Gynecologic Oncology xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

# Is human papillomavirus genotype important in predicting disease progression in women with biopsy-proven negative or CIN1 of atypical squamous cell of undetermined significance (ASC-US) cytology?☆

Woo Dae Kang, U Chul Ju, Seok Mo Kim *

*Department of Obstetrics and Gynecology, Chonnam National University Medical School, Gwangju, Republic of Korea*

## HIGHLIGHTS

- We estimate the risk of developing CIN2+ up to 5 years of follow-up in women with ASCUS.
- The risk of disease progression to CIN2+ is associated with being HPV-16, HPV-31, HPV-52, or HPV-58 among ASC-US cases.
- HPV-18 may not confer an incidence risk for CIN2+ that is greater than that for other HR-HPV genotypes among ASC-US cases

## ARTICLE INFO

*Article history:*
Received 11 August 2017
Received in revised form 16 November 2017
Accepted 20 November 2017
Available online xxxx

*Keywords:*
Atypical squamous cell of undetermined significance
Cervix
Cervical intraepithelial neoplasia
Human papillomavirus
Genotyping

## ABSTRACT

*Objectives.* Our aim was to estimate the risk of disease incidence in women with atypical squamous cell of undetermined significance (ASC-US) without histology-proven cervical intraepithelial neoplasia grade 2 or worse (CIN2+) by human papillomavirus (HPV) genotype.

*Methods.* Between January 2002 and September 2010, incidence of CIN2+ in 2880 women including 2172 with ASC-US and histology-proven negative and 708 with ASC-US with histology-proven CIN1 was investigated. Baseline HR-HPV status was determined by the hybrid capture II assay (HC2) and HR-HPV genotype by the HPV DNA chip test (HDC). Cumulative incidence and hazard ratios were estimated to explore differences between index data and associations with CIN2+.

*Results.* Of the 2880 women, the HC2 was positive in 1509 women (52.4%) and the HDC was positive in 1563 women (54.3%). The overall agreement between the HDC and HC2 was 97.4%. One hundred ninety (6.6%) patients developed CIN2+. The 5-year cumulative incidence rate of CIN2+ in HPV-16, HPV-31, HPV-52, and HPV-58 were 16.7%, 15.1%, 12.6%, and 12.9%, respectively. On multivariate analysis, being positive in HPV-16 (hazards ratio [HR] = 2.431; 95% CI, 1.789–3.332; $P < 0.01$), HPV-31 (HR = 2.335; 95% CI, 1.373–3.971; $P < 0.01$), HPV-52 (HR = 1.592; 95% CI, 1.031–2.458; $P = 0.03$), and HPV-58 (HR = 1.650; 95% CI, 1.132–2.407; $P < 0.01$) were significantly associated with developing CIN2+ compared to being negative for that type.

*Conclusions.* Among women with ASC-US, HPV-16, HPV-31, HPV-52, or HPV-58 positive women may need intensified follow-up as they have the highest risk of becoming CIN2+.



## 1. Introduction

Cervical cancer is the second most common type of cancer in women worldwide [1] and the sixth most common female malignancy in Korea [2]. Cervical intraepithelial neoplasia (CIN) is a premalignant cervical disease caused by high-risk human papillomavirus (HR-HPV) infection. CIN contains a spectrum of lesions with different severity. Low-grade disease, CIN1, has minimal potential for progressing to cervical cancer. CIN2 and CIN3 are regarded as high-grade lesions.

Atypical squamous cells of undetermined significance (ASC-US) account for approximately 3–5% of cervical screening of asymptomatic women based on cytology and comprise the majority of abnormal cytology results [3,4]. Patients with ASC-US exhibit a wide spectrum of histologic results, with most showing mild abnormalities of a grade less than CIN2 with spontaneous regression; however, invasive carcinoma is also diagnosed in rare cases [5].

The risk of developing pre-cancer and invasive cervical cancer is very low if the woman is HPV negative/ASC-US [4,5]. Women with HPV-

☆ W.D. Kang et al.

* Corresponding author at: Department of Obstetrics and Gynecology, Chonnam National University Medical School, 8 Hakdong, Dong-gu, Gwangju, Republic of Korea.
*E-mail address:* seokmo2001@hanmail.net (S.M. Kim).

https://doi.org/10.1016/j.ygyno.2017.11.025
0090-8258/

**Table 1**
Patient characteristics according to disease progression.

| | <CIN2 N = 2690 | CIN2+ N = 190 | *P* |
|---|---|---|---|
| Age (years) | | | <0.01 |
| Mean ± SD | 45.4 ± 11.5 | 41.9 ± 10.9 | |
| Range | 20–65 | 20–65 | |
| Menopause | | | 0.02 |
| No | 1988 | 156 | |
| Yes | 702 | 34 | |
| Baseline histology | | | <0.01 |
| Negative | 2051 | 121 | |
| CIN1 | 639 | 69 | |
| HC2 | | | <0.01 |
| Negative | 1350 | 21 | |
| Positive | 1340 | 169 | |
| HDC | | | |
| Negative | 1300 | 17 | <0.01 |
| Positive | 1390 | 173 | |
| HR-HPV genotype by HDC | | | 0.94 |
| Single infection | 1141 | 141 | |
| Multiple infection | 249 | 32 | |

HR, high risk; HPV, human papillomavirus; CIN, cervical intraepithelial neoplasia; CIN2+, cervical intraepithelial neoplasia grade 2 or worse; SD, standard deviation; HC2, Hybrid Capture II test; HDC, HPV DNA chip test.

negative/ASC-US had a similar 5-year risk for cervical intraepithelial neoplasia grade 2 or worse (CIN2+) as women with a normal Pap smear, and that the risk for HPV-positive/ASC-US women was as risky as the same as those with low-grade squamous intraepithelial lesions (LSIL) Pap results [4].

The risk of progression to cancer also varies between different HR-HPV genotypes and the strongest risk is associated with HPV-16, the most common genotype [6]. Of all HR-HPV types, types 16 and 18 are the two most frequently detected HR-HPVs in cervical cancer [7]. The association between the higher incidence of CIN3 in ASC-US with HPV-16 infection has been previously reported [8]. However, it has not been established how the risk of CIN2+ differs by other HR-HPV types among women with ASC-US.

In this study, we aim was to estimate the risk of developing CIN2+ up to 5 years of follow-up in order to provide in an insight into management of women with ASC-US without histology-proven CIN2+.

## 2. Methods

We retrospectively reviewed the records of all women with a cytologic sample interpreted as ASC-US at Chonnam National University Hospital (CNUH) between January 2002 and September 2010. Only patients with a first cytologic diagnosis of ASC-US were evaluated, and subjects with ASC-H were excluded. The pathology databases were queried to document all pathologic follow-up for each subject. All cytologic samples were liquid based preparations. Cytology was classified according to the 2001 Bethesda System terminology [9]. Histology was reported as negative, CIN1, CIN2, CIN3 or invasive cancer. The study protocol was evaluated and approved by the Institutional Review Board at CNUH.

2880 patients were considered eligible for the study if they fulfilled the following criteria: (a) Women aged from 20 to 65 years with an intact uterus; (b) patients having been histologically confirmed negative or CIN1 at a first cytologic diagnosis of ASC-US; (c) patients in whom HR-HPV test results from the HPV DNA chip test (HDC; MyGene Co., Seoul, South Korea) and the hybrid capture II assay (HC2; Digene Co., Gaithersburg MD, USA) were available; and (d) patients who had not received the HPV quadrivalent vaccine or the bivalent vaccine. For follow-up, women are examined by repeat cytology and HPV DNA tests (HC2 and HDC) every 6 months to detect CIN2+. Women who showed no abnormal cytology/HPV findings suggesting CIN in two consecutive visits exited the follow-up and returned for routine screening with cytology and HPV DNA test once a year. We excluded the women who were pregnant at initial evaluation, had malignant disease including cervical cancer, had a history of CIN being treated, had hysterectomy for any reason, and did not visit the hospital before returning for routine screening. Epidemiologic data, HR-HPV test data from the HDC and HC2, and pathology data were obtained from the medical records.

Each woman who needed to be followed up was informed by mail about the time of the next visit. Colposcopy was carried out if the HPV DNA test was positive or if repeat cytology revealed an ASC-US or greater. Colposcopic directed punch biopsies of the cervix were taken in the case of any suspected area after application of 5% acetic acid. When the lesion was not visible or was only partially visible, additional endocervical curettage was performed.

Criteria for CIN2+ were based on positive histology of colposcopy-directed biopsy or endocervical curettage. Histological evidence for the presence of CIN2+ was the endpoint of the study.

## 3. Hybrid capture II assay

The sample was collected by placing a cytobrush into the exocervix and rotating the brush 3 times; the sample was kept frozen at −20 °C in a collection tube (Digene Co) until needed. The denatured single-strand DNA was hybridized with a RNA researcher of the mixed HR-HPV group. This reaction mixture was placed in a microtiter well coated with antibodies for the RNA/DNA hybrid. After RNA/DNA hybrid-antibody bonding, the mixture was reacted with alkaline phosphatase-conjugated antibodies, washed, and lumi-Phospho 530 was added to react with the dioxetane-based chemiluminescent substrate. Alkaline phosphatase was added to obtain luminescent light, which was measured with a luminometer and expressed in relative light units. The solution containing 1 pg/mL of HPV-16 DNA was used as a positive control group for the HR-HPV group. The relative light units for all the samples were set to the degree of relative brightness in comparison with the positive control group. This ratio was considered positive when it was 1.0 or greater and negative when it was 1.0 or less. The samples were analyzed for the presence of 13 types of the HR-HPV groups (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68).

## 4. HPV DNA chip test

We used the HD-C, a PCR-based DNA microarray system as a HPV genotyping method for HPV typing. The HD-C contains 24 type-specific probes; 14 probes are HR types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) and 10 probes are LR types (6, 11, 34, 40, 42, 43, 44, 53, 54, and 70). Briefly, DNA was isolated from a swab sample using a DNA isolation kit (MyGene Co.), and then target L1 regions of HPV DNA were amplified and labeled by a single dye (indocarbocyanine-dUTP; NEN Life Science Products, Inc., Boston, MA, USA). The PCR products of all samples were detected by electrophoresis with a 2.5% agarose gel. The samples were mixed with a hybridization solution (MyGene Co.). Hybridization was performed at 43°C for 90 min. The hybridized HPV DNA was visualized using a DNA chip

**Table 2**
The level of concordance between HR-HPV tests.

| | No. of specimens (%) with HDC[a] | | Total no. of specimens (%) |
|---|---|---|---|
| | Negative | Positive | |
| HC2[a] | | | |
| Negative | 1306 | 65 | 1371 (47.63) |
| Positive | 11 | 1498 | 1509 (52.4) |
| Total | 1317 (45.7) | 1563 (54.3) | |

HR, high risk; HPV, human papillomavirus; HC2, Hybrid Capture II test; HDC, HPV DNA chip test.

[a] Absolute agreement = 97.4%, kappa = 0.947 ($P < 0.001$). Agreement between tests was assessed by Cohen's kappa statistic. *P* value was calculated using McNemar's test.

Please cite this article as: W.D. Kang, et al., Is human papillomavirus genotype important in predicting disease progression in women with biopsy-proven negative or CIN1 of atypi..., Gynecol Oncol (2017), https://doi.org/10.1016/j.ygyno.2017.11.025