High-risk Human Papillomavirus Testing in Young Japanese Women with Atypical Squamous Cells of Undetermined Significance

Takashi Mitamura1,2, Yosuke Konno2, Satomi Kikawa3, Yutaka Iwaki1, Kurumi Iwaki1, Fumie Tanuma1, Soromon Kataoka1

1Department of Obstetrics and Gynecology, Hakodate Central General Hospital, 33-2, Honcho, Hakodate, 2Department of Obstetrics and Gynecology, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, North 15, West 7, Kita-ku, Sapporo, 3Department of Obstetrics and Gynecology, Otaru General Hospital, Wakamatsu, Otaru, Hokkaido, Japan

Abstract

Introduction: The mortality due to uterine cervical cancer has been gradually increasing in women under 40 years of age (U40) in Japan. We investigated the effect of high-risk human papillomavirus (HR-HPV) on U40 subjects without any overt cytological abnormalities.

Materials and Methods: We retrospectively examined the clinical data, including the findings of a cobas 4800 HPV test that was approved in Japan in 2013 to triage women with atypical squamous cells of undetermined significance (ASC-US) and a histological examination in 589 Japanese women. Results: The overall prevalence rate of HR-HPV was 34.5%. Biopsy-confirmed cervical intraepithelial neoplasia (CIN) 2, or worse (CIN2+) was identified in 45.1% (23/51) of HR-HPV-positive women with ASC-US, who underwent colposcopy immediately. The mean period from the HPV test to the diagnosis of CIN2+ was 3.7 months. CIN2+ was more common (69.6%) in U40 patients. The rates of single or multiple infections of HPV-16, HPV-18, and 12 other HR-HPV (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) in CIN2+ U40 patients were 31.3%, 0%, and 81.3%, respectively. The relative risk for CIN 2+ among U40 women with HPV-16 was not significantly different from that of the patients with infection of any of the 12 other HR-HPVs. Conclusion: The results of this study suggest that the 12 other HR-HPVs have a potential to generate high-grade cervical lesions among young women, and the examination rate of colposcopy should be increased.

Keywords: Atypical squamous cells of undetermined significance, cobas 4800 HPV test, Human papillomavirus, young woman

Introduction: Cervical cancer is the fourth-most common cancer in women worldwide, and the most common cancer in women in many low- and middle-income countries.[1,2] In December 2003, the European Council adopted a recommendation to implement population-based screening for cancer of the uterine cervix for women in all member states of the European Union (EU).[3] A significant decline in the incidence and mortality has been seen in North America, parts of Europe, Australia, and New Zealand, where screening programs have been implemented.[4]

There is little doubt that well-organized cytology-based screening programs for cervical cancer have been effective in reducing the cancer incidence and preventing premature deaths. Potential reductions in disease of 60%–90% are possible within 3 years after implementing screening programs.[5,7] However, a previous study found that 47% of fully invasive cancers in women under the age of 70 occurred despite an apparently adequate screening history,[6] suggesting problems with the sensitivity of the test. Human papillomavirus (HPV) testing was subsequently found to be more sensitive than cytology, leading to a recommendation that HPV testing be introduced on a pilot basis for women with borderline and mild smears.[7] HPV testing is currently recommended to triage women with atypical squamous cells of undetermined significance (ASC-US) in the United States and as an adjunct to cytology (cotesting).[8] In Europe, the guidelines recommend the use of HPV testing to triage women with ASC-US, for surveillance after treatment.

Address correspondence: Dr. Takashi Mitamura, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, North 15, West 7, Kita-ku, Sapporo, Hokkaido, Japan. E-mail: takami@huhp.hokudai.ac.jp

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Mitamura T, Konno Y, Kikawa S, Iwaki Y, Iwaki K, Tanuma F, et al. High-risk Human papillomavirus testing in young Japanese women with atypical squamous cells of undetermined significance. J Cytol 2019;36:180-3.
of cervical intraepithelial neoplasia (CIN) and as a stand-alone primary screening test without cytology for cervical cancer screening (HPV primary screening).[9] The mortality due to uterine cervical cancer has been gradually increasing in women under 40 years of age (40) in Japan in recent years. To assess the correlation between ASC-US and HPV type, the ATHENA study, in which the cobas 4800 HPV test (Roche Molecular Systems, Pleasanton, CA, USA) was performed, showed that the relative risk (RR) for CIN2 or worse (CIN2+) among women who were HPV-16- and HPV-18-positive, including multiple infections, were 42.0 (95% CI, 20.1-87.5) and 5.8 (95% CI, 1.3-26.5), respectively, compared with those who were HR-HPV-negative (14 types). The RR for CIN2+ among women who were HPV-16-positive was 3.7 (95% CI, 2.4-5.7) compared with those who were positive for the 12 other HR-HPVs, excluding HPV-16 and HPV-18.[10] The cobas test was approved in Japan in 2013 to triage women with ASC-US.

To investigate whether or not HR-HPV is correlated with subsequent CIN2+ in young Japanese women with ASC-US, we retrospectively examined the results of cytology, the cobas 4800 HPV test, and follow-up data, including the findings from a histological examination.

### MATERIALS AND METHODS

#### Study population

This study retrospectively assessed a cohort of the women with both ASC-US and high-risk (HR) HPV at four centers in Hokkaido Prefecture, Japan. We enrolled 589 women ≥20 years of age undergoing cervical cancer screening from 2013 to 2016. The study inclusion criterion was no treatment for CIN in the past history. As a rule, we recommended women with HR-HPV-positive ASC-US undergo colposcopy. The vaccination history was not considered. The protocol was approved by the institutional review boards (Registration ID 2016-10).

#### Cytological testing, HPV testing, and colposcopy

Cytology was performed with either a Papanicolaou smear or liquid-based cervical cytology (ThinPrep, Hologic, Bedford, MA, USA). The cytology evaluation was conducted by full-boarded cytoscreeners and pathologists at each center. All HPV tests were performed within 6 weeks after cytology with a cobas 4800 HPV test. In brief, the cobas HPV test is according to two major processes: (1) automated specimen preparation to simultaneously extract HPV and cellular DNA; (2) PCR amplification of target DNA sequences using both HPV and beta-globin specific complementary primer pairs and real-time detection of cleaved fluorescent-labeled HPV and beta-globin specific oligonucleotide detection probes. A 2-ml aliquot removed from the liquid-based cervical cytology specimen was tested to detect HPV-16 individually, HPV-18 individually, and 12 pooled HR-HPV genotypes (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). For participants with only a Papanicolaou smear, a liquid-based specimen was obtained again after the results of cytology were revealed. The specimens for cytology were obtained by general gynecologists, and PCR was performed by laboratory medical technologists. The results of a cobas 4800 HPV were revealed within 3 weeks. We routinely recommend colposcopy with a simultaneous biopsy and/or endocervical curettage within 12 weeks after HPV testing in accordance with a standardized protocol that included a biopsy of all visible cervical lesions. For women with satisfactory colposcopy findings but no visible cervical lesions, a random biopsy at the squamocolumnar junction was performed. Biopsy specimens were evaluated by board qualified pathologists at each center and diagnosed using standard criteria and CIN terminology.

### RESULTS

#### Baseline characteristics

We retrospectively investigated 589 women with ASC-US cytology in this study and 64.0% of them were U40. The average age was 45.4 years (range: 20–82 years). The average gravidity and parity were 1.8 (range: 0–8) and 1.4 (range: 0–5), respectively.

#### Human papillomavirus test and colposcopy/biopsy results

The overall prevalence of HR-HPV (14 genotypes), HPV-16, HPV-18, and any of the 12 other HR-HPVs, including multiple infection, were 34.3% (202/589), 6.6% (39/589), 1.5% (9/589), and 27.2% (160/589), respectively. The prevalence of HR-HPV (14 genotypes) was 63.6%, 46.5%, 30.4%, 20.7%, 22.6%, and 17.8% for subjects in their 20s, 30s, 40s, 50s, 60s, and 70s or older, respectively, declining with increasing age. The most common type was any of the 12 other HR-HPVs, and the prevalence was 50.6%, 33.3%, 23.4%, 19.8%, 18.9%, and 15.6% for subjects in their 20s, 30s, 40s, 50s, 60s, and 70s or older, respectively [Table 1].

Fifty-one of the 202 subjects with positive HPV test results immediately underwent colposcopy. Biopsy-confirmed CIN2+ was identified in 45.1% (23/51) of HR-HPV positive women with ASC-US. CIN2+ was more common in the subjects U40 than in older ones (69.6% vs. 30.4%). The correlations between HPV type and CIN2+ by age in women with ASC-US are shown in [Table 1]. The most common type was any of the 12 other HR-HPVs without HPV-16 or HPV-18, especially in the subjects U40. The rates of single infection of HPV-16, HPV-18, and any of the 12 other HR-HPVs or multiple infections of HPV-16, HPV-18, and 12 other HR-HPV in CIN2+ patients were 21.7% (5/23), 0% (0/23), 60.9% (14/23), and 17.4% (4/23), respectively, in all women with biopsy -confirmed CIN2+. For women with biopsy-confirmed CIN2+ U40, the rates of single infection of HPV-16, HPV-18, and any of the 12 other HR-HPVs or multiple infections of HPV-16, HPV-18, and 12 other HR-HPV in CIN2+ patients were 18.8% (3/16), 0% (0/16), 68.8% (11/16), and 12.5% (2/16), respectively. The odds ratio for CIN2+ with single or multiple infections of HPV-16 versus single or multiple infections of any of the 12 other HR-HPVs...
Table 1: The correlation between the HR-HPV* type and age in women with ASC-US

| Age   | Overall | HR-HPV-positive | HPV-16 | HPV-18 | 12 other | HR-HPV-positive subtype NA* |
|-------|---------|-----------------|--------|--------|----------|-----------------------------|
| 20-29 | 114     | 63.6% (49)      | 3.9% (23) | 4.2% (8) | 0.0% (0) | 65.7% (387) |
| 30-39 | 182     | 53.5% (61)      | 2.7% (5) | 4.3% (8) | 0.0% (0) | 3.9% (23)     |
| 40-49 | 184     | 65.7% (387)     | 2.7% (5) | 4.3% (8) | 0.0% (0) | 3.9% (23)     |
| 50-59 | 59      | 53.5% (61)      | 2.7% (5) | 4.3% (8) | 0.0% (0) | 3.9% (23)     |
| 60-69 | 45      | 53.5% (61)      | 2.7% (5) | 4.3% (8) | 0.0% (0) | 3.9% (23)     |

HR-HPV* high-risk human papillomavirus; ASC-US: atypical squamous cells of undetermined significance; CIN2+: cervical intraepithelial neoplasia 2 or worse; NA*: not applicable, HPV-16 includes positivity for HPV-16 without HPV-18 or any of the 12 other HR-HPVs, HPV-18 includes positivity for HPV-18 without HPV-16 or any of the 12 other HR-HPVs, “12 other” includes positivity for any of the 12 other HR-HPVs without HPV-16 or HPV-18.
that some cervical lesions progress in young women without demonstrating any overt cytological abnormalities. Although HPV16 is strongly correlated with carcinogenesis of the female genital tract, the results of this study suggest that the 12 other HR-HPVs also have a great potential to generate high-grade cervical lesions in young women with low rates of cytological abnormalities. Further studies in larger cohorts are needed to clarify the mechanisms that induce the development of CIN in young women with HR-HPV infection and a history of being vaccinated for HPV.

**Conclusion**

Infections of HR-HPVs without HPV-16 or HPV-18 have a great potential to generate high-grade cervical lesions in young Japanese women with low rates of overt cytological abnormalities. The examination rate of colposcopy should be increased in Japanese women with HR-HPV-positive ASC-US.

**Acknowledgments**

Portions of this work were supported by Hiromasa Fujita (Cytology Center, Hokkaido Cancer Society, Sapporo, Hokkaido, Japan).

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

**References**

1. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11. Lyon, France: International Agency for Research on Cancer, 2013.
2. Mustafa RA, Santesso N, Khatib R, Mustafa AA, Wiercioch W, Kehar R, et al. Systematic reviews and meta-analyses of the accuracy of HPV tests, visual inspection with acetic acid, cytology, and colposcopy. Int J Gynaecol Obstet 2016;132:259-65.
3. Arbyn M, Raifu AO, Autier P, Ferlay J. Burden of cervical cancer in Europe: Estimates for 2014. Ann Oncol 2015;26:1708-15.
4. Chemoradiotherapy for Cervical Cancer Meta-Analysis Collaboration. Reducing uncertainties about the effects of chemoradiotherapy for cervical cancer: A systematic review and meta-analysis of individual patient data from 18 randomized trials. J Clin Oncol 2008;26:5802-12.
5. Screenin for squamous cervical cancer: Duration of low risk after negative results of cervical cytology and its implication for screening policies. IARC Working Group on evaluation of cervical cancer screening programmes. Br Med J (Clin Res Ed) 1986;293:659-64.
6. Sasieni PD, Cuzick J, Lynch-Farmery E. Estimating the efficacy of screening by auditing smear histories of women with and without cervical cancer. The National Co-ordinating Network for Cervical Screening Working Group. Br J Cancer 1996;73:1001-5.
7. Cuzick J, Sasieni P, Davies P, Adams J, Normand C, Frater A, et al. A systematic review of the role of human papilloma virus (HPV) testing within a cervical screening programme: Summary and conclusions. Br J Cancer 2000;83:561-5.
8. Wright TC Jr, Massad LS, Dunton CJ, Spitzer M, Wilkinson EJ, Solomon D. 2006 consensus guidelines for the management of women with abnormal cervical screening tests. J Low Genit Tract Dis 2007;11:201-22.
9. Arbyn M, Anttila A, Jordan J, Ronco G, Schenck U, Segnan N, et al. European Guidelines for Quality Assurance in Cervical Cancer Screening. Second edition—summary document. Ann Oncol 2010;21:448-58.
10. Stoler MH, Wright TC Jr, Sharma A, Apple R, Gutekunst K, Wright TL. High-risk human papillomavirus testing in women with ASC-US cytology: Results from the ATHENA HPV study. Am J Clin Pathol 2011;135:468-75.
11. Bergeron C, Giorgi-Rossi P, Cas F, Schiboni ML, Ghiringhelli B, Dalla Palma P, et al. Informed cytology for triaging HPV-positive women: Substudy nested in the NTCC randomized controlled trial. J Natl Cancer Inst 2015;107:pii: dju423. doi: 10.1093/jnci/dju423.
12. zur Hausen H. Molecular pathogenesis of cancer of the cervix and its causation by specific human papillomavirus types. Curr Top Microbiol Immunol 1994;186:131-56.
13. Arbyn M, Buntinx F, Van Ranst M, Paraskevaidis E, Martin-Hirsch P, Dillner J. Virologic versus cytologic triage of women with equivocal Pap smears: A meta-analysis of the accuracy to detect high-grade intraepithelial neoplasia. J Natl Cancer Inst 2004;96:280-93.
14. Castle PE, Eaton B, Reid J, Getman D, Dockter J. Comparison of human papillomavirus detection by Aptima HPV and cobas HPV tests in a population of women referred for colposcopy following detection of atypical squamous cells of undetermined significance by Pap cytology. J Clin Microbiol 2015;53:1277-81.
15. Gage JC, Hunt WC, Schiuffino M, Katki HA, Cheung LC, Cuzick J, et al. Risk Stratification using human papillomavirus testing among women with equivocally abnormal cytology: Results from a State-Wide Surveillance Program. Cancer Epidemiol Biomarkers Prev 2016;25:36-42.
16. Haldorsen T, Skare GB, Ursin G, Bjorge T. Results of delayed triage by HPV testing and cytology in the Norwegian Cervical Cancer Screening Programme. Acta Oncol 2015;54:200-9.
17. Siddiqi A, Spataro M, McIntire H, Akhtar I, Baliga M, Flowers R, et al. Hybrid capture 2 human papillomavirus DNA testing for women with atypical squamous cells of undetermined significance Papanicolaou results in SurePath and ThinPrep specimens. Cancer 2009;117:318-25.
18. Gnan P, Howell-Jones R, Li N, Bruni L, de Sanjose S, Franceschi S, et al. Human papillomavirus types in 115,789 HPV-positive women: A meta-analysis from cervical infection to cancer. Int J Cancer 2012;131:2349-59.
19. Arbyn M, Xu L, Verdoordt F, Cuzick J, Szareswki A, Belinson JL, et al. Genotyping for human papillomavirus types 16 and 18 in women with minor cervical lesions: A systematic review and meta-analysis. Ann Intern Med 2017;166:118-27.
20. Samimi SA, Mody RR, Goodman S, Luna E, Armylagos D, Schwartz MR, et al. Do infection patterns of human papillomavirus affect the cytologic detection of high-grade cervical lesions on papanicolaou tests? Arch Pathol Lab Med 2018;142:347-52.
21. Melnikow J, Nuovo J, Willan AR, Chan BK, Howell LP. Natural history of cervical squamous intraepithelial lesions: A meta-analysis. Obstet Gynecol 1998;92:727-35.
22. Petri J, Gilham C, Deacon J, Taylor C, Evans C, Binns W, et al. Cervical HPV infection and neoplasia in a large population-based prospective study: The Manchester cohort. Br J Cancer 2004;91:942-53.
23. Yost NP, Santosio JT, McIntire DD, Illya FA. Postpartum regression rates of antepartum cervical intraepithelial neoplasia II and III lesions. Obstet Gynecol 1999;93:359-62.
24. Vital Statistics Japan. Ministry of Health, Labour and Welfare; 2015.