9%. Other two- and three-marker panels showed 91.8-95.9% overall accuracy.

In diagnosis of ECAs, patterns of either negative staining of single ER or Vim revealed the highest sensitivity at 97.4% whereas 2-marker (Vim-/p16+), 3-marker (ER-/Vim-/p16+, ER-/mCEA+/p16+ and Vim-/mCEA+/p16+) and 4-marker (ER-/Vim-/mCEA+/p16+) panels had the highest specificity at 100%. The 2-

Table 4. Multivariable analysis of immunohistochemistry markers for distinguishing between primary endocervical and endometrial adenocarcinomas

Variables	ECAs				EMAs				*p*-value
	Coeff.	SE	aOR	95% CI	Coeff.	SE	aOR	95% CI	
Vim IHC score	-0.58	0.17	0.56	0.40-0.78	0.58	0.17	1.79	1.28-2.50	0.001*
p16 IHC score	0.53	0.14	1.70	1.28-2.26	-0.53	0.14	0.59	0.44-0.78	<0.001*
Constant	-3.39	1.49			3.39	1.49			

ECAs = Endocervical adenocarcinomas; EMAs = Endometrial adenocarcinomas; Coeff. = Coefficiency; SE = Standard error; aOR = Adjusted odds ratio; CI = Confidence interval; Vim = Vimentin; IHC = Immunohistochemistry
* Statistical significance at $p<0.05$

Table 5. Comparisons of the performance of each marker with the two-, three- and four-marker panels for distinguishing between primary endocervical adenocarcinoma and endometrial adenocarcinoma

IHC marker panels	ECAs (%)				EMAs (%)				Overall accuracy (%)
	Sn	Sp	PPV	NPV	Sn	Sp	PPV	NPV	
ER	97.4	79.3	76.0	97.9	79.3	97.4	97.9	76.0	86.6
Vim	97.4	84.5	80.9	98.0	84.5	97.4	98.0	80.9	89.7
mCEA	89.7	79.3	74.5	92.0	79.3	89.7	92.0	74.5	83.5
p16	94.9	87.9	84.1	96.2	87.9	94.9	96.2	84.1	90.7
ER/mCEA	89.7	93.1	89.7	93.1	65.5	97.4	97.4	65.5	91.8
ER/p16	94.9	96.6	94.9	96.6	70.7	97.4	97.6	69.1	95.9
Vim/mCEA	89.7	93.1	89.7	93.1	70.7	97.4	97.6	69.1	91.8
Vim/p16	94.9	100.0	100.0	93.1	72.4	97.4	97.7	70.4	97.9
ER/Vim/mCEA	89.7	94.8	92.1	93.2	60.3	97.4	97.2	62.3	93.8
ER/Vim/p16	94.9	100.0	100.0	96.7	63.8	97.4	97.4	64.4	97.9
ER/mCEA/p16	87.2	100.0	100.0	92.1	56.9	97.4	97.1	60.3	95.9
Vim/mCEA/p16	87.2	100.0	100.0	92.1	58.6	97.4	97.1	61.3	97.9
ER/Vim/mCEA/p16	87.2	100.0	100.0	92.1	51.7	97.4	96.8	47.7	97.9

IHC = Immunohistochemistry; ECAs = Endocervical adenocarcinomas; EMAs = Endometrial adenocarcinomas; Sn = Sensitivity; Sp = Specificity; PPV = Positive predictive value; NPV = Negative predictive value; ER = Estrogen receptor; Vim = Vimentin; mCEA = Monoclonal carcinoembryonic antigen

markers (Vim-/p16+) and the 3-markers (ER-/Vim-/p16+) panels exhibited the best performance at sensitivity of 94.9% and specificity of 100%. In diagnosis of EMAs, negative staining of single p16 (non-diffuse and strong pattern) manifested the highest sensitivity at 87.9%, and the four-marker panels had the lowest sensitivity at 51.7%. All patterns of panel expression revealed the same specificity of 97.4% except negative mCEA and negative p16 patterns (89.7% and 94.9%, respectively). Overall, these data indicated that the 2-marker panel of Vim and p16 tended to outperform the other 2-marker panels, and the 3- and 4-marker panels showed no improvement in performance when compared with the two-marker panels.

Discussion

In this study, the positive staining results for ER and Vim supported EMA whereas p16 and mCEA supported ECA. ER, Vim and mCEA performed well at the cut-off IHC score at 4. With respect to the German Semi-quantitative Score, the threshold for differentiating between final positive and negative immunostaining was set at cutoff value of 4 for interpretation, which has been widely accepted and used in previous studies[3,10-17]. However, p16 had a different expression from those markers, and required diffuse and strong

staining pattern to obtain good performance. Two-marker panels of p16 and Vim demonstrated the best performance in discriminating ECAs and EMAs. The continuing addition of 3- and 4-marker panels brought about no improvement in overall accuracy as compared with the 2-marker panels. In addition, the results of IHC marker staining show the better performance in diagnosis of ECAs than EMAs reflecting the variety of IHC patterns of EMAs.

EMAs typically exhibit diffuse nuclear ER positivity; however, well-differentiated ECAs occasionally reveal positive staining for ER. Several prior studies have investigated ER staining in ECAs and found positive staining varied from 4.2% to 38.5%[3,6,10,12,18], in contrast with the 2.6% observed in the present study. With regard to EMAs, previous studies reported 65.9-93.3% of positive ER staining[3,6,10,12,18] which those were comparable with 79.3% of the present study.

Although Vim is the most important marker of the mesenchymal cells, co-expression of Vim and cytokeratin is seen in the epithelial cells of most EMAs but not ECAs[16]. There were varying reports of the Vim expression in EMAs which observed that 61.4-96.7% of EMAs showed Vim positivity[3,6,10,12,17] which the present study found 84.5% of EMAs expressed Vim. These prior studies[3,6,10,12,17] identified Vim positivity in ECAs ranging from 6.9% to 12.5%, in contrast with the 2.6% observed in the present study.

Monoclonal CEA is expresses more commonly in ECAs than in EMAs. However, the squamous epithelium elements of endometrioid adenocarcinomas may express positive staining for CEA[6]. Previous studies had reported that mCEA stained positively in 6.8-26.7% of EMAs[3,10,12,17], which were consistent with the 20.7% of mCEA positivity found in the present study. On the other hand, there have been varying reports of the expression of mCEA in ECAs, with positive staining ranging from 30.8% to 96.2%[3,10,12,17] whereas in the present study positive mCEA staining was found in 89.7% of ECAs.

Cyclin-dependent kinase (CDK) inhibitor 2A or p16 is a surrogate biomarker of HPV infection (in particular high-risk oncogenic HPV types) used in evaluating HPV-associated squamous and glandular neoplasm of the lower gynecologic tract[19,20]. However, some tumor with overexpression of p16 regardless of HPV infection can also exhibit positive staining. The interpretation of p16 marker staining results is complicated because of the lack of optimized consensus for p16 IHC scoring. Staining of p16 can be demonstrated in both nuclear and cytoplasmic patterns, but the biologic significance of cytoplasmic staining is unclear. However, Koo et al[15] found that the mean of the sum of cytoplasmic plus nuclear German semi-quantitative score and nuclear score alone could help to distinguish between ECAs and EMAs, but cytoplasmic score alone was of no use in the process. In this study, the p16 scoring system appraised both nuclear and cytoplasmic stains in tumor cells. ECAs typically show diffuse p16 positivity due to the presence of high-risk HPV, while EMAs are generally negative or focally positive with a so-called mosaic pattern of immunoreactivity. McCluggage et al[7] stated that a diffuse and strong staining pattern of p16 involving nearly all tumor cells tended to be an ECA, whereas a focal, patchy staining pattern of p16 involving 0% to 50% of cells tended to be an EMA on routine tissue section stains in IHC. The present study found the same pattern as the aforementioned study of p16 expression in routine whole-sectioned tissue slides and used diffuse and strong pattern (IHC score 12) for distinguishing between ECAs and EMAs. Expression of p16 positively in 94.9% of ECAs compared with 12.1% of EMAs in the present study was consistent with prior studies[4,7,21] which used a threshold of diffuse strong pattern for interpretation (94.7-100% of ECAs vs. 0-10.3% of EMAs). In contrast with studies of Han et al[11-13] and Koo et al[15] which used IHC score 4 as the cutoff threshold for interpretation, the p16 marker staining expressed positivity in 71.4-78.6% of ECAs compared with in 12.5-29.2% of EMAs.

McCluggage et al[6] stated that the conventional three-marker panel of ER, Vim and mCEA is generally accepted for distinction between ECAs and EMAs. However, the p16 marker is currently the most promising single marker and carries more diagnostic ability than the others. The present study advocated a two-marker panel of Vim and p16 for use in discriminating between primary ECAs and EMAs without additional three- or four-marker panels. Han et al[11-13] and Liao et al[14] investigated the performance of five IHC markers ER, Vim, mCEA, p16 and progesterone receptor (PR) stained in tissue microarray from paraffin-embedded tissue of 14 ECAs and 21 EMAs. A two-marker panel of Vim and mCEA exhibited the most accuracy (78.3%) and was most appropriate for the diagnostic differentiation between ECAs and EMAs. Kong et al[22] evaluated IHC markers ER, PR, Vim, mCEA and HPV markers (p16, ProExC and HPV in situ hybridization or HPV-ISH) on 283 tissue microarray cores and 38 whole tissue sections and stated that the

3-marker panels including Vim, ER or PR, and HPV marker (p16, ProExC or HPV-ISH) were optimal for determining the site of origin for ECAs and EMAs (overall diagnostic accuracy ranged from 89% to 93%). However, these comparisons have limitations because of the heterogeneity of tissue slides, the IHC scoring system and the IHC markers.

Although this study has some limitation since its retrospective nature makes the results more susceptible to bias, it reflects routine practice, which introduces performance of IHC markers in pre-operative specimens with whole tissue sections. Additionally, the interpretation bias is limited by the blinding of other diagnostic results. Molecular studies for HPV may yield benefits in problematic cases. Usual ECAs typically contain high-risk HPV infection, whereas EMAs are negative. However, some unusual histologic types of ECAs are HPV-negative and p16-negative including mesonephric, clear cell, and minimal-deviation adenocarcinomas[23]. Moreover, serous carcinomas are consistently strongly and diffusely positive for p16, whereas some FIGO grade 3 and clear cell carcinomas may show moderately to strongly positive staining[24]. Therefore, the ER, Vim, mCEA and p16 panel should be used with a great deal of caution when the tumor in question could be of mesonephric, minimal deviation, serous, clear cell, FIGO grade 3 endometrioid or undifferentiated type. In this context, clinical and radiologic findings should be considered accompanied with IHC data.

Conclusion

In summary, the two-marker panel of Vim and p16 is recommended for use in differentiating primary ECAs versus EMAs. A pattern of negative Vim and positive p16 (diffuse and strong stain) expression suggests diagnosis of ECAs, whereas positive Vim and negative p16 (non-diffuse and strong stain) points to diagnosis of EMAs.

What is already known on this topic ?

Primary endocervical and endometrial adenocarcinoma have some degree of clinical and histopathology overlapping. Immuno-histochemistry technique is an ancillary method used in practice, which involves antibody staining of estrogen receptor, vimentin, monoclonal carcinoembryogenic antigen and p16. Using this panel, endometrial adenocaricinomas are characterized by an ER positivity, Vim positivity, mCEA negativity, and p16 negativeity; in contrast, endocervical adenocarcinomas are characterized by an ER negativity, Vim negativity, mCEA positivity, and p16 positivity.

What this study adds ?

The two-marker panel of Vim and p16 is recommended for use in distinguishing between primary endocervical adenocarcinoma and endometrial adenocarcinoma, and the use of three-marker and four-maker panel does not bring about any improvement in performance. Diffuse and strong staining pattern of p16 (immunohistochemistry semiquantitative score = 12) is appropriate for this process.

Potential conflicts of interest

None.

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การวินิจฉัยแยกโรคระหว่างมะเร็งปากมดลูกและมะเร็งเยื่อบุโพรงมดลูกปฐมภูมิชนิดอะดิโนคาร์สิโนมาโดยการย้อมทางอิมมูโนพยาธิวิทยาด้วย estrogen receptor, vimentin, carcinoembryonic antigen และ p16

มรุต ญาณารณพ, สุชา อายุวัฒน์, ศรัญญู นาครังษี

ภูมิหลัง: เมื่อการประเมินทางคลินิกและจุลพยาธิวิทยาไม่เพียงพอในการแยกมะเร็งปฐมภูมิชนิดอะดิโนคาร์สิโนมาที่ปากมดลูก (ECAs) และเยื่อบุโพรงมดลูก (EMAs) วิธีทางอิมมูโนพยาธิโดยการย้อมด้วย estrogen receptor (ER), vimentin (Vim), monoclonal carcinoembryonic antigen (mCEA) และ p16 เป็นวิธีการที่ใช้อย่างสม่ำเสมอ

วัตถุประสงค์: เพื่อประเมินความสามารถของการตรวจทางอิมมูโนพยาธิด้วย ER, Vim, mCEA และ p16 ในการแยกมะเร็งปฐมภูมิชนิด ECAs และ EMAs และเพื่อเปรียบเทียบระหว่างการตรวจโดยสอง, สาม และสี่ตัวย้อม

วัสดุและวิธีการ: สตรีโรคมะเร็งปากมดลูกและมะเร็งเยื่อบุโพรงมดลูกที่ได้รับการวินิจฉัยเป็น mucinous หรือ endometrioid adenocarcinoma หรือ adenocarcinoma, non-otherwise specified จากการตัดชิ้นเนื้อที่ปากมดลูก, การตัดชิ้นเนื้อเยื่อบุโพรงมดลูกหรือการขูดมดลูกและเข้ารับการผ่าตัดแบบนัดที่โรงพยาบาลราชวิถีระหว่างวันที่ 1 มกราคม พ.ศ. 2554 ถึง วันที่ 30 มิถุนายน พ.ศ. 2555 ได้รับการทบทวนย้อนหลัง สิ่งส่งตรวจในพาราฟินได้จากก่อนผ่าตัดได้รับการทบทวนและย้อมด้วย ER, Vim, mCEA และ p16 สไลด์ทางพยาธิวิทยาหลังผ่าตัดได้ถูกทบทวนและใช้เป็นเกณฑ์อ้างอิงมาตรฐาน

ผลการศึกษา: ผู้ป่วย 110 ราย แบ่งเป็นมะเร็งปฐมภูมิชนิด ECAs 44 ราย และชนิด EMAs 66 ราย ER และ Vim ย้อมติดอย่างมีนัยสำคัญใน EMAs ($p<0.001$) ในขณะที่ mCEA และ p16 ย้อมติดอย่างมีนัยสำคัญใน ECAs ($p<0.001$) การวิเคราะห์พหุปัจจัยพบว่า Vim และ p16 เป็นตัวย้อมที่มีนัยสำคัญในการแยกระหว่าง ECAs และ EMAs เมื่อเปรียบเทียบระหว่างการรวมกันหลายๆ แบบพบว่าแผง Vim/p16, ER/Vim/p16, Vim/mCEA/p16 และ ER/Vim/mCEA/p16 มีความแม่นยำรวมสูงสุดที่ร้อยละ 97.9

สรุป: Vim และ p16 เป็นตัวย้อมทางอิมมูโนพยาธิที่มีความสำคัญและการย้อมด้วยสองตัวย้อม Vim และ p16 ถูกแนะนำสำหรับการแยกระหว่างมะเร็งปฐมภูมิ ECAs และ EMAs ซึ่งรูปแบบผลลบต่อ Vim และผลบวกต่อ p16 ใช้สำหรับการวินิจฉัย ECAs ในขณะที่ผลบวกต่อ Vim และผลลบต่อ p16 ใช้สำหรับการวินิจฉัย EMAs