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ORIGINAL RESEARCH

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Clinical trial of HPV mRNA testing for uterine cervical cancer screening in Kitakyushu city

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Abstract

Background: We investigated the clinical usefulness of HPV (Human Papillomavirus) mRNA testing for uterine cervical cancer screening in Kitakyushu City. **Methods**: Based on the guidelines, cervical cancer screening through cytology was conducted for women aged 20 and above. Among those screened, HPV mRNA testing (Aptima HPV) was performed on 763 women aged 30–69 years who consented to the screening between 23 June and 06 October 2022. **Results**: Of the 2456 patients who underwent cervical cancer screening, 24 (0.98%) showed abnormal cytological results: ASC-US (atypical squamous cells of undetermined significance) (9 cases), LSIL (low-grade squamous intraepithelial lesion) (8 cases), HSIL (high-grade squamous intraepithelial lesion) (3 cases), ASC-H (atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion) (3 cases) and AGC (atypical glandular cells) (1 case). Among the 763 individuals who underwent HPV mRNA testing, 35 (4.6%) tested positive for HPV. Statistical analysis revealed a significant difference in HPV mRNA positivity rates across age groups ($p < 0.001$). The positivity rates were highest in the 30s age group (13.4%) and lowest in the 60s age group (2.2%). The HPV mRNA positivity rate observed in this study was lower compared to HPV DNA testing methods reported in other studies. This suggests that the HPV mRNA test may have different performance characteristics compared to traditional HPV DNA tests. **Conclusions**: Based on these findings, Kitakyushu City is considering the introduction of HPV mRNA testing for cervical cancer screening, as it may offer a unique perspective on HPV detection and could potentially enhance detection rates and screening coverage.

Keywords

Cancer screening; HPV mRNA testing; Uterine cervical cancer; Kitakyushu city

1. Introduction

Uterine cervical cancer is the most common primary gynecological malignant disease in Japan, with an annual estimate of 10,000 new cases and 2800 deaths. Since 1990, both the number of patients and the mortality rate have been on the rise [1]. The disease progresses slowly from preinvasive cervical intraepithelial neoplasia to invasive cancer [2]. Screening asymptomatic women with regular Papanicolaou (Pap) smears allows the diagnosis of the readily treatable preinvasive phase [3]. Since 1998, the Japanese guidelines for cancer screening have been developed and revised by a research group funded by the Ministry of Health, Labor and Welfare. Accordingly, biennial cervical cytologic testing has been recommended as a uterine cervical cancer screening test for women aged $\geq$20 years since 2004. However, the incidence of early cervical neoplasms and invasive cancers has gradually increased among young women [1]. Unfortunately, the consultation rate for cervical cancer screening in Japan remains markedly low, at 30–40% of prescribed individuals, in comparison to screening in other developed countries (70–80%) [4]. In 2001, only 15,501 women (6.8%) underwent a Pap test in Kitakyushu City, which was less than half of the national average. The introduction of free coupons and promotional activities has extended the scope of cervical cancer screening almost nationwide. However, the number of attendees has plateaued in recent years, with approximately 10,000 people remaining below the peak number of approximately 35,000 people (Fig. 1). Cervical cancer screening targets individuals aged 20 and above, with approximately 280,000 people falling into this category. However, the screening rate for the relevant year is about 8%.

Human papillomavirus (HPV) is one of the most common causes of sexually transmitted diseases in men and women worldwide. Numerous studies have established a correlation between HPV, squamous intraepithelial lesions, and uterine cervix conditions [2, 5–7]. HPV testing has already been incorporated as a part of screening in various countries, and some local governments in our nation have implemented screenings that combine cytology and HPV testing [8] (Table 1). In July

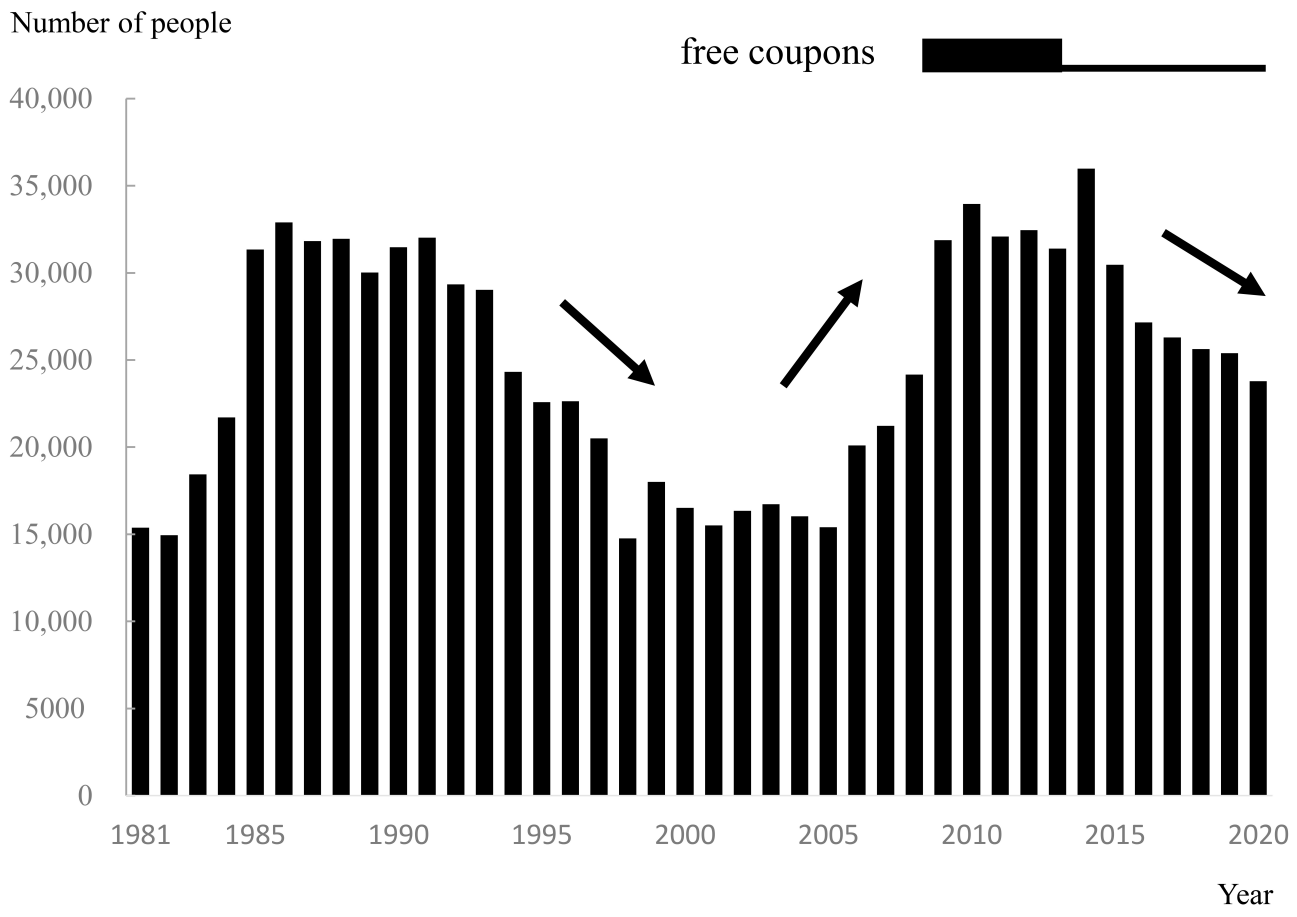

FIGURE 1. The number of the women receiving cervical tests in Kitakyushu city.

TABLE 1. Cervical cancer screening guidelines across countries.

Country	Population	Year	HPV test
Netherlands	1770	2017	HPV test alone (30~35 every 5 yr 40~60 every 10 yr)
Australia	2601	2017	HPV test alone every 5 yr (25~74)
Portugal	1041	2017	HPV test alone every 5 yr (25~60)
Spain	4778	2019	Cytology alone every 3 yr (25~30), HPV test alone every 5 yr (30~65)
Italy	5894	2020	Cytology alone every 3 yr (25~64), HPV test alone every 5 yr (30~64)
UK	6697	2020	HPV test alone (25~49 every 3 yr 50~64 every 5 yr)
Germany	8380	2020	Cytology alone every 1 yr (20~34), HPV + Cytology every 3 yr (35~65)
USA	33,330	2020	Cytology alone every 3 yr (25~65), HPV + Cytology every 5 yr (25~65) HPV test alone every 5 yr (25~65)
New Zealand	512	2023	HPV test alone every 5 yr (25~69)
Canada	3893	2021	Cytology alone every 3 yr (25~69)
Mexico	12,750	2023	Cytology alone every 3 yr (25~64)
Sweden	1049	2023	HPV test alone every 5 yr (23~70)
Norway	546	2023	HPV test alone every 5 yr (25~69)
Denmark	590	2023	HPV test alone every 3 or 5 yr (23~64)

(Ten thousand persons)

HPV: human papillomavirus; yr: year; UK: United Kingdom; USA: United States of America.

2020, the National Cancer Center of Japan, a national research and development institution, released the "Cervical Cancer Screening Guidelines Based on Effectiveness Evaluation". In these guidelines, both cytology alone and HPV testing alone are classified as Grade A recommendations [9].

The Japanese guidelines for uterine cervical cancer screening have been revised in April 2024. Traditional cytology-based screening has been supplemented with screening using HPV testing alone. However, currently, no municipalities have yet started using the HPV testing alone method. In our country, cervical cancer screening is particularly recommended for individuals aged 20 to 69 years old. In the new guidelines, HPV testing is recommended for women aged 30 and older, with testing conducted once every five years. Under the current Japanese guidelines for cervical cancer screening, if cytology results indicate ASC-US, an HPV test is conducted. Colposcopy and punch biopsy are performed when the HPV test is positive. If the colposcopy shows no abnormalities, endocervical curettage is conducted. If the histopathology results are negative, it is common in Japan to follow up every six months. Cytology and HPV testing are recommended one year later when the HPV test is negative. In the HPV testing alone method, if the HPV test result is negative, the next screening will be conducted five years later. If the HPV test result is positive, a cytology test will be performed. If the cytology test shows ASC-US or higher, colposcopy and punch biopsy will be conducted. If the cytology test result is negative, the next HPV test will be conducted one year later.

In Japan, five HPV testing kits are covered by insurance. Each testing kit comes with unique features, including HPV type, result display method, measurement site, and HPV DNA or mRNA. The Aptima HPV Assay, for instance, uses HPV E6/E7 mRNA as the target gene to enhance specificity and reduce the occurrence of false positives. This assay allows detection of 14 high-risk human papillomavirus (HPV) RNA types in cervical specimens (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) [10]. This approach has the potential to identify patients with a higher risk of developing cervical cancer while minimizing false-positive rates.

This study aimed to investigate the clinical usefulness of HPV mRNA testing for uterine cervical cancer screening in Kitakyushu City, because few papers about uterine cervical cancer screening with Aptima (HPV mRNA Testing) have been reported in Japan.

We will confirm the HPV test positivity rate, identify the number of people referred for further testing, and determine the number of individuals who, with both negative cytology and HPV tests, can extend their next screening to five years later. This information will be used to consider the future cost-effectiveness.

2. Materials and methods

Based on the guidelines, cervical cancer screening through cytology was conducted for women aged 20 and above. Among those screened, the HPV test was explained to participants aged 30 to 69, and for those who consented, the HPV test was also performed on a trial basis simultaneously. The target population consisted of women aged 30–69 years, excluding those who were pregnant and had undergone cervical cancer screening under the Health Promotion Act (population-based screening) at four facilities in Kitakyushu City between 23 June and 06 October 2022. These women were provided with explanations of HPV testing using Aptima HPV (Hologic Japan, Inc.) during their visits, and consent was obtained. Following the current system of the Kitakyushu Medical Association, cytology specimens (glass slides) were submitted to the Kitakyushu Cytologic Examination Association. Further, HPV test samples were submitted to SRL, Inc., where they were determined to be either positive or negative.

Cervical cancer screening is conducted at primary healthcare facilities, and if abnormalities are detected through cytology, colposcopy and punch biopsy are performed at specialized healthcare facilities. Since this study focuses on data from primary healthcare facilities where cervical cancer screening was conducted, we did not have access to biopsy results.

Pearson's chi-squared test and an agreement test with the Cohen's Kappa coefficient were carried out. These statistical analyses were performed using the SPSS software program for Windows, version 28.0.1 (IBM, New York, NY, USA). A p-value $< 5\%$ was considered to be statistically significant.

3. Results

During the specified period, a total of 2456 women aged $\geq$20 underwent cervical cancer screening. Among these, 24 (0.98%) had abnormal cytological results. The content of the 24 cases was as follows: ASC-US (9 cases), LSIL (8 cases), HSIL (3 cases), ASC-H (3 cases), AGC (1 case). Abnormal cytology results were detected in 1.3% (4/307) individuals in their 20s, in 2.4% (8/340) individuals in their 30s, in 0.7% (4/587) individuals in their 40s, in 0.9% (5/585) individuals in their 50s, and in 1.0% (3/315) individuals in their 70s and above. The highest rate was observed in the 30s age group (Table 2).

Table 3 presents the screening results outcomes from a combination of cytology and HPV testing among 763 individuals aged 30–69. Among women who underwent cervical cancer screening and were eligible for HPV testing (aged 30–69), 1834 individuals were identified. Of this group, 763 (41.6%) underwent HPV testing, and 35 (4.6%) tested positive for HPV. Positive HPV test results were detected in 13.4% (17/127) individuals in their 30s, in 4.8% (12/251) individuals in their 40s, in 1.2% (3/251) individuals in their 50s, in 2.2% (3/134) individuals in their 60s. The rate of HPV positivity was highest among women aged 30–39 years and it reached 13.4% (17/127). When categorized by age, there was a significant difference in the number of individuals testing positive for HPV, as evidenced by Pearson's chi-squared test ($p < 0.001$). An agreement test with the Cohen's Kappa coefficient was 0.234.

A total of 0.8% (6/763) individuals exhibited abnormal cytological results. Among them, one tested negative for HPV (indicating endometrial cancer), whereas five individuals tested positive for HPV. Within the test group, 30 individuals (3.9%) had negative cytology results but positive HPV test results (HPV+/Pap−). Notably, 727 of 763 people (95.3%) tested negative for both cytology and HPV (HPV−/Pap−),

TABLE 2. Pap test results in the age bracket.

Age Group, yr	NILM	Abnormal Pap	ASC-US	LSIL	HSIL/ASC-H	AGC
20–29 (n = 307)	303 (98.7%)	4 (1.3%)	2	1	1	0
30–39 (n = 340)	332 (97.6%)	8 (2.4%)	2	3	3	0
40–49 (n = 587)	583 (99.3%)	4 (0.7%)	1	2	1	0
50–59 (n = 585)	580 (99.1%)	5 (0.9%)	2	2	0	1
60–69 (n = 322)	322 (100.0%)	0 (0.0%)	0	0	0	0
Above 70 (n = 315)	312 (99.0%)	3 (1.0%)	2	0	1	0
Total = 2456	2432 (99.02%)	24 (0.98%)	9	8	6	1

NILM: negative for intraepithelial lesion or malignancy; Pap: Papanicolaou; ASC-US: atypical squamous cells of undetermined significance; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; ASC-H: atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion; AGC: atypical glandular cells.

TABLE 3. The screening results using a combination of cytology and HPV testing.

Age Group, yr	HPV−/Pap−	HPV+/Pap−	HPV−/Pap+	HPV+/Pap+
20–29	-	-	-	-
30–39 (n = 127)	110 (86.6%)	15 (11.8%)	0 (0.0%)	2 (1.6%)
40–49 (n = 251)	239 (95.2%)	11 (4.4%)	0 (0.0%)	1 (0.4%)
50–59 (n = 251)	247 (98.4%)	1 (0.4%)	1 (0.4%)	2 (0.8%)
60–69 (n = 134)	131 (97.8%)	3 (2.2%)	0 (0.0%)	0 (0.0%)
Above 70	-	-	-	-
Total = 763	727 (95.3%)	30 (3.9%)	1 (0.1%)	5 (0.7%)

HPV: human papillomavirus; Pap: Papanicolaou.

indicating an extremely low risk of progression to invasive cervical cancer. Therefore, extending the screening intervals for these individuals may be considered.

4. Discussion

In 2005, the World Health Organization (WHO) recommended that countries establish national cancer control programs to reduce the number of deaths attributed to preventable cancers and improve the quality of life for cancer patients and their families. Cervical cancer is well understood in terms of processes leading to invasive cancer, and HPV is evidently involved in its development [2, 5–7]. The prevention of cervical cancer is drawing attention as a eradicable "cancer" through primary prevention with HPV vaccination before sexual activity and secondary prevention through early detection of precancerous lesions via cancer screening [11]. Conventional cervical testing has been shown to be extremely effective in reducing cervical cancer incidence and mortality [3]. Nevertheless, the incidence of early cervical neoplasms and invasive cancers has been gradually increasing among young women in Japan [1]. The population with cervical cancer screening experience remains comparatively small compared to that in other developed countries [4].

Prophylactic HPV vaccination for young girls has been implemented in most developed countries. Recognizing the significance of cervical cancer and other HPV-related diseases as global health problems, the WHO recommends the inclusion of routine HPV vaccinations in national immunization programs [12, 13]. In Japan, the Ministry of Health, Labor and Welfare suspended the active recommendation for HPV vaccination from June 2013 to April 2022 following highly publicized cases of alleged adverse events (complex regional pain syndrome (CRPS)) in girls who had been vaccinated. Consequently, the vaccination rates are extremely low, posing a significant issue.

The involvement of high-risk HPV types, including HPV 16 and 18, in the development of cervical cancer is well-established [2, 14, 15]. According to the current US cervical cancer screening guidelines (American Cancer Society), the recommended age to commence screening is 25 years. For women aged 25–65 years, practitioners may choose to perform cytologic and HPV co-testing or opt for HPV testing alone every 5 years, or alternatively, perform cytologic testing alone every 3 years [16]. Furthermore, findings from four European randomized controlled trials showed that HPV-based screening provided 60–70% greater protection against invasive cervical carcinomas than cytology [17]. Consequently, HPV testing has become a mainstream technique for cervical cancer screening, and HPV testing is adopted in each country according to its specific circumstances [18].

The detection rate of abnormal smears (ASC-US+) in this study, at only 0.98%, was notably lower than typically reported in similar studies [19–21]. In Kitakyushu City, cervical cancer screening by cytology is conducted annually, and due to a high number of repeat attendees, such as those with a history of previous screenings, the cytology positivity rate is believed to be lower compared to other reports. In the current screening

trial using a combination of cytology and HPV testing, the HPV positivity rate was 4.6%. This study marks the first report in Japan using data from cervical cancer screening with Aptima. Notably, this rate was lower than that reported in previous studies using other testing methods targeting HPV DNA (positive rate: 5.1~15.6% by HC2 (hybrid capture II) [19, 22–30] (Table 4). The Aptima HPV Assay targets HPV mRNA, and its high specificity suggests the potential to reduce the occurrence of false positives, allowing the identification of patients at a higher risk of developing cervical cancer [29, 31–34]. With the combined method of cytology and HPV testing, an increase of 101 false positives is observed per 1000 screenings (Out of 1000 women, 101 were HR (high risk) HPV positive with negative cytology) [9]. However, in the current study, the use of HPV testing led to only a 3.9% (HPV+/Pap−). In other words, since Aptima targets the mRNA of HPV, it results in a lower positivity rate and is likely capable of identifying true risk cases. A positive HPV DNA test indicates the presence of HPV DNA above a certain level in infected cervical cells, and HPV infections are usually transient. In contrast, the presence of high risk HPV E6/E7 mRNA in cervical cells more accurately detects patients at risk for developing precancerous high-grade cervical intraepithelial lesions and invasive cervical cancer than the presence of high risk HPV DNA [35]. According to the current American Society for Colposcopy and Cervical Pathology (ASCCP) guidelines, patients with normal cytology results may be referred for colposcopy according to their high-risk HPV test results. A higher positive predictive value (PPV) of high-risk HPV has significance for preventing unnecessary colposcopic examinations [36]. On the other hand, women with negative results in both cytology and HPV tests exhibit an extremely low likelihood of developing invasive cervical cancer. Therefore, as followed in many overseas settings, subsequent screenings are scheduled five years later [16, 17, 37]. Adjusting screening intervals based on results to anticipate risk is considered highly useful in terms of cost-effectiveness. When HPV testing is introduced, managing individuals who test positive for HPV becomes extremely important. It is anticipated that the burden on gynecologists will increase due to the rise in the number of patients requiring colposcopies and biopsies. However, currently, screenings using cytology are conducted annually in Kitakyushu City. If HPV testing is introduced, those who test negative will have their next screening scheduled for five years later, potentially leading to budget savings for the city. Considering that screening targets asymptomatic individuals, high specificity in HPV testing would be particularly beneficial.

The most crucial consideration in the introduction of HPV testing for cervical cancer screening lies in the management of women testing positive for HPV. HPV testing detects “infection” rather than “lesions”, indicating the absence of evidence of lesions at the time of testing. Although most infections are transient, a small percentage of individuals may experience persistent infection, leading to the potential development of disease several years later, categorizing them as “risk carriers”. This raises concerns about psychological burdens and disadvantages and the possibility of overdiagnosis. To address these concerns, providing a thorough explanation and establishing a follow-up management system (algorithm) are crucial. In this context, screening management tailored to individual risks has been proposed, considering cytology results and HPV test outcomes [11, 37–39]. Aside from cervical cancer, HPV-related diseases include oropharyngeal cancer and penile cancer, which are increasing in various countries. Moving forward, it is essential to globally reduce HPV-related diseases through primary prevention with HPV vaccination for both men and women, and secondary prevention using molecular biological methods such as HPV testing [40].

In Kitakyushu City, the introduction of HPV testing for cervical cancer screening is under consideration after examining various challenges, with the goal of identifying individuals who have not undergone screening. To increase the screening rate, disseminating accurate information and supporting appropriate decision-making are crucial.

TABLE 4. HPV tests positive rate.

Study	Ref.	Country	Target Age (yr)	Year	HPV test	Positive rate (%)
Artistic	[22]	UK	20–64	2009	HC2	15.6
NTCC	[23]	Italy	25–60	2010	HC2	9.4
Katki *et al.*	[19]	USA	Above 30	2011	HC2	5.1
CITRUS study	[24]	Japan	30–64	2017	HC2	11.7
HPV FOCAL	[25]	Canada	25–65	2018	HC2	8.1
Kurokawa *et al.*	[26]	Japan	25–69	2018	HC2	6.8
AMED cohort	[27]	Japan	30–49	2021	HC2	7.6
ATHENA	[28]	USA	Above 30	2011	COBAS	6.7
CLEAR	[29]	USA	Above 30	2015	Aptima	4.7
Rad *et al.*	[30]	Norway	25–69	2023	PreTec HPV-Proofer	3.2
Present study			30–69	2022	Aptima	4.6

HPV: human papillomavirus; UK: United Kingdom; NTCC: New Technology in Cervical Cancer; USA: United States of America; HC2: hybrid capture II.

5. Limitations

The limitations of this study include a relatively short duration, a small number of cases, and limited geographic coverage. Furthermore, the study lacks a gold standard comparison as it does not utilize histologically confirmed high-grade lesions (CIN2+) as an endpoint, relying solely on cytology and HPV test results. While the results provide valuable insights into the feasibility of introducing HPV testing in Kitakyushu City for cervical cancer screening, the absence of biopsy-confirmed endpoints leaves the clinical relevance of HPV positivity uncertain. Therefore, more comprehensive studies incorporating biopsies and longer follow-up periods are essential to fully evaluate the effectiveness of the Aptima system in different regions.

6. Conclusions

In the Aptima HPV assay, the HPV-positivity rate was lower than that reported for other testing methods targeting HPV DNA. The Aptima HPV Assay, which uses HPV mRNA as the target gene, is considered highly useful. In Kitakyushu City, we aim to introduce HPV testing (Aptima) for cervical cancer screening after consulting with the relevant parties.

ABBREVIATIONS

HPV, human papillomavirus; NILM, negative for intraepithelial lesion or malignancy; Pap, Papanicolaou; ASC-US, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; ASC-H, atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion; AGC, atypical glandular cells; WHO, World Health Organization; CRPS, complex regional pain syndrome; UK, United Kingdom; USA, United States of America; HC2, hybrid capture II.; HR, high risk; ASCCP, American Society for Colposcopy and Cervical Pathology; NTCC, New Technology in Cervical Cancer; PPV, positive predictive value.

AVAILABILITY OF DATA AND MATERIALS

The data that support the findings of this study are available from the corresponding author (Yusuke Matsuura), upon reasonable request.

AUTHOR CONTRIBUTIONS

YM and KY—conceptualization; validation; formal analysis. YM, TK and KY—methodology. YM—software; data curation; writing–original draft preparation; visualization. TK and KY—writing–review and editing. KY—supervision. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All patients have provided written informed consent for this study. This study was conducted in accordance with the Declaration of Helsinki, and the protocol was accepted by the Medical Ethics Committee of the University of Occupational and Environmental Health, Japan (UOEHCRB21-178).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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