Letter to the Editor

Assessing the Effectiveness of a Cervical Cancer Screening Program in a Hospital-based Study[*]

YANG Yi[1], LANG Jing He[1], WANG You Fang[1], CHENG Xue Mei[1],
CAI Yu Pin[2], LI Hui[2], ZHU Bao Li[2], and ZHANG Rui Fen[2,#]

This study compared HPV testing and liquid-based cytology (LCT) as performance indicators for cervical cancer screening in a hospital-based study. A total of 61,193 outpatients were screened initially by LCT. Samples with screening results showing atypical squamous cells of undetermined significance (ASC-US) or worse were referred for colposcopy, and some samples were tested for high-risk HPV types with the Hybrid Capture II system (HC II). Data on LCT (*n*=61,193) and HC II (*n*=1056) results were analysed. Overall test positivity for LCT was 2.53% using an ASC-US threshold, 3.11% using a low-grade squamous intraepithelial lesion (LSIL) threshold, and 0.67% using a high-grade squamous intraepithelial lesion (HSIL) threshold. A total of 1839 women (84% of the 3893 patients with abnormal cytology) underwent colposcopy-directed biopsy. HPV was positive in 80.3% of women with cervical intraepithelial neoplasia 1 (CIN1), 88.3% of those with CIN2, 79.2% of women with CIN3 and 50% (2 of 4) of women with invasive cancer. There was a significant increase in the detection of CIN2 or worse with adjunct HPV testing of women with ASC-US and LSIL. However, there were no differences in the detection of CIN2+ cases with adjunct HPV testing of women with HSIL. The results indicate that HPV testing for HSIL triage should not be recommended in cervical cancer screening.

Cervical cancer is the second most common cancer among women worldwide; persistent infection with high-risk types of human papillomaviruses (HR-HPV) is the primary cause of cervical carcinogenesis[1]. Systematic cervical screening has significantly decreased the incidence of and death rate from cervical cancer in developed countries. Screening strategies include single or combination-test strategies for the identification of women with positive screening results who require follow-up procedures. Single-test strategies include cytology or HR-HPV DNA testing. Combination-test strategies include HR-HPV DNA testing as an adjunct to cytology, or co-testing with both cytology and HR-HPV DNA test[2]. HPV testing as an adjunct to cytology could resolve the ambiguous cytology category of atypical squamous cells of undetermined significance (AS-CUS) and increase the sensitivity for detecting cervical intraepithelial neoplasia (CIN).

Studies have suggested that cervical cancer incidence rates have been increasing in recent decades in China[3]. The disease burden of cervical cancer and the screening programs vary greatly in China due to the imbalance in economic development. Cervical cancer screening programs are inconsistent and cytology-based in most regions of China; only women living in urban regions have access to HPV DNA or cytology-based screening. Although a few excellent cervical cancer screening studies have focused on rural and high-incidence areas in China[4], few large studies have been based on the settings of a hospital in an urban area using histologically confirmed cervical lesions. To better examine cervical cancer screening outcomes in the urban setting, we performed a cross-sectional cervical cancer screening study in Beijing, China.

This study was a hospital-based cervical cancer screening study that was conducted in Beijing from 2004 through 2006. The study protocol was approved by the Ethics Committee of Peking Union Medical College Hospital. A total of 61,193 outpatients aged 21-66 years (mean age: 40.4±9.2; median age: 41.0) attending the Department of Obstetrics and Gynaecology were included. At the

initial baseline visit, cervical samples were taken using an endocervical cytobrush and placed into 2 mL of PreservCyt Solution (Cytyc Corp., Boxborough, MA, USA) for cytological examination. Women were included for further study if screening results showed ASC-US or worse cytological screening results.

HPV testing and colposcopy were offered within 6-12 months to women with abnormal cytology. During the follow-up visit, two cervical samples were taken; the first was collected for histological specimen examination. The second sample was taken using a cervical sampler brush included in the HC II test kit (Digene, Gaithersburg, MD, USA), and the brush was placed into a tube containing HC II transport medium. The samples were transported and stored at 4 °C until further testing.

Cytological examination was performed by senior cytopathologists according to the Bethesda System 2001, which classifies cervical samples as follows: a) within normal limits (WNL); b) ASC-US; c) low-grade squamous intraepithelial lesion (LSIL); d) high-grade squamous intraepithelial lesion (HSIL); e) squamous cell carcinoma (SCC) or adenocarcinoma (ACC)[5]. The threshold for a cytological abnormality was AS-CUS. Women with ASC-US or worse were further allocated to follow-up study according to the protocol shown in Figure 1.

Ectocervical biopsy specimens were taken from all lesions that were acetowhite by visual inspection after application of 5% acetic acid to the cervix close to the squamocolumnar junction. If the colposcopy was normal (i.e., no acetowhite lesions were observed), four biopsy specimens were taken at the 3-, 6-, 9-, and 12-o'clock positions on the ectocervix close to the squamocolumnar cell junction and subjected to histopathologic analysis. Specimens were fixed in 10% formalin and processed for paraffin embedding. Histological sections were cut to 4 μm thickness and stained with haematoxylin and eosin. Histological diagnosis of biopsy specimens was performed according to a three-tiered designation for CIN, in which CIN1 is mild dysplasia, CIN2 is moderate dysplasia, CIN3 is severe dysplasia, and carcinoma in situ (CIS), SCC or ACC are confirmed cases of cervical cancer. All confirmed cases of cervical cancer and CIN3 were treated.

The sample tube for the HPV test was processed using the supplies and reagents of the HC II assay according to the manufacturer's instructions. This test targets 13 HR-HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). The test cut-off and internal quality assurance procedures were based on the manufacturer's instructions. The results of the HPV DNA detection assay are expressed as a ratio of relative light units (rlu ratio) to the average of three positive controls, with 1.00 (equivalent to an HPV DNA concentration of 1 pg/mL) as the cut-off for test positivity (i.e., an rlu ratio ≥1.00 was considered HPV DNA positive, and an rlu ratio <1.00 was considered HPV DNA negative). All tests were performed blindly with regards to the cytological results.

The chi-squared test was used to assess between-group and within-group comparisons. A $P<0.05$ was considered statistically significant.

During the 3-year period, 61,193 women were evaluated for cervical cytology, and 6.4% (3893) of the population examined had a cytological screening result of ASC-US or worse; more specifically, 1550 (2.53%) women were diagnosed with ASC-US, 1906 (3.11%) were diagnosed with LSIL, 411 (0.67%) were diagnosed with HSIL, and 26 (0.05%) were diagnosed with carcinoma. Among the 3893 patients with abnormal cytology, 1839 women (47.2%) consented to colposcopy-directed biopsy procedures, and 1056 women consented to an HPV DNA test. The summary results of the outcome of cytologically abnormal categories and HPV testing are shown in Figure 1. Approximately 28.8% (529/1839) of women were diagnosed with ASC-US, 48.7% (895/1839) were diagnosed with LSIL, and 22.5% (415/1839) were diagnosed with HSIL.

Not all CIN3+ lesions had high-risk HPV infections in our study; Results of the HC-II test were positive for 80.3% of the women with CIN1, 88.3% of those with CIN2, 79.2% of women with CIN3/CIS, and in 2 of 4 (50%) women with invasive cancer. Approximately 69.7% of women with normal cytology or cervicitis had a positive HC II test result (Table 1). The incidence of HPV in women with abnormal cytology categories was similar, ranging from 74.1% in women with ASC-US to 78.6% in women with HSIL (Table 2).

It is notable that patients with LSIL and ASC-US could be more precisely diagnosed with CIN2 or worse with adjunct HPV testing. There was a significant increase in the detection of CIN2 or worse in the ASC-US and LSIL groups with adjunct HPV testing. However, there were no differences of CIN2+ cases within HSIL patients (Supplementary Table 1).
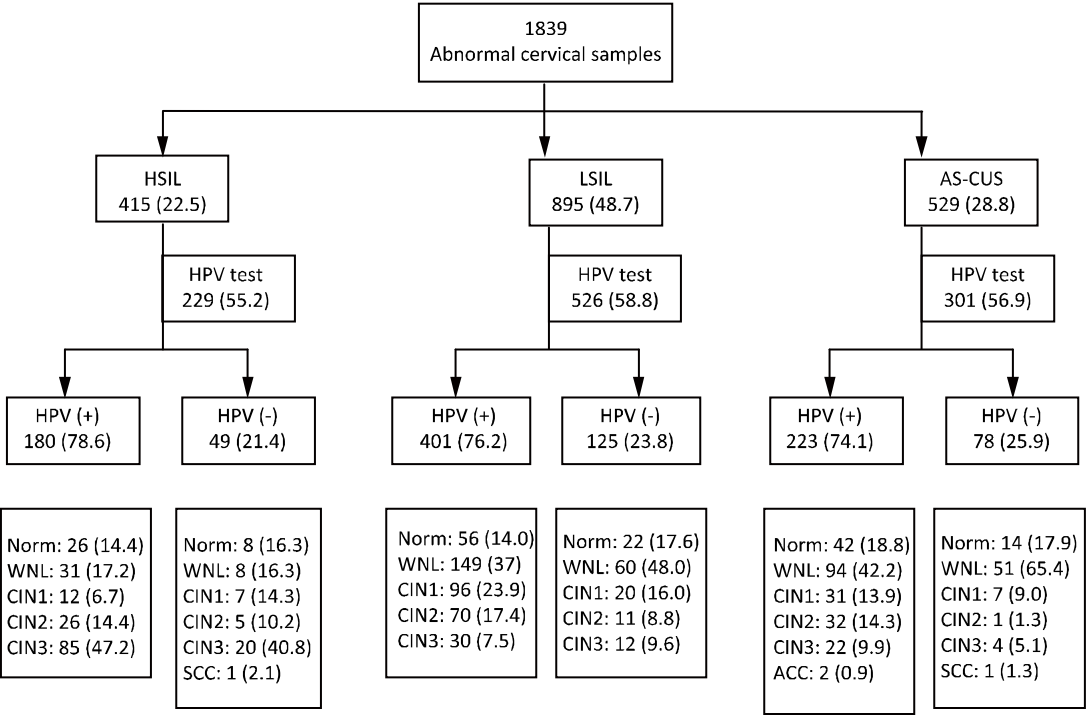
Figure 1. Flow chart of the cervical cancer screening protocol using a combination of cervical cytology and HPV-DNA testing. Data are *n* (%). HPV, human papillomavirus; Norm, healthy; WNL, within normal limits (including metaplasia, cervicitis, etc.); ASC-US, atypical squamous cells of undetermined significance LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; ICC, invasive cervical cancer; CIN, cervical intraepithelial neoplasia; SCC, squamous cell carcinoma; ACC, adenocarcinoma.

Table 1. HPV DNA Test and Histology for Women with Abnormal Cervical Cytology

HC II	WNL	CIN1	CIN2	CIN3	SCC	ACC	Total
HC II +	274 (69.7)	139 (80.3)	128 (88.3)	137 (79.2)	0 (0.0)	2 (100.0)	680
negative	119 (30.3)	34 (19.7)	17 (11.7)	36 (20.8)	2 (100.0)	0 (0.0)	208
Total	393	173	145	173	2	2	888

Note. Data are *n* (%). Abbreviations: HC II, Hybrid Capture 2; HPV, human papillomavirus; WNL, within normal limits (including metaplasia, cervicitis, etc.); CIN, cervical intraepithelial neoplasia; SCC, squamous cell carcinoma; ACC, adenocarcinoma.

Table 2. HPV DNA Test for 1056 Women with Abnormal Cervical Cytology

HC II	AS-CUS	LSIL	HSIL	Total
HC II +	223 (74.1)	401 (76.2)	180 (78.6)	804
negative	78 (25.9)	125 (23.8)	49 (21.4)	252
Total	301	526	229	1056

Note. Data are *n* (%). Abbreviations: HC II, Hybrid Capture 2; HPV, human papillomavirus; ASC-US, atypical squamous cells of undetermined significance LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion.

HPV infection is the primary cause of precancerous lesions of the cervix. Consequently, precancerous lesions might very well be found in women who test positive for HPV infection. The potential role for HPV testing among the abnormal cytology groups could be revealed by the relationships between HPV detection and severity of CIN shown by the biopsy. In this study, we found that there was a much larger risk of CIN2+ in women with positive HPV DNA compared to women with negative HPV DNA in the AS-CUS or LSIL subset, whereas the HPV DNA test did not make any difference in diagnosing CIN2+ cases for patients with HSIL. These findings enhance our knowledge about appropriate HPV testing and confirm the concept that HPV testing should not be recommended to guide the colposcopy triage of women with cervical abnormalities of higher grade than LSIL[6]. Inappropriate cervical cancer diagnostic strategies would waste health care resources in developing countries such as China. Misuse of the HPV test might reflect confusion about cervical cancer screening recommendations. According to the recent findings reported by Saccardi et al, the challenge of cervical screening is to reach an optimum balance between benefits, harm and affordability[7]. The detection of HR-HPV could be considered an appropriate and cost-effective clinical tool in AS-CUS-LSIL triage because of the increased detection of CIN2+ cases compared to HR-HPV testing based on HSIL triage.

Our results did not show that an increase in HPV prevalence is related to a higher cytology grade, whereas some studies have observed high rates of HPV positivity in cases of severe cervical dysplasia. Furthermore, not all CIN3+ lesions harboured high-risk oncogenic HPV type infections in our study; 36 CIN3 and 2 invasive cancer cases were HPV DNA negative. The reason for this difference may be that in our study, HPV test screening was not performed for women with negative cytology, and these missing data could bias our results. HPV testing is expensive and is not covered by Chinese medical insurance plans. Economic considerations and lack of public awareness of the causal association between HPV infection and cervical cancer have prevented the widespread use of HPV testing. Additionally, in our study, the colposcopy-directed biopsy was used as gold standard to determine a patient's true disease state. The skill and experience of the gynaecologist performing the colposcopy procedure could impact the test results[8]. The diagnosis of CIN3 may be falsely positive when benign atypical changes or lesser-grade CIN is over-interpreted or misclassified. There might have been a few incident (new) cases of cervical pre-cancer that as false-negative non-high-risk HPV as shown by Castle[9]. Finally, the interval from screening to colposcopy examination and biopsy was 6-12 months in our study. Other studies have included lesions detected within 2 months from screening to colposcopy[10]. It is possible that late referral led to the detection of a lower proportion of HPV negative cases that otherwise would have appeared as HPV positive and thus decreased HPV detection in some women with CIN3+ lesions.

Our study shows that HPV testing for HSIL triage should not be recommended as part of cervical cancer screening. The study was performed in a high-quality hospital in Beijing, and the results might not apply to all hospitals in China. However, the findings of our study provide evidence to support the appropriate use of HPV testing in cervical cytological screening programs in China.

#Correspondence should be addressed to ZHANG Rui Fen, Tel: 86-10-64807433, Fax: 86-10-64807358, E-mail: zhangrf@im.ac.cn

Biographical note for the first author: YANG Yi, male, born in 1970, MD, PhD, associate professor, specialising in obstetrics and gynaecology.

REFERENCES

1. Ngan HY, Garland SM, Bhatla N, et al. Asia oceania guidelines for the implementation of programs for cervical cancer prevention and control. J Cancer Epidemiol, 2011; 794861.

2. Wright TC Jr, Schiff man M, Solomon D, et al. Interim guidance for the use of human papillomavirus DNA testing as an adjunct to cervical cytology for screening. Obstet Gynecol, 2004; 103, 304-9.

3. Yang L, Parkin DM, Li L, et al. Time trends in cancer mortality in China: 1987–1999. Int J Cancer, 2003; 106, 771-83.

4. Zhao FH, Lin MJ, Chen F, et al. Performance of high-risk human papillomavirus DNA testing as a primary screen for cervical cancer: a pooled analysis of individual patient data from 17 population-based studies from China. Lancet Oncol, 2010; 11, 1160-71.

5. Solomon D, Nayar R. The Bethesda system for reporting cervical cytology. 2nd ed.New York: Springer Verlag, 2004; p67-87. www.fda.gov/cdrh/mda/docs/p890064s009.html. [2006-05-26].

6. Irwin K, Montano D, Kasprzyk D, et al. Abnormal Cytology Management, and Counseling Practices in the United States. Obstet Gynecol, 2006; 108, 397-409.

7. Saccardi C, Gizzo S, Noventa M, et al. High-risk human

papillomavirus dna test: could it be useful in low-grade cervical lesion triage? five-year follow-up. Reprod Sci, 2014; 21, 198-203.
8. Baseman JG, Kulasingam SL, Harris TG, et al. Evaluation of primary cervical cancer screening with an oncogenic human papillomavirus DNA test and cervical cytologic findings among women who attended family planning clinics in the United States. Am J Obstet Gynecol, 2008; 199, 26e1-8.

9. Castle PE, Cox JT, Jeronimo J, et al. An analysis of high-risk human papillomavirus DNA-negative cervical precancers in the AS-CUS-LSIL Triage Study (ALTS). Obstet Gynecol, 2008; 111, 847-56.
10.Pan QJ, Hu SY, Zhang X, et al. Pooled Analysis of Performance of Liquid Based Cytology in Population-Based Cervical Cancer Screening Studies in China-Cancer Cytopathol, 2013; 121, 473-82.

Supplementary Table 1. Histology Diagnosis in relation to HPV DNA test for Women with abnormal Cytology

	ASC-US		LSIL		HSIL	
	≥CIN2	<CIN2	≥CIN2	<CIN2	≥CIN2	<CIN2
HPV (+)	56	167	100	301	111	69
HPV (-)	6	72	23	102	26	23
P value	0.001		0.132		0.276	

Note. Data are *n*. Abbreviations: CIN, cervical intraepithelial neoplasia; HPV, Human Papillomavirus; ASC-US, atypical squamous cells of undetermined significance LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion.