DNA methylation for cervical cancer screening: A validation trial in China

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Research

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Abstract

**Background** Based on published results from a training set, EPB41L3 and JAM3 gene methylation status had favorable results compared with high-risk human papillomavirus (hrHPV) testing. This validation trial evaluated the DNA methylation status and genotyping of hrHPV in unselected patients with gynecologic diseases.

**Methods** This study enrolled all patients in the study center from June 1, 2019 to September 1, 2019. Liquid-based samples were collected from cervical swabs for methylation assays and hrHPV testing one day before surgery. The primary endpoint was the diagnostic accuracies of DNA methylation and hrHPV genotyping for cervical intraepithelial neoplasia (CIN) 2 or more severe lesions (CIN2+).

**Results** In total, 307 patients were included in the final analysis, with a median age of 46 years. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the methylation assay for CIN2+ were 68.7%, 96.7%, 0.933 and 0.712, respectively, and the methylation assay achieved similar diagnostic accuracies to those in the training set and to hrHPV testing. Even in patients with negative hrHPV results, DNA methylation was significantly able to identify CIN2+ (odds ratio 39.857, 95% confidence interval 7.137-222.577). The methylation assay was sensitive for CIN2/3 and cervical squamous cell carcinoma without residual lesions and was not positive in this context.

**Conclusion** For the diagnosis of cervical adenocarcinoma, methylation assessment, hrHPV testing and their combination had favorable results in all subtypes.

Background

Uterine cervical cancer is one of most common causes of cancer-related deaths among women globally[1] and in China.[2] Chinese patients account for 28% of the total number of new cases of cervical cancer worldwide.[3] Robust and standard screening programs would significantly decrease the prevalence of cervical cancer.[4] Currently, cervical cytology and/or high-risk human papillomavirus (hrHPV) are the mainstream screening methods,[5, 6] however, both cytology and hrHPV have limitations in terms of their diagnostic accuracies.[7, 8] Thus, a cost-benefit analysis is essential for decision-making in cervical cancer screening.[9] A new screening strategy with high accuracy and feasibility is urgently needed in developing countries such as China. DNA methylation is an epigenetic mechanism that results in heritable silencing of genes without changes to their coding sequences.[10, 11] More than 100 human (host) genes have been reported to be possible methylation biomarkers of cervical cancer.[12] Genotyping and methylation markers are objective and can be used with self-obtained samples, offering great advantages in low- and middle-income settings.[9] Numerous studies have shown that methylation has a favorable screening sensitivity for cervical intraepithelial neoplasia (CIN) 2 or more severe lesions (CIN2+, or high-grade intraepithelial lesions [HSIL]) as a triage method in women with positive hrHPV status. Multiple panels have been utilized as classifiers consisting of dozens of candidate host genes, viral genes or both as well as various combinations thereof.[13]

In our previous exploratory study,[14] assessment of the combination of EPB41L3 and JAM3 methylation had a similar diagnostic accuracy to that of hrHPV testing for detecting CIN2+, which suggested that DNA methylation may be the preferred screening method regardless of hrHPV status. In this validation set, EPB41L3 and JAM3 gene methylation assessment and hrHPV genotyping were used to evaluate cervical liquid-based samples before major surgery from unselected patients with various diseases in a gynecologic oncology unit. The diagnostic accuracies of various screening strategies were compared.

Methods

**Ethical approval**

The institutional review board from the study center approved the study (No. JS-1954). All patients provided their consent before enrollment. The registration number is NCT03960879 (clinicaltrials.gov, registered on May 23, 2019). All procedures in
the study involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Study design

The study was performed in a prospective cohort with patients who were ready for conization, the loop electrosurgical excision procedure (LEEP) or hysterectomy for various gynecologic diseases. In such situations, the cervical histology could be evaluated sufficiently. Before surgery, liquid-based samples was collected from cervical swabs and analyzed for both DNA methylation and hrHPV genotyping. The accuracies of DNA methylation, hrHPV genotyping and the combination were compared for various surgically confirmed cervical pathological types. The primary endpoints were the diagnostic accuracies of DNA methylation and hrHPV in liquid-based cytology specimens for CIN2+. The secondary endpoints were the diagnostic accuracies of DNA methylation and hrHPV for CIN2+ with or without residual lesions and for specific pathological types, including cervical adenocarcinomas (ADCs) and endometrial and ovarian tumors.

Patient enrollment

This study enrolled all patients who were ready for surgery in one gynecologic oncology unit of the study center from June 1, 2019, to September 1, 2019. Data regarding the patients' epidemiological characteristics and medical histories were obtained from the medical records and supplemented by interviews with the patients. The inclusion criteria were as follows: aged 18 or older; confirmed CIN2/3 or with definite cytology and/or hrHPV screening results without intervention according to the screening guidelines of the American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology [5] and its updated version[6]; consent for conization, LEEP or hysterectomy, with relevant pathological results obtained; negative HIV results; no history of organ transplantation or use of immunosuppressive therapy; and willingness to participate in the study. Cases not meeting all the criteria were excluded. All histological materials were re-evaluated by two pathologists (YY and HW). For patients with CIN2/3 or cervical cancer as the primary diagnosis, pathological results before and after surgery were checked meticulously to confirm whether there were residual lesions, since biopsy or excision for diagnosis before the last surgery would eliminate the primary lesion.

Collection and assays of study materials

One day before surgery, a liquid-based sample was collected from the cervical swabs and stored in PreservCyt Solution (Thinprep Pap Test; Hologic, Marlborough, MA, USA) at room temperature.

The assays for DNA methylation and hrHPV have been described previously.[14] Methylation of the EPB41L3 and JAM3 genes was evaluated using TaqMan-based technologies with the Methylated Human EPB41L3 and JAM3 Gene Detection kit (real-time fluorescent polymerase chain reaction [PCR]) (Beijing SinoMDgene Technology Co., LTD, China) and an ABI 7300 Real Time Fluorescence Quantitative PCR system (Life Tech, USA). The methylation level of each gene was determined by the ΔCt value (the target gene Ct value subtracts the reference gene Ct value). Positive status was defined as a ΔCt value less than 7.945 and 9.250 for EPB41L3 and JAM3, respectively. Total methylation status was defined as positive EPB41L3 and/or JAM3 methylation.

No matter whether the patients previously received hrHPV testing or not, hrHPV genotyping was uniformly performed with TaqMan-based technology using an ABI 7500 Real Time Fluorescence Quantitative PCR system (Life Tech, USA) or a Stratagene Mx3000p Fluorescence Quantitative PCR system (Stratagene, USA) with an HPV nucleic acid genotyping diagnostic kit (Real time Fluorescent PCR) (Beijing SinoMDgene Technology Co., Ltd, China). The diagnostic kit detects a pooled result for hrHPV types, including HPV 16, 18, 31, 33, 45, 52, 6, 11, 35, 51, 39, 59, 68, 56, 58, and 66, with type-specific probes.

Statistics
Nonnormally distributed variables and categorical data were compared between different screening groups by using nonparametric tests. The specificity, sensitivity, negative predictive value (NPV) and positive predictive value (PPV) were also calculated for various screening groups. The odds ratio (OR) and 95% confidence intervals (95% CIs) of the positive ratios of different screening methods for various histological types were calculated with Logistic regression models. Unless otherwise stated, all analyses were performed with a two-sided significance level of 0.05 and were conducted with the use of Statistical Product and Service Solutions Statistics 20.0 (IBM Corporation, Armonk, NY, USA).

**Results**

**Patient characteristics**

A flow diagram of the study is provided in Figure 1. From June 1, 2019, to September 1, 2019, 368 eligible patients were recruited, and 307 were included in the final analysis. The median age was 46 years (range 22 to 77). Regarding primary diagnoses, there were 41 cases of benign ovarian or uterine disease, 101 of CIN2/3, 66 of cervical cancer, and 99 of ovarian or uterine malignancies, precancerous lesions, or borderline tumors (Table 1 and Supplement 1). The surgeries comprised 84 cases of conization or LEEP and 223 hysterectomies. The final cervical pathology results are listed in Table 1 and Figure 1. The median interval from biopsy to conization/LEEP or from conization/LEEP to hysterectomy/radical hysterectomy was 40 days (range 10 to 51).

Table 1
Cervical procedures and pathology before and after sampling for DNA methylation. Three cases were excluded from the table, including one cervical mucinous adenocarcinoma, one uterine clear cell carcinoma, and one vaginal melanoma. ADC, adenocarcinoma. CIN, cervical intraepithelial lesion. LEEP, loop electrosurgical excision procedure. SCC, squamous cell carcinoma.

| Primary diagnosis | Cervical methylation procedures before DNA methylation (n) | Surgeries after DNA methylation (n) | Actual cervical pathology after DNA methylation (n) |
|------------------|-------------------------------------------------------------|-----------------------------------|-----------------------------------------------|
| Benign (n=41)    | None (41) Biopsy (0) Conization or LEEP (0) | Conization LEEP (0) or Hysterectomy (41) | Inflammation (41) |
| CIN2/3 (n=101)   | 0 84 17                                                | 84 17                            | Inflammation (16) CIN1 (n=1) CIN2 (n=17) CIN3 (n=55) |
| Cervical carcinomas (n=66) | 0 37 29                                                | 0 65                            | Inflammation (n=21) CIN1 (n=1) SCC (n=25) ADC, villoglandular type (n=3) ADC, in situ (n=6) ADC, endocervical type (n=9) ADC, mucinous type (n=1) |
| Others* (n=99)   | 99 0 0                                                 | 0 99                            | Inflammation (96) CIN1 (n=1) Endometrial clear cell carcinoma (n=1) Vaginal melanoma (n=1) |

* Others includes gynecological malignancies, precancerous lesions, or borderline tumors rather than cervical lesions.

DNA methylation and hrHPV assays were successfully performed for all 307 patients. There were 68 (22.1%), 103 (33.6%), 109 (35.5%), 131 (42.7%) and 72 (23.5%) cases with positive EPB41L3, JAM3, EPB41L3 or JAM3, hrHPV and methylation plus hrHPV, respectively. The details are listed in Supplementary Table 1. In the whole cohort, patients with positive and negative methylation had similar ages (45.9±10.0 versus 47.9±12.1 years, p=0.143); however, patients with positive hrHPV were significantly younger than patients with negative hrHPV (42.8±10.5 versus 50.4±11.1 years, p<0.001).

**Diagnostic accuracies for cervical lesions**

The diagnostic accuracies of the various screening methods are listed in Table 2. The positive methylation status rates for normal pathology in the whole cohort and in the cohort with exclusions for gynecological malignancies, precancerous lesions, and borderline tumors were 26.0% (25/96) and 3.9% (3/77), respectively (Table 2). Therefore, the normal pathology of benign diseases served as a reference. As shown in Table 2, methylation, hrHPV status and their combination were all significantly related to the severity of the cervical lesion. However, compared with the reference, DNA methylation and methylation plus
hrHPV achieved only marginal differences in CIN2 (with p values of 0.055 and 0.065), probably due to the small sample size for this pathology.

### Table 2

The diagnostic accuracies of DNA methylation, hrHPV and their combinations for the final cervical pathology. ADC, adenocarcinoma. CIN2+, cervical intraepithelial neoplasia (CIN) 2 or more severe lesion. hrHPV, high-risk human papillomavirus. N/A, not available. NPV, negative predictive value. OR, odds ratio. 95% CI, 95% confidence interval. PPV, positive predictive value. SCC, squamous cell carcinoma.

| Cervical pathology | DNA methylation | hrHPV | HPV16 or HPV18 | DNA methylation + hrHPV |
|--------------------|-----------------|-------|---------------|------------------------|
|                   | n (%) OR CI p   | n (%) OR CI p | n (%) OR CI p | n (%) OR CI p |
| Normal in benign diseases* (n=77) | 3 (3.9) Reference | 18 (23.4) Reference | 11 (14.3) Reference | 1 (1.3) Reference |
| Normal in others † (n=96) | 25 (26.0) (2.511-30.405) 0.001 | 6 (6.5) (1.982-21.683) 0.002 | 5 (6.6) (3.273-10.460) 0.049 | 2 (2.2) Reference |
| CIN1 (n=15) | 0 (0.0) N/A | 10 (66.7) (6.556) | 5 (33.3) (3.000) | 0 (0.0) Reference |
| CIN2 (n=17) | 3 (17.6) (5.286) 0.055 | 13 (76.5) (10.653) | 6 (35.3) (3.273) | 2 (11.8) Reference |
| CIN3 (n=55) | 38 (69.1) (55.137) 0.001 | 50 (90.9) (32.778) | 33 (60.0) (9.000) | 35 (63.6) Reference |
| SCC (n=25) | 24 (96.0) (592.00) 0.001 | 22 (88.0) (24.037) | 21 (84.0) (31.500) | 22 (88.0) Reference |
| ADC (n=18) | 14 (77.8) (17.392-428.568) 0.001 | 14 (77.8) (3.353-39.255) | 12 (66.7) (3.726-38.646) | 11 (61.1) Reference |

* These cases excluded gynecological malignancies, precancerous lesions, or borderline tumors rather than cervical lesions.
† These cases only included gynecological malignancies, precancerous lesions, or borderline tumors rather than cervical lesions.

For the diagnosis of CIN2+, the sensitivities, specificities, PPVs and NPVs of the methylation assay in the previous training set [14] and in the current trial were all equal: 72.1% versus 68.7% (p=0.525), 91.5% versus 96.7% (p=0.120), 0.679 versus 0.712 (p=0.331), and 0.679 versus 0.863 (p=0.002), respectively.

For the diagnosis of CIN2+, assays for methylation assessment, hrHPV testing, HPV 16/18 testing and their combination all had favorable results, with ORs of 69.105 (95% CI 19.294-219.611), 14.143 (7.094-28.197), 7.953 (4.118-15.362) and 141.556 (19.043-1052.225), respectively (Table 2).

- The sensitivity values of methylation assessment and the combination were lower than that of hrHPV testing (68.7%, 60.9% versus 86.1, p = 0.002 and <0.001), but that of methylation assessment was similar to that of HPV 16/18 testing (68.7% versus 62.6, p=0.331).
- The specificity values of both methylation assessment and the combination were significantly higher than that of hrHPV testing (96.7% and 98.9% versus 6.96, p <0.001 and <0.001), and that of methylation assessment was also significantly higher than that of HPV 16/18 testing (96.7% versus 82.6, p=0.002).
- The PPVs of both methylation assessment and the combination were significantly higher than that of hrHPV testing (0.963 and 0.986 versus 0.780, p <0.001 and <0.001), and that of methylation assessment was also significantly higher
than that of HPV 16/18 (0.963 versus 0.818, p=0.003).

- The NPV of methylation assessment, but not the combination, was similar to that of hrHPV (0.712 and 0.669 versus 0.800, p = 0.158 and 0.039), and that of methylation assessment was also similar to that of HPV 16/18 (0.712 versus 0.639, p=0.221).

Even in patients with negative hrHPV results, DNA methylation assessment still had a significant discrepancy for CIN2+ (OR 39.857, 95% CI 7.137-222.577). In this population, the sensitivity, specificity, PPV and NPV were 56.3%, 96.9%, 0.818 and 0.899, respectively.

Further analysis of CIN2/3 and cervical squamous cell carcinoma (SCC) is presented in Table 3. Regarding SCC with or without residual lesions, significant differences in the methylation assay (p<0.001) and in hrHPV testing (p=0.004) were evident; regarding CIN2/3 with or without residual lesions, significant differences in the methylation assay (p<0.001) and in hrHPV testing (p=0.004) were evident. Regarding CIN2/3 and SCC without residual lesions after biopsy or excision, their methylation statuses were similar to those of benign uterine and ovarian lesions (p = 0.999 and 0.998). Regarding cervical diseases with or without residual lesions, the positive hrHPV ratios were all significantly higher than those of benign uterine and ovarian lesions (all p values less than 0.05, Table 3).

Table 3
The diagnostic accuracies of DNA methylation, hrHPV and their combination for cervical SCC and CIN2/3 with or without residual lesions. CIN, cervical intraepithelial neoplasia. hrHPV, high-risk human papillomavirus. N/A, not available. OR, odds ratio. 95% CI, 95% confidence interval. SCC, squamous cell carcinoma.

| Cervical pathology                  | DNA methylation | hrHPV | DNA methylation + hrHPV |
|------------------------------------|-----------------|-------|-------------------------|
|                                    | n (%)           | OR (95% CI) | p       | n (%)           | OR (95% CI) | p       | n (%)           | OR (95% CI) | p       |
| Normal* (n=41)                     | 3 (7.3)         | Reference  | -      | 2 (4.9)         | Reference  | -      | 1 (2.4)         | Reference  | -      |
| SCC without residual lesions (n=16)| 0 (0.0)         | 0.000 (0.000-N/A) | 0.999 | 7 (43.8)       | 15.167 (2.687-85.598) | 0.002 | 0 (0.0)       | 0.000 (0.000-N/A) | 0.999 |
| SCC with residual lesions (n=25)   | 24 (96.1)       | 304.000 (29.871-3093.870) | <0.001 | 22 (88.0)       | 143.000 (22.173-922.234) | <0.001 | 22 (88.0)      | 293.333 (28.764-2991.426) | <0.001 |
| CIN2/CIN3 without residual lesions (n=29)| 0 (0.0) | 0.000 (0.000-N/A) | 0.998 | 18 (62.1) | 31.909 (6.399-159.128) | <0.001 | 0 (0.0) | 0.000 (0.000-N/A) | 0.998 |
| CIN2/CIN3 with residual lesions (n=72)| 41 (56.9) | 16.753 (4.730-59.332) | <0.001 | 63 (87.5) | 136.500 (28.021-664.933) | <0.001 | 37 (51.4) | 42.286 (5.513-324.365) | <0.001 |

* These cases only included uterine or ovarian benign diseases

For the diagnosis of cervical ADC, methylation, hrHPV and their combination all had favorable results (Table 4). According to various subtypes of ADC, compared with uterine or ovarian benign diseases, methylation (OR 44.333, 95% CI 6.230-315.499), hrHPV (156.000, 12.575-1935.197) and their combination (80.000, 7.111-900.008) had the highest ORs in endocervical ADC, the most common pathology in this cohort. However, due to the limited sample size of ADC, the differences between the various subtypes of ADC were not significant.

Table 4
The diagnostic accuracies of DNA methylation, hrHPV and their combination for cervical adenocarcinoma. ADC, adenocarcinoma. hrHPV, high-risk human papillomavirus. N/A, not available. NPV, negative predictive value. OR, odds ratio. 95% CI, 95% confidence interval. PPV, positive predictive value. SCC, squamous cell carcinoma.
A number of studies have explored the role of a panel that includes EPB41L3, JAM3, and both 8, 26-30 for the screening or triage of HSIL and/or cervical cancer. In this validation trial, the performance of DNA methylation assessment based on the EPB41L3 and JAM3 genes was similar to that of our training set, 14 and the assessment showed favorable results in identifying CIN2+ or ADC. The sensitivities, specificities, PPVs, and NPVs of the methylation assay for CIN2+ in our

**Discussion**
precancerous lesions. Overexpression of DNA methyltransferase 1 microRNA is mainly observed in endometrial carcinomas in addition, 7 cases of EIN were negative for methylation. There is very limited evidence of methylation in uterine or ovarian malignancies, an intrauterine tool may be able to obtain higher number methylation-positive cells than a cervical swab. In limitations of cytological pathology in the diagnosis of endometrial or ovarian tumors. To assess methylation in uterine however, DNA methylation was detected in more than half (55.0%) of endometrial carcinomas. These differences reflect the

ovarian tumors (Table 5). Regarding ovarian tumors, only 12.2% of patients with EOC had positive methylation expression;[42-47] ovarian cancer tissues,[48-50] or both.[51, 52] In our current study, we enrolled more patients with endometrial and ovarian tumors (Table 5). Regarding ovarian tumors, only 12.2% of patients with EOC had positive methylation expression; however, DNA methylation was detected in more than half (55.0%) of endometrial carcinomas. These differences reflect the limitations of cytological pathology in the diagnosis of endometrial or ovarian tumors. To assess methylation in uterine malignancies, an intrauterine tool may be able to obtain higher number methylation-positive cells than a cervical swab. In addition, 7 cases of EIN were negative for methylation. There is very limited evidence of methylation in uterine or ovarian precancerous lesions. Overexpression of DNA methyltransferase 1 microRNA is mainly observed in endometrial carcinomas
rather than normal tissues or EINs.[53] In the study by Marichereda et al.,[54] methylation of the SFRP2 gene in the endometrial tissues of patients with hyperplastic processes was above 20-25%. However, gene ontology analysis has shown differential methylation at linked CpG sites between low-grade serous carcinomas and serous borderline tumors.[55]

The strength of this study is its relatively large cohort with unselected gynecologic diseases. The detailed pathological results before and after major surgery (also before and after methylation assessment) provide robust validation of EPB41L3 and JAM3 as tools for cervical and even endometrial lesion screening. However, there are several limitations in our study. First, we did not compare the methylation assay results to the cytology results. As most patients with cervical lesions underwent cervical procedures before the last surgery (median time 40 days), we doubted that the results of the cytology assay would be unaffected by surgery. DNA methylation analysis of HPV-positive self-obtained samples was not inferior to cytology triage in the detection of CIN2+. [56] The effectiveness of methylation assessment versus cytology with or without hrHPV testing should be assessed in community-based population in the future. Second, we did not follow up on the prognosis of the patients after major surgery, limiting the interpretation of DNA methylation results in the carcinogenesis and progression of cervical cancer.[57] Third, we excluded patients who were positive for HIV from the training and validation sets. This limitation would hamper the extrapolation of the DNA methylation assay to a large cohort, as reported previously.[58, 59] Lastly, the relatively low prevalence of positive methylation in CIN2/3 indicates that further pathological and molecular analysis is needed for these pathological types.

Conclusion

In this validation trial from China, we discovered that methylation of EPB41L3 plus JAM3 had a favorable diagnostic accuracy for CIN2+, which is consistent with our previous training set. Our findings suggest the methylation assessment of the EPB41L3 and JAM3 genes could be an independent screening method regardless of hrHPV status. Specifically, methylation assessment of liquid-based samples is favorable for identifying cervical ADCs and endometrial carcinomas.

Abbreviations

95% CIs - 95% confidence intervals
ADCs - cervical adenocarcinomas
CIN - cervical intraepithelial neoplasia
CIN2+ - CIN2 or more severe lesions
hrHPV - high-risk human papillomavirus
HSIL - high-grade intraepithelial lesions
LEEP - loop electrosurgical excision procedure
NPV - negative predictive value
OR - odds ratio
PCR - polymerase chain reaction
PPV - positive predictive value
SCC - squamous cell carcinoma

Declarations
**Ethics approval and consent to participate**

The institutional review board of Peking Union Medical College Hospital has approved this study (No. JS-1954). The registration number is NCT03960879 (clinicaltrials.gov, registered on May 23, 2019).

**Consent for publication**

Consent for publication has been obtained from every patient.

**Availability of data and materials**

All data for this study are included in the supplemental file.

**Competing interests**

All authors declare that they have no conflicts of interest to disclose.

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**Authors' contributions**

PL and LL conceived of the original idea for the study, interpreted the results, carried out the statistical analysis, edited the paper and were the overall guarantors. LK, LW, ZW, XX and LL obtained ethical approval, collected study samples, contributed to the preparation of the data set, interpreted the results and contributed to drafting the paper. LW, ZW and PL carried out the DNA methylation analysis. YY and HW conducted the pathological evaluations. XX and MW contributed to the study design and interpretation of results and commented on drafts of the paper. All authors have approved the final version of the manuscript.

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None

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**Figures**
Patients were recruited from June 1 to September 1, 2019 (n=368)

Exclusion (n=61):
- Not meeting the inclusion criteria (n=8)
- Not willing to participate the study (n=48)
- Unable to provide cytology sample (n=5)

In total 307 cases were included
- Benign (n=41)
- CIN2/CIN3 (n=101)
- Cervical cancer (n=66)
- Other tumors (n=99)

Surgeries including:
- Conization or LEEP (n=84)
- Hysterectomy (n=223)

Final cervical pathology:
- Normal or inflammation (n=173)
- CIN1 (n=16)
- CIN2 (n=17)
- CIN3 (n=55)
- SCC (n=25)
- ADC (n=19)
- Endometrial CCC (n=1)
- Vaginal melanoma (n=1)

**Figure 1**

Flow diagram of the study. ADC, adenocarcinoma. CCC, clear cell carcinoma. CIN, cervical intraepithelial neoplasm. LEEP, loop electrosurgical excision procedure. SCC, squamous cell carcinoma.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- Supplement1.xlsx