# Clinical impact of age-specific distribution of combination patterns of cytology and high-risk HPV status on cervical intraepithelial neoplasia grade 2 or more

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**Abstract.** To the best of our knowledge, the present study is the first to elucidate the significance of cytology and high-risk human papillomavirus (hrHPV) status in different age groups for the detection of cervical intraepithelial neoplasia (CIN)2, CIN3 and squamous cell carcinoma (SCC). There were 12 combinations based on cytology and hrHPV status [cytology: Atypical squamous cells (ASC) of undetermined significance, low-grade squamous intraepithelial lesion, ASC not excluding high-grade squamous intraepithelial lesion (HSIL) and HSIL; hrHPV status: HPV16/18-positive (16/18+), hrHPV positive for subtypes other than 16/18 (others+) and hrHPV-negative (hrHPV-)]. All patients were categorized into four groups based on age (18-29, 30-39, 40-49 and ≥50 years). For patients with CIN2, CIN3 and SCC (CIN2+) (n=107), the distribution of cytology and hrHPV was investigated in each age group. In addition, for all patients (n=446), the occurrence of CIN2+ in each of the 12 combinations was investigated in each age group. In the 18-29-year age group, the most common combination was HSIL and 16/18+, followed by HSIL and others+, which accounted for 73% of CIN2+ cases. The occurrence of HSIL and 16/18+ decreased with increasing age, and no cases occurred in the 50-year age group. In the 18-29-year age group, all patients with HSIL and 16/18+ were diagnosed with CIN2+. CIN2+ was predominantly detected in patients with HSIL in the 18-29-year age group, as well as hrHPV- and others+. This definite distinction was not observed in any other age group. For CIN2+, the distribution patterns of cytology and hrHPV status combinations varied significantly among different age groups. Accordingly, the clinical impact of the combination of cytological findings and hrHPV status can vary among age groups.

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*Key words:* human papillomavirus, cytology, cervical intraepithelial neoplasia, squamous cell carcinoma, cervical cancer, human papillomavirus vaccine

## Introduction

Cervical cancer is a common gynecological cancer worldwide (1-3) and squamous cell carcinoma (SCC) accounts for ~75% of cervical cancer cases (4). SCC of the cervix usually develops from cervical intraepithelial neoplasia (CIN); high-grade CIN (CIN2 or CIN3) is considered to be a precursor (5-7). To date, screening for CIN2, CIN3 and SCC (CIN2+) has been widely performed using cytological tests (8,9). For several years, cytology has been the first choice for screening. The presence of high-risk human papillomavirus (hrHPV) is also associated with CIN2+ because CIN and cancer can develop due to hrHPV infection (10,11). HPV16 and HPV18 are two major carcinogenic subtypes of hrHPV (12). Moreover, several other hrHPV subtypes, including 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68, are associated with a high frequency of cervical cancer development (13). Therefore, tests for detecting hrHPV infections are widely used for screening (13); however, hrHPV infection does not always indicate CIN or cervical cancer. Therefore, hrHPV status should be correlated with cytological findings.

Screening for high-grade squamous intraepithelial lesion (HSIL) and cervical cancer is associated with a controversy regarding the age at which it should be performed and variation in screening content with age. In Japan, SCC is frequently observed even in individuals >60 years of age. Several reports have described different distribution patterns of hrHPV subtypes and cytologies among different age groups (14-17). If the distribution of hrHPV and/or cytology results differs among age groups, the interpretation of cytological results and hrHPV status combinations should vary among age groups. However, few reports have comprehensively observed the

cytological background and hrHPV status of patients with CIN2+, and examined the clinical impact of screening among different age groups from younger to older patients (18,19).

The present study aimed to elucidate the significance of cytological results and hrHPV status in patients with CIN2+ across a wide age group. In addition, the clinical impact of HPV vaccination was evaluated. In Japan, a public HPV vaccination program for 13-16-year-olds began in 2010. By 2013, the vaccination rate in this generation was ~70% (20,21). However, due to repeated media reports of various symptoms following HPV vaccination, the government announced the suspension of active vaccine recommendations in April 2013, which led to a decline in vaccination rates to <1% (22). Therefore, individuals born between 1994 and 1999 are called the 'vaccination generation', as vaccination rates in earlier generations were 0% and those in the later generations are also very low (23). In the present study, the 'vaccination generation' corresponds to individuals in the 18-24-year age group.

## Materials and methods

*Study design and patients*. The present case-control study aimed to investigate the relationship among cytological results, hrHPV status and clinicopathological findings. The patient examination flow chart is shown in Fig. 1. Patients who underwent cytological tests at the Department of Obstetrics and Gynecology, Toyooka Public Hospital (Toyooka, Japan) between April 2016 and January 2019 were first included, which revealed the presence of atypical squamous cells (ASC) of undetermined significance (ASC-US), low-grade squamous intraepithelial lesion (LSIL), ASC not excluding HSIL (ASC-H) or HSIL. For patients with ASC-US cytology, a qualitative hrHPV test was performed. If the test results were positive, hrHPV typing and colposcopy-directed biopsy were performed. For patients with LSIL, ASC-H or HSIL cytologies, hrHPV typing and colposcopy-directed biopsy were performed simultaneously. The biopsy results were categorized as malignancy (SCC), CIN3, CIN2, CIN1 or no malignancy. CIN2, CIN3 and SCC were then grouped as CIN2+.

*Collection of clinical data, and cytological and hrHPV results*. Data pertaining to cytological results, hrHPV status and histopathological diagnoses were extracted from the medical records of the patients. Patient age, and history of pregnancy and childbirth at the time of examination were confirmed from the medical records or by interviewing the patient. Cervical cytology was performed using a cervix brush (Rovers Medical Devices B.V., Netherlands) with the conventional method of mounting cells directly on a glass slide. Cytological results were analyzed and classified according to the Bethesda system (24).

Qualitative hrHPV assessment of patients with ASC-US cytology was performed using the Cobas HPV test (Roche Diagnostics K.K.). Quantitative PCR was used to determine the subtype of HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). hrHPV typing was performed using the PCR-rSSO method (MEVGEN HPV kit; cat. no. GS-B0702; Medical & Biological Laboratories Co., Ltd.). The test results were reported as 'positive for HPV16/18' or 'positive for an HPV subtype other than 16/18'.

The pathological diagnoses of the biopsy specimens were determined in consultation with a gynecological oncologist and a pathologist specializing in gynecology.

*Statistical analysis*. For continuous variables, one-way analysis of variance was first performed to assess the presence of a significant difference in the overall distribution. When it was present, Bonferroni correction for multiple comparisons was applied to compare two groups. For categorical data, Fisher's exact test was first used to determine whether there was a significant difference overall, after which the Bonferroni correction was performed to compare two groups. For calculation of relative risk (RR) and 95% confidence interval (CI), Fisher's exact test was used. $P<0.05$ was considered to indicate a statistically significant difference. GraphPad Prism version 9.0 (Dotmatics) was used for statistical analysis.

## Results

*Association between age, different cytological findings, hrHPV status and pathology in all patients*. In total, 446 patients with the following cytological findings were included in the present study: ASC-US, n=310; LSIL, n=44; ASC-H, n=38; and HSIL, n=54. hrHPV status was categorized into three groups: hrHPV-negative (hrHPV-), n=263; hrHPV positive for subtypes other than 16/18 (others+), n=137; and HPV 16/18-positive (16/18+), n=46. The age of patients and frequency of multiparity in each group are shown in Table I.

The present study first examined the age distribution of patients with a particular cytological and hrHPV status. Significant differences were observed with respect to both cytological results and hrHPV status. Specifically, patients with ASC-US and ASC-H were significantly older, whereas those with LSIL and HSIL were significantly younger (Fig. S1A). Regarding hrHPV status, patients with 16/18+ were significantly younger than those with others+ and hrHPV-(Fig. S2A). There were no significant differences in age distribution among patients with different histopathological findings (Table SI; Fig. S1B). Next, the influence of the HPV vaccine was compared among all patients (n=446) by dividing the entire group into nine groups based on age (18-24 years, n=12; 25-29 years, n=36; 30-34 years, n=46; 35-39 years, n=52; 40-44 years, n=84; 45-49 years, n=70; 50-54 years, n=42; 55-59 years, n=31; ≥60 years, n=73; Tables SII and SIII; Fig. S2B). Regarding the influence of the HPV vaccine, the occurrence of 16/18+ was considerably lower at 8.3% in the 18-24-year age group, which corresponded to the 'vaccination generation' (Table SII; Fig. S2B). By contrast, the occurrence of others+ was considerably higher in the 'vaccination generation' at 66.7% (Table SIII; Fig. S2B). The details of the age distribution of patients with a particular cytological and hrHPV status, and the results of the influence of the HPV vaccine are described in Appendix SI.

*Comparison of hrHPV status among different age groups in patients with CIN2+*. Next, the present study focused on patients with CIN2+ (n=107). The age distribution and parity for each pathological status are described in Table SI. The present study first examined the hrHPV status and found significant differences in age distribution among the three

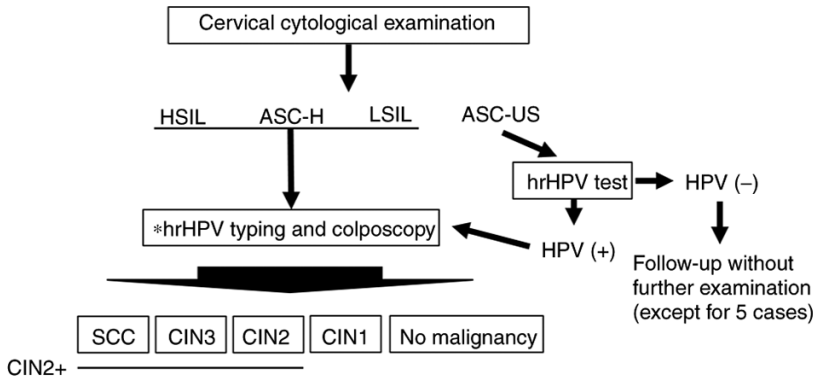


Figure 1. Schematic diagram of the present study. A total of 446 patients underwent cervical cytological examination. For patients with HSIL, ASC-H, and LSIL cytology, colposcopy-directed biopsy and hrHPV typing were performed. For patients with ASC-US cytology, an hrHPV test was performed. If positive, colposcopy-directed biopsy and hrHPV typing were performed. Additionally, for clinical reasons, five patients with ASC-US and hrHPV-underwent colposcopy-directed biopsy and hrHPV typing. *HPV typing test: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 types were examined. ASC-US, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; ASC-H, atypical squamous cells not excluding HSIL; HSIL, high-grade squamous intraepithelial lesion; hrHPV, high-risk human papillomavirus; hrHPV-, hrHPV-negative; SCC, squamous celll carcinoma; CIN, cervical intraepithelial neoplasia grade; CIN2+, SCC, CIN3 and CIN2.

groups (P=0.0157; Table II; Fig. 2A). Patients with 16/18+ were relatively younger than those with others+ and hrHPV-(16/18+ vs. others+, P=0.0092; 16/18+ vs. hrHPV-, P=0.056; Table II; Fig. 2A).

The present study evaluated the effects of the HPV vaccine. As aforementioned, patients with CIN2+ were categorized into nine groups by age (18-24 years, n=2; 25-29 years, n=13; 30-34 years, n=15; 35-39 years, n=16; 40-44 years, n=25; 45-49 years, n=16; 50-54 years, n=5; 55-59 years, n=5; ≥60 years, n=10) and the occurrence of 16/18+ and others+ was calculated for each age group (Tables SII and SIII; Fig. 2B). In the 'vaccination generation', the occurrence of 16/18+ was considerably lower at 0%, whereas that of others+ was 100% (Tables SII and SIII; Fig. 2B). In addition, the occurrence of others+ tended to be higher in the ≥40-years age groups (Table SIII; Fig. 2B).

*Comparison of cytology among different age groups in patients with CIN2+.* The present study examined the differences in cytological findings and age distribution, and significant differences in age distribution were revealed among patients with different cytological findings (P=0.028; Table II; Fig. 3A). Subsequently, the distribution of cytological findings were compared among the 107 patients with CIN2+ by dividing the entire group into four groups based on age (age groups, 18-29 years, n=15; 30-39 years, n=31; 40-49 years, n=41; ≥50 years, n=20; Fig. 3B). The distribution pattern differed significantly among the four age groups (P=0.0037). In the 18-29-year age group, the occurrence of HSIL was considerably higher (80.0%) and was much lower in the 50-year age group (25.0%) (Fig. 3B). By contrast, the occurrence of ASC-US was high in the ≥40-years age groups (18-29 years, 6.7%; 30-39 years, 22.6%; 40-49 years, 36.6%; 50 years, 30.0%; Fig. 3B).

*Comparison of cytology and hrHPV combinations in different age groups among patients with CIN2+.* As both background cytology and hrHPV findings in patients with CIN2+ differed significantly among the different age groups, the present study examined the association between cytological findings and hrHPV status in each age group. There were 12 groups based on cytology and hrHPV combinations, and all patients with 11 combinations (with the exception of ASC-US and hrHPV-) underwent biopsy. The occurrence of CIN2+ was calculated in each age group (Fig. 3C-F).

In the 18-29-year age group, the most common combination was HSIL and 16/18+, followed by HSIL and others+; these two combinations accounted for 73% of total CIN2+ cases. Only one case of hrHPV-was detected with HSIL cytology (Fig. 3C).

This definite contrast was not observed in any other age group. More than half of patients with CIN2+ in the 30-39-year group exhibited 16/18+, but their cytological findings were not highly skewed (ASC-H and 16/18+, n=6; HSIL and 16/18+, n=4; LSIL and 16/18+, n=4; ASC-US and 16/18+, n=2; Fig. 3D).

In the 40-49 and 50-year age groups, the majority of patients with CIN2+ had a hrHPV status of others+; the most common combination was ASC-US and others+, with a proportion of 31.7% in the 40-49-year age group and 25% in the 50-year age group (Fig. 3E and F). In the 50-year age group, no patient had a combination of HSIL and 16/18+ (Fig. 3F).

*A screening perspective: Different clinical impacts of cytology and hrHPV combinations for each age group.* Subsequently, the present study investigated perspectives on screening for CIN2+. The occurrence of CIN2+, according to cytological findings and hrHPV status, was compared in all participants with the exception of the population whose cytological findings and hrHPV combination was ASC-US/hrHPV-. As a result, 232 cases were included in the analysis. As expected, the occurrence of CIN2+ differed significantly depending on the cytological findings and hrHPV status (Fig. S3A and B). In addition, CIN2+ occurrence was more precisely reflected by a combination of cytological findings and hrHPV status (Fig. S3C). The details are described in the Appendix SI.

The present study investigated the occurrence of CIN2+ in the 12 groups in which cytological findings and hrHPV status were assessed according to each age group (Fig. 4).

Table I. Clinical background of patients.

| Patient characteristic | Number | Median age, years (IQR) | Multiparity (%) |
|---|---|---|---|
| Total | 446 | 44.0 (36.0-53.0) | 82.10 |
| Cytology | | | |
| ASC-US | 310 | 46.0 (39.0-56.0) | 84.20 |
| LSIL | 44 | 35.5 (30.0-46.5) | 72.70 |
| ASC-H | 38 | 46.0 (38.0-58.3) | 76.30 |
| HSIL | 54 | 39.0 (29.0-45.0) | 81.50 |
| hrHPV status | | | |
| Negative | 263 | 47.0 (40.0-57.5) | 84.80 |
| Others+ | 137 | 43.0 (35.0-50.0) | 78.10 |
| 16/18+ | 46 | 37.0 (32.75-43.0) | 80.40 |

ASC-US, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; ASC-H, atypical squamous cells cannot exclude HSIL; HSIL, high-grade squamous intraepithelial lesion; hrHPV, high-risk human papillomavirus; others+, hrHPV positive for subtypes other than 16/18; 16/18+, HPV16/18-positive.

Table II. Age of patients with CIN2+ according to cytology and hrHPV status.

| CIN2+ patients | Number | Median age, years (IQR) |
|---|---|---|
| Patient characteristic | 107 | 41.0 (33.0-47.0) |
| Cytology | | |
| ASC-US | 29 | 43.0 (39.0-49.0) |
| LSIL | 12 | 34.5 (32.25-45.0) |
| ASC-H | 23 | 44.0 (38.0-58.0) |
| HSIL | 43 | 39 (29.0-44.0) |
| hrHPV status | | |
| Negative | 18 | 44 (38.75-46.5) |
| Others+ | 56 | 43 (36.0-49.5) |
| 16/18+ | 33 | 37 (32.0-41.0) |

ASC-US, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; ASC-H, atypical squamous cells cannot exclude HSIL; HSIL, high-grade squamous intraepithelial lesion; hrHPV, high-risk human papillomavirus; others+, hrHPV positive for subtypes other than 16/18; 16/18+, HPV16/18-positive; CIN2+, squamous cell carcinoma, CIN3 and CIN2; CIN, cervical intraepithelial neoplasia.

In the 18-29-year age group, the occurrence of CIN2+ was very high in patients with HSIL and very low in patients with ASC-US (Fig. 4A). However, this definite distinction was not observed in patients aged ≥30 years. Nonetheless, in the 30-39 and 40-49-year age groups, CIN2+ was detected at a relatively high frequency in patients with 16/18+, regardless of cytological findings, and in those with HSIL, regardless of hrHPV status (Fig. 4B and C). However, this distinction was unclear in the 50-year age group (Fig. 4D). In this age group, CIN2+ was detected to some extent in all hrHPV and cytological combinations. The details are described in Appendix SI.

Table III. Comparison of CIN2+ detection in the cytology and hrHPV status combinations among different age groups.

A, Analysis of ASC-H, HSIL and 16/18+ combinations.

| Age group, years | CIN2+/Total | RR | 95% CI |
|---|---|---|---|
| 18-29 | 6/6 | Ref. | Ref. |
| 30-39 | 10/11 | 0.91 | 0.62-1.51 |
| 40-49 | 7/7 | 1.0 | 0.65-1.64 |
| 50 | 1/2 | 0.50 | 0.095-1.06 |

B, Analysis of ASC-US and others+ combination

| Age group, years | CIN2+/Total | RR | 95% CI |
|---|---|---|---|
| 18-29 | 1/10 | Ref. | Ref. |
| 30-39 | 5/21 | 2.38 | 0.46-14.70 |
| 40-49 | 13/30 | 4.33 | 0.98-24.97 |
| 50 | 5/22 | 2.27 | 0.43-14.05 |

C, Analysis of LSIL, ASC-H and hrHPV-combinations

| Age group, years | CIN2+/Total | RR | 95%CI |
|---|---|---|---|
| 18-29 | 0/7 | Ref. | Ref. |
| 30-39 | 1/8 | 2.00 | 0.16-26.6 |
| 40-49 | 4/8 | 8.00 | 0.99-82.9 |
| 50 | 2/12 | 2.67 | 0.26-30.4 |

ASC-US, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; ASC-H, atypical squamous cells cannot exclude HSIL; HSIL, high-grade squamous intraepithelial lesion; hrHPV, high-risk human papillomavirus; others+, hrHPV positive for subtypes other than 16/18; 16/18+, HPV16/18-positive; CIN2+, squamous cell carcinoma, CIN3 and CIN2; CIN, cervical intraepithelial neoplasia; RR, relative risk; 95% CI, 95% confidence interval.

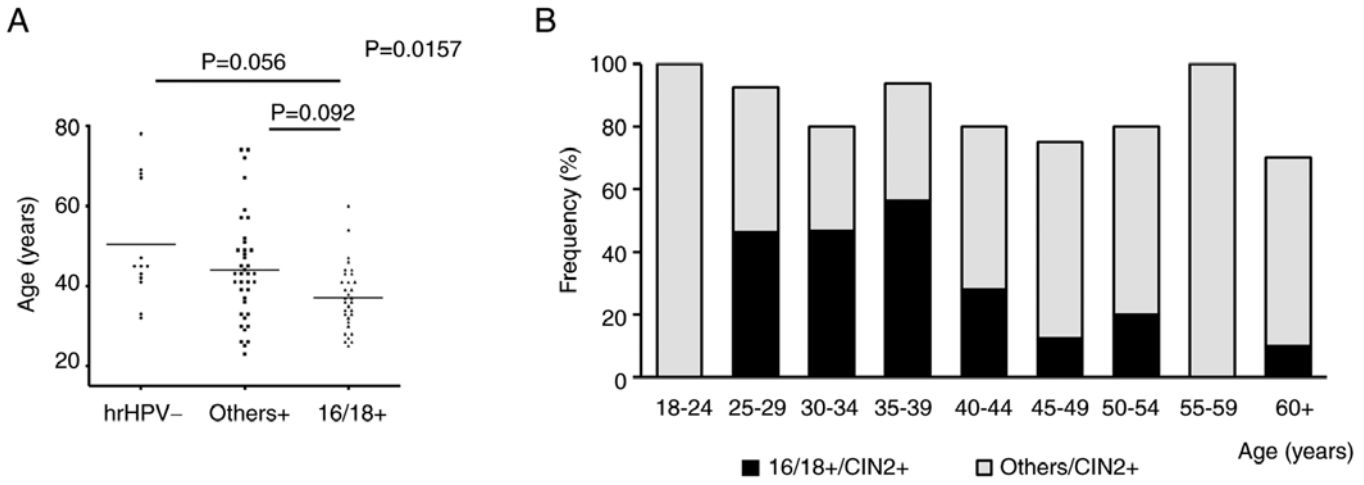


Figure 2. Comparison of hrHPV findings with respect to age in patients with CIN2+ (n=107). (A) Comparison among different hrHPV statuses. One-way analysis of variance was performed, and in case of a significant difference, the results were compared between the two groups. If the difference was significant, an adjusted P-value was calculated. (B) Frequency of occurrence of 16/18+ and others+ in each age group. hrHPV, high-risk human papillomavirus; hrHPV-, hrHPV-negative; others+, hrHPV positive for subtypes other than 16/18; 16/18+, HPV16/18-positive; CIN2+, squamous cell carcinoma, CIN3 and CIN2; CIN, cervical intraepithelial neoplasia.

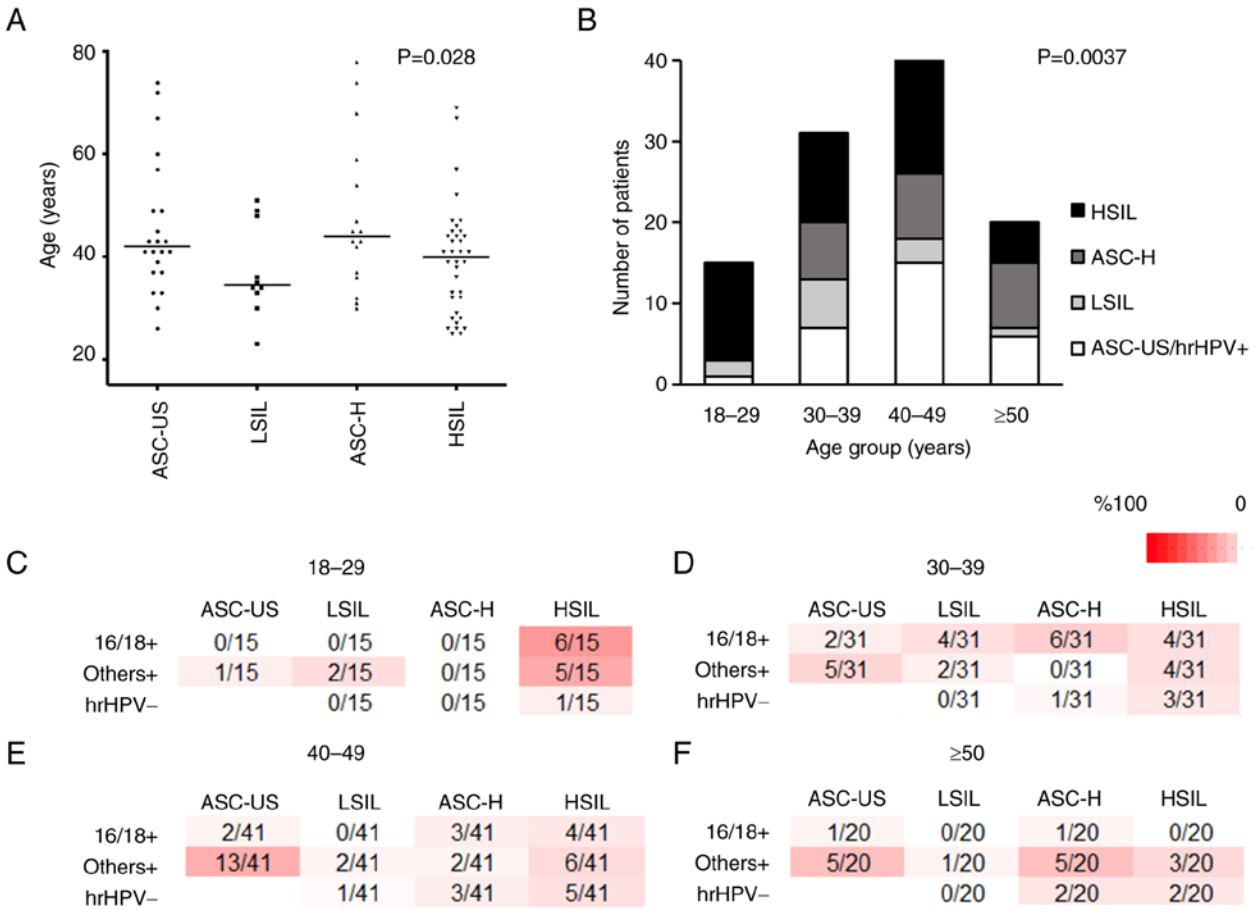


Figure 3. Comparison of cytology findings with respect to age, and distribution of cytology and hrHPV status combinations in patients with CIN2+ (n=107). (A) Comparison among different cytology findings. (B) Comparison of distribution of cytology in patients with CIN2+ in different age groups (age groups, 18-29 years: n=15; 30-39 years: n=31; 40-49 years: n=41; ≥50 years: n=20). (C-F) Distribution of combination of cytological results and hrHPV status in each age group. There were 12 combinations of cytological results and hrHPV status, and all patients with the 11 combinations (with the exception of ASC-US and hrHPV-) underwent a biopsy. The numerator/denominator indicates the number of patients included in each combination/total patients with CIN2+, respectively. (C) 18-29-year age group. The combination of HSIL and 16/18+ accounted for 40.0% of all CIN2+ cases, followed by that of HSIL and others+ at 33.3%. (D) 30-39-year age group. (E) 40-49-year age group. The combination of ASC-US and others+ accounted for >25.0% of all CIN2+ cases. (F) ≥50-year age group. The combination of ASC-US and others+ accounted for 25.0% of all CIN2+ cases. There were no cases of HSIL and 16/18+. ASC-US, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; ASC-H, atypical squamous cells not excluding HSIL; HSIL, high-grade squamous intraepithelial lesion; hrHPV, high-risk human papillomavirus; hrHPV-, hrHPV-negative; others+, hrHPV positive for subtypes other than 16/18; 16/18+, HPV16/18-positive; CIN2+, squamous cell carcinoma, CIN3 and CIN2; CIN, cervical intraepithelial neoplasia.

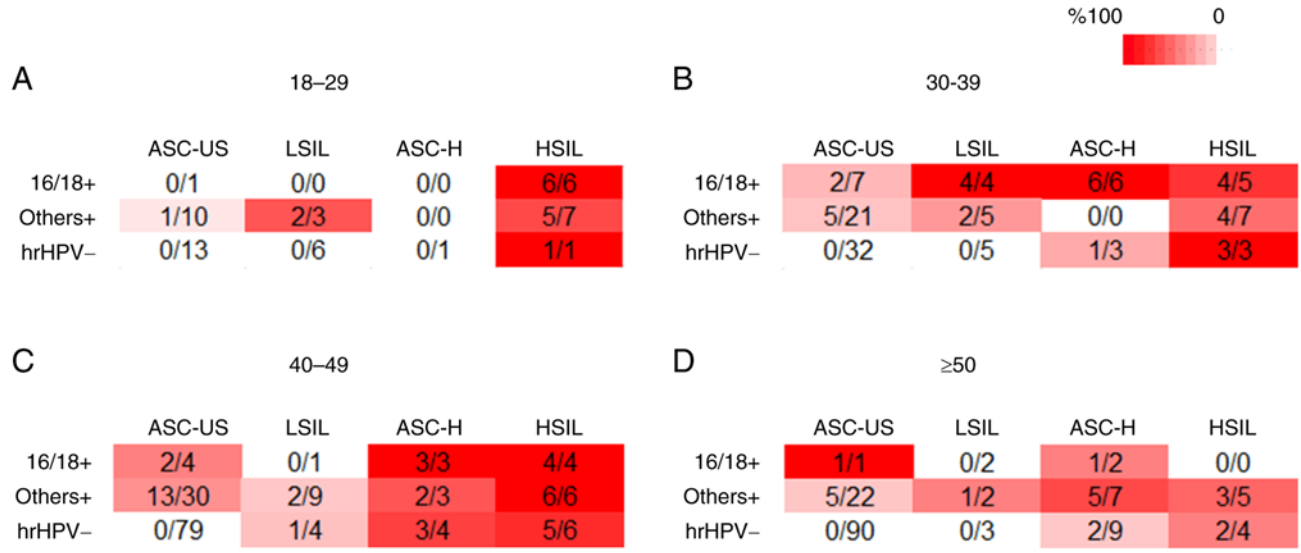


Figure 4. Frequency of occurrence of CIN2+ was calculated for each combination of cytological results and hrHPV status among the different age groups (age groups, 18-29 years: n=48; 30-39 years: n=98; 40-49 years: n=153; ≥50 years: n=147). The numerator/denominator indicates patients with CIN2+/number of patients included in each combination. (A) 18-29-year age group. The occurrence of CIN2+ was predominant in patients with HSIL, whereas it was limited in patients with ASC-US. (B) 30-39-year age group. The frequency of occurrence of CIN2+ was relatively high in patients with HSIL and/or 16/18+. (C) 40-49-year age group. The frequency of occurrence of CIN2+ was relatively high in patients with HSIL and/or 16/18+. (D) ≥50 year age group. The distribution of CIN2+ was unclear. ASC-US, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; ASC-H, atypical squamous cells not excluding HSIL; HSIL, high-grade squamous intraepithelial lesion; hrHPV, high-risk human papillomavirus; hrHPV-, hrHPV-negative; others+, hrHPV positive for subtypes other than 16/18; 16/18+, HPV16/18-positive; CIN2+, squamous cell carcinoma, CIN3 and CIN2; CIN, cervical intraepithelial neoplasia.

Finally, the occurrence of CIN2+ in these cytological and hrHPV status combinations was compared among different generations. While it would be ideal to validate all combinations, due to the limited number of cases, there were not enough cases to analyze every combination in every age group; therefore, several classifications were combined. In patients with ASC-H/HSIL and 16/18+, CIN2+ was detected at a high frequency in all generations (Table IIIA). By contrast, in patients with a combination of ASC-US and others+, although the difference was not significant, CIN2+ was more frequently detected in other age groups than in the 18-29-year age group, especially in the 40-49-year age group, with a very high relative risk (RR) of 4.33 [95% confidence interval (CI): 0.98-24.97] (Table IIIB). Additionally, in patients with a combination of LSIL/ASC-H and hrHPV-, CIN2+ was more frequently detected in other age groups than in the 18-29-year age group, especially in the 40-49-year age group with a very high RR of 8.00 (95% CI: 0.99-82.9) (Table IIIC).

## Discussion

The present study comprehensively analyzed the pathological status of cervical lesions for combinations of cytological results and hrHPV status in different age groups. To the best of our knowledge, the present study is the first in Japan to examine the background cytology and hrHPV status of patients with CIN2+ among different age groups.

First, it was confirmed that the background hrHPV types differed significantly among age groups in patients with CIN2+. Specifically, a greater number of younger patients had HPV16/18 types, whereas more older patients had others+ or hrHPV-. Regarding the clinical impact of HPV vaccination, the occurrence of 16/18+ was considerably lower at 0% in patients of the 'vaccination generation' with CIN2+.

Notably, cytological findings were significantly different among the age groups and hrHPV statuses, even in patients with CIN2+. Younger patients tended to be HSIL-dominant, whereas older patients tended to be ASC-US-dominant.

Furthermore, the distribution patterns of cytology and hrHPV status combinations varied significantly among age groups in patients with CIN2+. The combinations of HSIL and 16/18+, as well as HSIL and others+ were predominant in the <29-year age groups, whereas this definite distinction was not seen in other age groups. The analysis in terms of screening perspective also reflected this result, with the cytology and hrHPV combination being more important at younger ages, especially in the <29-year age groups.

It was hypothesized that this difference may be due to the hrHPV type and time to carcinogenesis. Previous reports have indicated that the CIN2+ background hrHPV type differs among different age groups. Onuki *et al* (25) reported that the prevalence of HPV16/18 in patients with CIN2-3 or SCC was highest in the 20-29-year age group (SCC: 90% and CIN2-3: 53.9%), and the occurrence of SCC decreased with age to 56.3% and of CIN2-3 to 25.0% in the 60+ age group. Giannella *et al* (17) also reported that the occurrence of CIN3 with hrHPV types other than HPV16/18 increased with age, with a significant difference observed when the age range was 30-45 years (<30: 23.6%, 30-44: 32.1%, >45: 38.0%; P=0.0004). This previous study reported that younger patients had a greater probability of HPV16/18 infection, whereas older patients had a higher probability of others+ or HPV-. The present results are consistent with those of previous studies. CIN2+ cases in the <29-year age groups, which are mainly caused by persistent HPV16/18 infection, followed by others+, may require a relatively short time for carcinogenesis, resulting in uniform characteristics (HSIL-dominant). By contrast,

in older patients with CIN2+, the time required for carcinogenesis differs significantly (16). Therefore, the carcinogenic background of patients with CIN2+ is not as consistent as that of younger patients, which may be reflected in the heterogeneous combination pattern of cytology and hrHPV status in older age groups. Several reports have referred to the association between age and prognosis in cervical cancer (16,26-29), and have indicated differences in features of cervical cancer between older and younger patients. The present findings of a more heterogeneous cytology and hrHPV status in older patients with CIN2+ may reflect its unique characteristics, which require a longer time from HPV infection to carcinogenesis.

Since the present study was a retrospective study conducted at a single center, the limitations, including the limited number of cases and patient backgrounds, should be considered. Additionally, it has been reported that colposcopy-directed biopsies do not extract all CIN lesions (30,31); therefore, pathological assessments may be insufficient. However, because this was a single-center study, the diagnostic criteria were consistent.

The influence of HPV vaccination on hrHPV infection status cannot be ignore, thus the present study also evaluated this factor. There are a number of reports that refer to the marked reduction in the occurrence of CIN2-3 and SCC due to HPV vaccination in foreign countries, as well as in Japan (32,33). In Japan, large-scale vaccination (either bivalent or quadrivalent) at public expense started in 2010, mainly for 12-16-year-old women. That generation is called the 'vaccination generation' and corresponds to the 18-24-year age group in the present study. Epidemiological studies have estimated that ~70% of this population is vaccinated (18,19). Although the direct influence of HPV vaccination cannot be verified in each patient, it was hypothesized that large-scale vaccination had a considerable influence on the apparent change in the occurrence of 16/18+ in the 18-24-year group.

Finally, the present study determined the definite frequency of occurrence of CIN2+ for each combination of cytological results and hrHPV status. It was hypothesized that the results of the present study may be useful in clinical practice. Based on the results, further investigations should be conducted for each age group. First, cytology was revealed to be relatively more important in younger patients; there was a very high frequency of occurrence of CIN2+ in patients with HSIL; conversely, the frequency of occurrence of CIN2+ was very low in patients with ASC-US. When considering the indications of conization in young patients, their cytological results and hrHPV status should be considered because conization is significantly related to a high incidence of preterm birth (34,35). However, the presence of CIN2+ should be considered if cytological results or hrHPV status are abnormal in older patients. In case of a clinical suspicion of CIN2+, diagnostic conization should be considered in older patients.

In Japan, where the application of the HPV vaccine has not progressed due to social factors and the rapidly aging population, early detection of cervical cancer is extremely important. The current study determined the specific frequency of occurrence of CIN2+ for every combination of cytology and hrHPV status. In addition, the distribution of cytology and hrHPV status varied widely among different age groups. Based on these results, we propose screening protocols based on age that can detect CIN2+ more efficiently. In patients with CIN2+, the clinical impact of the combination of cytology and hrHPV status can vary among age group and age-specific perspectives are important for screening CIN2+.

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**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Authors' contributions**

KY, MiS, NM, TO, HS, TK, YI, SS and MaS were involved in the conception and design, case enrollment, data interpretation and manuscript writing. MiS collected the data, and KY and MiS analyzed the data. KY and MiS prepared the manuscript. KY and MiS confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

**Ethics approval and consent to participate**

Patients were not required to provide informed consent for the present study because anonymous clinical data were obtained after each patient agreed to treatment by written consent. The study design was approved by the Toyooka Public Hospital's Ethics Committee (approval no. 226).

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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