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International Endocervical Adenocarcinoma Criteria and Classification: Validation and Interobserver Reproducibility

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Abstract

The current World Health Organization (WHO) classification for endocervical adenocarcinoma (EA) is based on descriptive morphological characteristics; however, it does not fully reflect our current knowledge of the diverse pathogenesis of cervical glandular neoplasia. A novel classification system, the International Endocervical Adenocarcinoma Criteria and Classification (IECC), which incorporates etiology and biologic behavior into the morphologic scheme, has been recently proposed. We aimed to validate the IECC by assessing its interobserver reproducibility in comparison to the WHO system. A cohort of 75 EAs was reviewed independently by 7 gynecologic pathologists and categorized following IECC and WHO criteria based on hematoxylin and eosin (H&E) material alone and after immunohistochemistry results for p16, PR, p53, Napsin-A, vimentin, CDX2 and GATA3 were provided. HPV in situ hybridization (ISH) and polymerase chain reaction (PCR) results were compared with consensus diagnoses. IECC was superior to WHO in terms of interobserver agreement with K=0.46 vs 0.3 respectively on H&E review and K=0.51 vs 0.33 respectively with immunohistochemistry. Under the IECC, 73 (97%) of EAs had

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majority agreement (4 reviewers in agreement) while 42 (56%) had perfect agreement (7/7 reviewers in agreement). Conversely, WHO showed majority agreement in 56 (75%) and perfect agreement in only 7 (10%) EAs. Reproducibility was poor in HPV-related WHO types (usual K=0.36, mucinous NOS K=0.13, intestinal K=0.31, villoglandular K=0.21) and good in major HPV-unrelated categories (gastric type K=0.63, clear cell K=0.81, mesonephric K=0.5). Classification as per the IECC had excellent correlation with HPV status (by RNA ISH or PCR). We have shown that the IECC has superior interobserver agreement compared to the WHO classification system, and that distinction between HPV-related and HPV-unrelated EA can be made with good reproducibility and excellent prediction of HPV status. WHO morphologic variants of HPV-related EA are poorly reproducible. Conversely, agreement is high among important high-risk HPV-unrelated subtypes. Thus, our results further support replacing the current WHO classification with the IECC.

Keywords

Cervix; endocervical; adenocarcinoma; HPV; gastric type endocervical adenocarcinoma; clear cell carcinoma; mesonephric carcinoma; serous carcinoma; villoglandular carcinoma; stratified mucin producing carcinoma; mucinous adenocarcinoma; classification; IECC; reproducibility; interobserver agreement

INTRODUCTION

In contrast to cervical squamous cell carcinoma which shows a rate of high-risk HPV detection approaching 100%, endocervical adenocarcinoma (EA) shows a variable prevalence of HPV depending on several factors including geographic region, tumor subtype, and detection method¹⁻⁵. Indeed, it is now known that several EA subtypes are unrelated to HPV infection⁶⁻⁸ and have distinct clinico-pathologic features and prognosis compared to HPV-related tumors^{8,9}.

The current World Health Organization (WHO) classification of tumors of female genital organs classifies EA based on morphological characteristics, particularly cytoplasmic features¹⁰. The practicality of this classification system has been questioned, as the reproducibility of the terminology has not been thoroughly explored and tumor type definitions are vague. In addition, the lack of robust biological basis of the current classification has led to further questioning of its clinical value. Notably, classification of epithelial neoplasia of the vulva¹¹⁻¹⁴ and oropharynx^{15,16} has shifted towards a system based on pathogenesis, as it has been found to be more clinically informative and reproducible.

The International Endocervical Adenocarcinoma Criteria and Classification (IECC), which categorizes malignant glandular endocervical lesions based on underlying etiology and biologic behavior, was recently proposed by an international group of experts in gynecologic pathology (Table 1)¹⁷. The system, applied to a well-annotated cohort of 409 invasive EAs, showed reliable segregation of HPV-related EA (HPVA) and HPV-unrelated EA (NHPVA) by morphology alone, and was further improved by the use of a selective immunohistochemical panel (p16, p53, PR, vimentin). Under the IECC, EA is classified as

HPVA and NHPVA based on the presence or absence of HPV-related features (easily identifiable apical mitoses and apoptotic bodies). NHPVA was further subdivided into known categories with updated and improved definitions. Finally, the category of EA-not otherwise specified (EA-NOS) was assigned when a tumor could not be classified by IECC criteria. This classification is a promising step towards categorizing EA in a more consistent and clinically meaningful manner. While treatment algorithms for cervical cancer incorporating HPV status do not exist currently for EA¹⁸, a precedent for such approach has been set in other organ systems like head and neck^{15,16,19,20}.

Adoption of the IECC is justified by its more biologically congruent approach and simple pathologic definitions. However, its interobserver reproducibility has yet to be addressed. We therefore aimed to evaluate the IECC by documenting its interobserver agreement in comparison to the WHO system and its predictive value of the HPV status. In this context, we also intended to study the reproducibility of HPVA and NHPVA subcategories as defined by WHO and IECC and to compare the reproducibility of EA diagnoses in biopsy versus excision material.

MATERIALS AND METHODS

Case selection and review

Material selected for independent review was obtained from consecutive invasive EA specimens collected between 2002 and 2017 at Sunnybrook Health Sciences Centre (Toronto, Canada). Specimens with all histologic slides and at least one suitable tissue block available were included; others were excluded from this study. Cases further selected included cervical biopsy and/or excision material (loop electrosurgical excision procedure (LEEP), cold knife cone, trachelectomy, hysterectomy). All original slides were reviewed by one gynecologic pathologist (CPH), and the most representative slides were selected for independent review by all the participants (1 to 4 slides per case).

Tissue microarray construction

A representative paraffin-embedded tissue block from each tumor was retrieved. Tissue microarrays (TMAs) were constructed using two 2-mm cores of representative tumor tissue per case. Normal tissue controls were included in each TMA block.

Immunohistochemistry

TMA sections were stained with p16, p53, PR, vimentin, CDX2, Napsin-A, and GATA3 (Table 2). Stains were interpreted by a gynecological pathologist (CPH) and results recorded using the following definitions: p16 staining was considered “overexpressed” if diffuse block-like nuclear and cytoplasmic staining was seen in 80% of tumor cells, “patchy” if positivity was patchy (<80% of tumor cells) or cytoplasmic only, or “absent” if no staining was seen; p53 was scored as “overexpressed” if 80% of tumor nuclei were strongly positive, “null” when no staining was seen in tumor cells in the presence of an intact internal control, or “wild type” when nuclear expression was heterogeneous in intensity. For p16 and p53, results on TMA slides were compared to those from available archival immunohistochemistry whole-section slides. Scores for PR (nuclear), vimentin (cytoplasmic

or membranous), Napsin-A (cytoplasmic), CDX2 (nuclear), and GATA3 (nuclear) were based on the percentage of positive tumor cells (0–100%) and the average intensity (weak, moderate, or strong).

HPV detection studies

In situ hybridization (ISH) for high-risk HPV detection was performed on TMA slides. In situ hybridization (ISH) with a chromogen was performed using the Advanced Cell Diagnostics (ACD) (Hayward, CA) RNAscope[®] system (catalogue no. 312598). The RNAscope[®] Probe “HPV HR18” contains probes targeting E6 and E7 mRNA for the following high risk subtypes: HPV16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82. Historic tumors known to contain high risk-HPV were used as positive controls, while those known to be high risk-HPV negative were used as negative controls during assay optimization. Subsequently, a negative control slide lacking application of the probes was prepared and examined, particularly when adjudicating tumors with rare or equivocal signals. A full range of cytoplasmic and nuclear signals were encountered, as has been previously described²⁶.

HPV detection by polymerase chain reaction (PCR) was performed in all tumors with negative ISH results, and in tumors with undetermined ISH results and residual tissue available. We used the Roche Cobas[®] 4800 system (Pleasanton, California) which detects the presence of 14 high-risk HPV types. The HPV DNA is detected by real-time PCR with specific fluorescent-labelled DNA probes. Qualitative results are generated separately for HPV Types 16 and 18, and a combined results for 12 other high-risk types of 31,33,35,39,45,51,52,56,58,59,66,68. Each run included a no-template negative control and a positive control supplied by the system; both control results were required to be valid in each run prior to reporting. In addition, the β -globin gene in each human sample was amplified in the same reaction tube as internal control.

To determine the IECC predictive value of HPV status, the majority diagnosis from the 7 reviewers for each case was compared to the HPV results as determined by RNA ISH and / or PCR.

Pathologist independent review

Seven gynecologic pathologists from 6 major institutions in Canada, the United States and Romania were recruited to participate in the study. Four reviewers (KJP, EO, SS, RS) were part of the original group that developed the IECC¹⁷. Reviewers were blinded to all clinical features and HPV status of each case. Written instructions were distributed to each reviewer summarizing the WHO and IECC terminology and criteria in tabular and graphic formats. Slide review and diagnosis assignment were done independently. The review process was first based on evaluation of routine hematoxylin and eosin (H&E) stained slides only. Subsequently, the immunohistochemical profile for each case was provided and the reviewers rendered a revised diagnosis.

The IECC classification system has been thoroughly described in the original study¹⁷. Briefly, the diagnosis of HPVA requires apical mitotic figures and apoptotic bodies appreciable at scanning magnification; if those features are not identified a cursory

examination at 200× is performed to detect mitoses and/or apoptoses (Figure 1) and in their absence a diagnosis of NHPVA is warranted. NHPVAs are then classified based on established criteria as endometrioid²¹, gastric including minimal deviation adenocarcinoma^{5,9}, serous, clear cell or mesonephric (Figure 2). Finally, a designation of EA-NOS is used when a tumor cannot be classified into HPVA or NHPVA categories. In addition to the above diagnoses, adenocarcinoma in situ (AIS) was available as a possible choice, as it has been noted that distinguishing between florid AIS and certain forms of invasive EA can be challenging^{22,23}. Reviewers were asked to sub-classify HPVA following WHO cytoplasmic morphologic criteria as usual type, villoglandular, mucinous NOS, mucinous intestinal type, mucinous signet ring cell type, and invasive stratified mucin-producing carcinoma^{24,25}. Within the HPVA category, the reviewers were also given the option to select “adenosquamous carcinoma” as a diagnosis.

Statistical analysis

All statistical analyses were performed using the SPSS software 24.0 (IBM Corporation, New York, NY, United States). The interobserver agreement of classification among individual pathologists, based on histology alone as well as based on histology and results of immunohistochemistry studies was assessed using Fleiss' kappa (K) statistics. Kappa analysis was also applied to compare pathologist diagnoses as HPVA or NHPVA with HPV ISH results. Kappa values as well as the 95% confidence interval (CI) were calculated, and P values of less than 0.05 were considered to be statistically significant. Kappa was interpreted categorically following Fleiss guidelines for comparisons between >2 raters as follows: poor (K<0.4), fair/good (K=0.4–0.75) and perfect (K>0.75)²⁷.

RESULTS

A total of 83 EA were identified and reviewed. Of these, 75 were successfully tested for HPV by ISH and/or PCR, and constitute the final study cohort. 47/75 cases (63%) had material from excision +/- biopsy specimens, while the remaining 28 cases in the cohort (37%) were composed of biopsy material only (including 13 from patients with advanced stage at presentation who underwent primary radiotherapy +/- chemotherapy without surgical treatment). A total of 73 tumors were successfully sampled in TMAs and were evaluable by immunohistochemistry (IHC). Of the 75 EAs, 64 (85%) were positive for high-risk HPV types by either ISH (63) or PCR (1). The remaining 11 (15%) were negative for high-risk HPV types either by PCR (3) or ISH (9 cases; PCR confirmed a negative result in 4 and resulted undetermined in 5).

Interobserver agreement

Individual tumor classification by each reviewer following the IECC based on H&E review only (Figure 3) showed superior interobserver reproducibility compared to classification as per the WHO system (Table 3). Interobserver agreement was higher for IECC with both H&E review (IECC K=0.46 vs WHO K=0.3) and after revision with IHC results (IECC K=0.51 vs. WHO K=0.33). Restricting the analysis to a three-tier classification (NHPVA vs. NHPVA vs. EA-NOS) also showed good reproducibility (K=0.45 on H&E and K=0.51 with

IHC). Of note, reproducibility of the EA-NOS category was poor ($K=0.2$ and 0.19 by H&E and after IHC review, respectively).

Table 4 shows the different levels of agreement among the classification systems. Level of agreement was determined by the number of reviewers who gave the same diagnosis in each case. Overall, when using WHO criteria, a majority diagnosis was not achieved in a significant number of tumors (19, 25%) and among these, only 3 or fewer reviewers concurred with any given diagnosis. Similarly, under the WHO system, perfect agreement (7 out of 7 reviewers) was only reached only 7 (10%) cases. In contrast, levels of concordance in diagnosis as per IECC were significantly higher, with perfect agreement in 42 (56%) cases and lack of majority diagnosis in only 2 (3%) tumors. A simplified approach looking at HPVA vs. NHPVA vs EA-NOS showed similarly high levels of agreement.

Table 5 shows interobserver agreement among HPVA morphologic categories as per the WHO classification. Reproducibility among HPVA subtypes was poor and did not improve significantly with IHC. Poor reproducibility was also observed in exceedingly rare NHPVA categories (endometrioid and serous carcinoma) as shown in Table 6. Conversely, gastric, clear cell and mesonephric types showed good reproducibility both on H&E and after IHC review.

Interobserver agreement for both classification systems was evaluated in biopsy and resection specimens separately. In biopsy material, the IECC showed higher concordance than the WHO ($K=0.41$ versus $K=0.23$, respectively). However, agreement in biopsies was lower compared to that of excision specimens for both systems ($K=0.43$ for IECC, $K=0.34$ for WHO).

IECC / HPV status correlation and immunohistochemical data

In order to determine the value of IECC criteria in predicting the HPV status, the majority diagnosis (4 reviewers) for each tumor was correlated with HPV ISH and PCR results. There was near perfect correlation between IECC diagnosis and HPV status: all 64 HPV-positive tumors were classified as HPVA, whereas the 9 of the 11 EAs negative for HPV testing were classified into one of the HPV-negative categories. The two remaining cases negative for HPV testing were classified as EA-NOS by consensus. p16 was overexpressed in 58 (92%) of the 63 HPVAs with p16 IHC available, while all 8 NHPVAs that were tested for p16 showed either negative or patchy p16 staining.

Immunohistochemical results are displayed graphically in Figure 4. p53 was overexpressed in 4% of tested EA (3/73, 2 of which had majority agreement for NHPVA). PR staining of any intensity was detected in 13% of tested tumors (9/72), ranging from 5–90% positively stained nuclei. Vimentin expression (5–100%) was detected in 14% of tested EA (10/71). Staining was membranous in all positive cases; a basolateral staining pattern was not observed. CDX2 staining (5 to 100%) was seen across the majority of tested tumors (59%, 40/68), while GATA3 expression (5 to 100%) was detected in 43% tumors (30/69). Napsin-A (any staining) was seen in 6% of tested tumors (4/70). Tissue microarray p16 and p53 results were concordant with archival whole tissue section immunohistochemical stains in all 12 cases with archival stains available. The addition of IHC data resulted in a change of

diagnosis by at least one reviewer in 18 out of 73 cases (25%); but only in 22% (4/18) of them the diagnosis was changed by 2 reviewers. In the remaining 55 EAs (75%), review of IHC data resulted in no change of H&E diagnosis.

DISCUSSION

To the best of our knowledge, this is the first study to explore interobserver reproducibility of the traditional WHO and novel IECC systems in the classification of EA. Despite the broad spectrum of histologic subtypes within EA, classification by the IECC demonstrated higher levels of concordance. Our results validate the IECC as a more reproducible approach to classify EA compared to WHO, showing better Fleiss' kappa values and higher proportion of cases with perfect or near perfect agreement among reviewers. Importantly, agreement improved when a selected panel of IHC was used to add in interpretation, as previously demonstrated in the seminal study describing the IECC¹⁷.

Distinction between HPVA and NHPVA is important given the differences in their pathogenesis and clinical behavior^{9,28–32}. The latter in particular applies to gastric-type EA, which has significantly higher rates of adverse clinical features and outcome compared to HPVA³⁰. Moreover, different treatment algorithms have been developed in other organ systems where tumors are dichotomized based on HPV status^{15,16,33} and it is conceivable that such an approach could also be implemented in EA for treatment purposes. We demonstrate that distinction between HPVA and NHPVA, the first step in the IECC categorization, has good levels of interobserver agreement among pathologists. Furthermore, the majority HPVA or NHPVA IECC diagnosis in each case had perfect correlation with HPV detection results, which were not provided to the reviewers. This observation further validates the IECC as an accurate estimator of HPV status and EA subtype.

The current WHO classification contains several morphologic categories within the spectrum of HPV-related endocervical glandular neoplasia. These categories include usual type (mucin depleted, intracellular mucin in <50% of tumor cells), mucinous NOS (intracellular mucin in 50% of tumor cells), intestinal type and villoglandular carcinoma. All these tumor types display the distinctive features of HPVA defined in the IECC, namely easily identifiable apical mitoses and apoptotic bodies. An additional HPVA variant not included in WHO is the recently described invasive stratified mucin-producing carcinoma²⁴. As initially conceived, the IECC allows for the subdivision of HPVA into the above WHO categories, mostly to provide consistency with the pre-existing WHO classification system¹⁰. However, the present study shows that such sub-categorization of HPVA suffers from poor reproducibility. In fact, the high interobserver variability in the diagnosis of HPVA morphologic subtypes largely explains the overall lower reproducibility of the WHO classification. Our findings question the value of sub-categorization of HPVA, which should be addressed in future large clinical studies.

Among NHPVA, clinically important types have overall good reproducibility (gastric, clear cell and mesonephric). These types are rare, but can behave aggressively if presenting at advanced stage^{34,35}. Our study shows that the diagnosis of these biologically and clinically relevant entities is reproducible. Endometrioid and serous EA, on the other hand, showed

poor interobserver agreement. This can be explained by the rarity and arguable non-existence of these tumor types in the cervix. As per IECC, the term endometrioid carcinoma is reserved for tumors with low-grade endometrioid glands and confirmatory endometrioid features (squamous metaplasia or endometriosis). Under this definition, endometrioid carcinoma is rare, representing ~1% of cervical adenocarcinomas¹⁷. Primary cervical serous carcinoma has been rarely described in the literature^{36,37}; nonetheless, it has been postulated that serous carcinoma, when strictly defined, does not occur in the cervix^{38,39}. When considering this diagnosis, endometrial and/or tubo-ovarian primary tumors must be rigorously excluded. Further exploration of the arguable existence of primary cervical serous carcinoma requires additional patient cohort studies.

The use of a targeted immunohistochemical panel improved interobserver agreement in diagnosis. However, it is important to note that closer observation to rates of change in diagnosis based on IHC data showed that IHC more frequently confirmed, rather than changed, the initial interpretation made on morphologic grounds. The immunohistochemical markers used in this study are commonly used in the workup of lesions of the gynecologic tract including of the uterine cervix^{40,41}, and are likely to be available in most pathology laboratories.

p16 immunohistochemistry showed high correlation with HPV results in our study. p16 overexpression in EA is mostly due to the oncogenic effects of HPV^{42,43}; of note, loss of heterozygosity and methylation have been shown to result in loss of p16 staining even in HPV positive tumors^{44,45}. None of the tumors classified as NHPVA showed p16 overexpression. We also explored the role of GATA3, CDX2 and Napsin-A in improving interobserver agreement, in addition to the initial panel of p16, p53, PR and vimentin suggested by *Stolnicu et al*¹⁷. We found significant rates of expression for CDX2 and GATA3 irrespective of EA type in our cohort, indicating the limited value of these markers. For example, positivity for GATA3, a sensitive and specific marker of both benign and malignant mesonephric lesions in the lower female genital tract⁴⁶, was detected in over 40% of our cohort, but only 1 case resulted in a majority diagnosis (4 reviewers) of mesonephric carcinoma.

In the original IECC study, 2.4% of EA were unclassifiable, i.e. EA-NOS¹⁷. A similar proportion was found in our cohort (2 EAs, 3%). It was reported that this group of tumors contain both HPVA and NHPVA, based on immunophenotype and HPV ISH status¹⁷. In our study, the 2 EAs with majority classification as EA-NOS were HPV negative by ISH. Interestingly, of the 18 tumors where at least one reviewer changed the original diagnosis after IHC evaluation, 4 (22%) were changed from a HPVA or NHPVA diagnosis to EA-NOS. This finding is in keeping with previous observations recommending caution in the use of immunohistochemistry, in particular p16 which can be overexpressed in NHPVA and occasionally be patchy or negative in HPVA, as seen in our cohort. As previously recommended, a tumor classified as EA-NOS may benefit from HPV ISH testing in the clinical setting¹⁷.

Biopsy specimens are often poorly represented in reproducibility studies. The standard treatment of locally advanced cervical cancer, commonly defined as stage IB2 to IVA, is

upfront chemoradiation, i.e. primary surgery is usually not indicated^{47–51}. For this reason, biopsy material is often the only tissue available from these patients. We included a subset of EAs represented in biopsy material only to assess reviewer agreement in this subgroup. As might be expected, overall agreement based on biopsy evaluation is lower compared to that achieved upon examination of excision material. This finding highlights the inherent limitation of accurate diagnosis in biopsy specimens and the need for reliable biomarkers for different EA subtypes that can be used in the evaluation of scant tumor material.

A potential limitation of our study is that all of the cases originated from a single institution; however, this institution is a tertiary care center for gynecologic oncology that provides care to a wide spectrum of referral patients in the largest metropolitan area in Canada. We also recognize that our reviewers are all experts in gynecologic pathology, and thus the generalization of our results in the wider pathology community needs to be explored.

Future directions in this field include the search for reliable biomarkers for major EA histologic types and eventually, the development of a combined histologic-molecular-based classification for EA. While the reproducibility of IECC is superior to the current WHO, none of the comparisons made in our study reached perfect Kappa values, indicating a clear need for better criteria and ancillary tools to further optimize the reproducibility of the IECC. In this regard, molecular testing is a promising tool. Recent insights into the genomic landscape of HPVA show that prevalent mutations (*KRAS*, *PIK3CA*) correlate with morphology⁵² and have prognostic as well as therapeutic implications, suggesting a potential genomic role in the ultimate classification of EA. Finally, the IECC may benefit from the incorporation of other clinically useful systems such as the pattern-based classification for HPVA⁵³. This classification provides useful prognostic information and can complement or even replace the current morphologic subtypes of HPVA outlined in the WHO.

In summary, the recently described IECC is a more reproducible system to categorize EA compared to the current WHO classification. As defined, the morphologic definitions provided by IECC allow for higher interobserver agreement and have excellent correlation with tumor HPV status. Our results support previous recommendations to replace the WHO classification with the IECC. The need remains to improve the value and reproducibility of the classification by identifying sensitive, specific and biologically significant biomarkers of endocervical glandular neoplasia.

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Figure 1. HPV-related endocervical adenocarcinoma (HPVA). A. Well differentiated HPVA with hyperchromatic nuclei and mucin depletion. B. Multiple hyperchromatic figures can be appreciated at scanning (10 $\times$) magnification (yellow arrows). C. At higher magnification (20 $\times$), these figures are confirm to be apoptoses (blue arrows) and apical mitoses (red arrows). D. HPVA, invasive stratified mucin producing variant, also with distinguishable apoptoses and mitoses at scanning (10 $\times$) magnification (yellow arrows).

Figure 2.

HPV-unrelated (NHPVA) and unclassifiable (EA-NOS) endocervical adenocarcinoma. A. Gastric-type NHPVA with infiltrative growth and clear mucinous cytoplasm. B. Clear cell NHPVA with high grade nuclear features and "hobnail" pattern. C. Mesonephric NHPVA with pseudo-endometrioid glands (left aspect) and dilated glands with eosinophilic luminal material (right aspect). D. High grade adenocarcinoma not amenable for classification into IECC categories by reviewers; HPV ISH was negative in this case.

CASE	REVIEWER							HPV status	IECC CATEGORY		HPV ISH RESULT	
	A	B	C	D	E	F	G					
EA2										HPV-related EA		
EA4												
EA5										Gastric type EA		
EA7												
EA9										Clear cell EA		
EA11												
EA14										Mesonephric EA		
EA16												
EA19												
EA22										EA-NOS		
EA23												
EA24										Endometrioid EA		
EA27												
EA29										Serous EA		
EA30												
EA31												
EA33												
EA36												
EA37												
EA39												
EA40												
EA41												
EA42												
EA44												
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EA47												
EA43												
EA69												
EA45												
EA46												
EA15												
EA78												
EA32												
EA63												
EA53												
EA20												
EA74												
EA3												
EA12												
EA73												
EA17												
EA52												
EA51												

Figure 3.
Diagram illustrating the IECC diagnosis provided by each reviewer.

Case	Diagnosis	p16	p53	PR	Vimentin	Napsin A	CDX2	GATA3	IHC result	
EA01	HPV/A									Positive *
EA02	HPV/A									Positive *
EA04	HPV/A									Negative **
EA05	HPV/A									Negative **
EA07	HPV/A									Not done
EA09	HPV/A									Not done
EA10	HPV/A									
EA11	HPV/A									
EA14	HPV/A									
EA15	HPV/A									
EA16	HPV/A									
EA18	HPV/A									
EA19	HPV/A									
EA21	HPV/A									
EA22	HPV/A									
EA23	HPV/A									
EA24	HPV/A									
EA25	HPV/A									
EA27	HPV/A									
EA28	HPV/A									
EA29	HPV/A									
EA30	HPV/A									
EA31	HPV/A									
EA32	HPV/A									
EA33	HPV/A									
EA35	HPV/A									
EA36	HPV/A									
EA37	HPV/A									
EA39	HPV/A									
EA40	HPV/A									
EA41	HPV/A									
EA42	HPV/A									
EA43	HPV/A									
EA44	HPV/A									
EA45	HPV/A									
EA46	HPV/A									
EA47	HPV/A									
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EA50	HPV/A									
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EA66	HPV/A									
EA67	HPV/A									
EA68	HPV/A									
EA69	HPV/A									
EA70	HPV/A									
EA71	HPV/A									
EA72	HPV/A									
EA75	HPV/A									
EA76	HPV/A									
EA77	HPV/A									
EA78	HPV/A									
EA79	HPV/A									
EA83	HPV/A									
EA08	Gastric									
EA12	Gastric									
EA20	Gastric									
EA53	Gastric									
EA68	Gastric									
EA74	Gastric									
EA78	Clear cell									
EA17	Mesonephric									
EA51	EA-NOS									
EA82	EA-NOS									

Figure 4.

Diagram illustrating *consensus* IECC diagnosis and immunohistochemical results. *For p16 and p53, positive means overexpressed as defined in text. **For p16, negative means patchy or absent expression; for p53, negative means wild type expression as defined in text.

Table 1

International Endocervical Adenocarcinoma Criteria and Classification (IECC) and World Health Organization (WHO) classification systems

International Endocervical Adenocarcinoma Criteria and Classification (IECC)		World Health Organization (WHO)
Main categories	Morphologic subcategories (optional)	
1. HPV-related endocervical adenocarcinoma	HPV adenocarcinoma, usual type	1. Usual type adenocarcinoma
	HPV adenocarcinoma, villoglandular type	2. Villoglandular adenocarcinoma
	HPV adenocarcinoma, mucinous NOS type	3. Mucinous adenocarcinoma NOS
	HPV adenocarcinoma, mucinous intestinal type	4. Mucinous adenocarcinoma, intestinal type
	HPV adenocarcinoma, signet-ring cell type	5. Mucinous adenocarcinoma, signet-ring cell type
	HPV adenocarcinoma, stratified mucin producing type	
2. Non-HPV adenocarcinoma, gastric type		6. Mucinous adenocarcinoma, gastric type
3. Non-HPV adenocarcinoma, endometrioid type		7. Endometrioid carcinoma
4. Non-HPV adenocarcinoma, clear cell type		8. Clear cell carcinoma
5. Non-HPV adenocarcinoma, mesonephric type		9. Mesonephric carcinoma
6. Non-HPV adenocarcinoma, serous type		10. Serous carcinoma
7. Invasive adenocarcinoma, NOS		11. Invasive adenocarcinoma, NOS

Table 2

Immunohistochemical antibody information

Antibody	Clone	Vendor	Protocol	Detection system
P16	JC8	Ventana	CC1-24,8	Ultraview
P53	DO-7	Ventana	CC1-76,32,+AMP	Ultraview
PR	16	Vector	CC1-64,32	Ultraview
Vimentin	V9	Ventana	CC1-36,16	Ultraview
CDX2	EPR2764Y	Ventana	CC136,16	Ultraview
GATA-3	L50-423	Ventana	CC1-32,32	Optiview
Napsin A	MRQ-60	Ventana	CC1-16,8	Optiview

Table 3

Interobserver agreement (Kappa) in the classification of Endocervical Adenocarcinoma as per IECC and WHO systems

All EA categories	H&E only*	H&E + IHC*
WHO	0.3 (0.28–0.33)	0.33 (0.31–0.35)
IECC	0.46 (0.42–0.49)	0.51 (0.47–0.55)
HPVA vs NHPVA	0.45 (0.42–0.48))	0.5 (0.47–0.53)
HPVA	0.46 (0.41–0.51)	0.54 (0.49–0.59)
NHPVA	0.53 (0.48–0.58)	0.64 (0.59–0.69)
EA–NOS	0.2 (0.16–0.26)	0.19 (0.14–0.24)

* Mean (95% confidence interval)

Table 4

Levels of agreement in the diagnosis of endocervical adenocarcinoma

Level of agreement (# reviewers)	WHO	IECC	HPVA vs NHPVA vs EA-NOS
None (3 or less)	19 (25%)	2 (3%)	2 (3%)
4	18 (24%)	4 (5%)	4 (5%)
5	18 (24%)	12 (16%)	12 (16%)
6	13 (17%)	15 (20%)	14 (19%)
Perfect (7)	7 (10%)	42 (56%)	43 (57%)

WHO=World health organization; IECC=International endocervical adenocarcinoma criteria and classification; HPVA= HPV-related adenocarcinoma; NHPVA=HPV-unrelated adenocarcinoma; EA-NOS=Endocervical adenocarcinoma, no otherwise specified

Table 5

Interobserver agreement (Kappa) among WHO HPVA subcategories

	H&E only*	H&E + IHC*
Usual	0.36 (0.31–0.41)	0.38 (0.33–0.43)
Villoglandular	0.22 (0.17–0.27)	0.22 (0.17–0.27)
Mucinous NOS	0.13 (0.1–0.18)	0.14 (0.09–0.19)
Intestinal	0.32 (0.27–0.37)	0.29 (0.24–0.34)
Signet ring cell	0.08 (0.03–0.13)	0.08 (0.03–0.13)
Stratified mucin producing	0.28 (0.23–0.33)	0.26 (0.21–0.31)

* Mean (95% confidence interval)

Table 6

Interobserver agreement (Kappa) among NHPVA categories

	H&E only*	H&E + IHC*
Gastric	0.63 (0.58–0.68)	0.76 (0.71–0.81)
Clear cell	0.81 (0.76–0.86)	0.5 (0.45–0.56)
Mesonephric	0.5 (0.45–0.55)	0.5 (0.45–0.55)
Serous	–0.008 (–0.06–0.04)	0.06 (0.007–0.11)
Endometrioid	0.012 (–0.06–0.04)	–0.012 (–0.06–0.04)

* Mean (95% confidence interval)