

Analysis of one year follow-up of women with cervical cytology report of atypical squamous cells and the diagnostic role of high-risk HPV infection

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Summary

Objective: To investigate the risk of developing cervical intraepithelial neoplasia grade 2 (CIN2) or greater disease in patients with cytology report of atypical squamous cells of undetermined significance (ASC-US) or cannot exclude high-grade atypical squamous cells (ASC-H) in one year follow-up. **Study design:** Analysis of colposcopy-directed multiple cervical biopsies in all patients. Patients without CIN2 or greater diseases were tested for human papillomavirus (HPV) DNA at the enrollment and at 12th month and followed up by cytology at the 6th and 12th month. Patients with repeated abnormal results were subjected to colposcopy-directed biopsy. **Results:** A total of 894 ASC-US and 101 ASC-H patients were enrolled. The rate of CIN2 or greater disease was 14.2% in ASC-US group and 46.5% in ASC-H group, at the first test respectively. A total of 65.0% of patients in ASC-US have completed the study and 47.5% repeatedly showed abnormal cytology, while the same rates in ASC-H were 62.7% and 50%. Only four cases were diagnosed with CIN2 in ASC-US group. The rate of HPV DNA becoming negative was 54.9% and 51.5% for ASC-US and ASC-H, respectively. **Conclusions:** The diagnosis rate of CIN2 or greater lesions in ASC-US and ASC-H patients was about 15% and 46.5%, respectively, within one year.

Key words: Cervical intraepithelial neoplasia; Atypical squamous cell; HPV; Follow-up.

Introduction

The number of deaths worldwide in women with cervical cancer is just after that in women with breast cancers. Each year, new occurrence of cervical cancers is about 466,000 cases globally, among which one-third are from China. As the incidence of cervical cancer has risen in recent years, it shows a trend of affecting younger patients [1, 2]. Cervical smear is the widely-used method in screening precancerous lesions and cervical cancers. The highest proportion of cells in the Bethesda system (TBS) is atypical squamous cells, which show certain atypia and can be divided into two subtypes: atypical squamous cells of undetermined significance (ASC-US) and high-grade atypical squamous cells (ASC-H) [3]. ASC-US accounts for a huge number of patients but has lower chance to develop cervical intraepithelial neoplasia (CIN2) or greater lesions (about 10%) [4, 5]; although it is rare in cytology report, ASC-H shows higher potential of developing CIN2 or greater lesions (about 40%) [6]. The current concern is how to effectively detect cancer from these atypical squamous cells without over-diagnosis and over-treatment of the disease. After the establishment of relationship between high-risk human papillomavirus (HR-HPV) infection and cervical precancerous lesion and cancer, HPV detection has been applied in the primary screening of cervical cancer and precancerous lesions, the ASC-US triage management, and the follow-up management after treatment of CIN disease [7]. Although there are extensive forward-thinking studies with a large number of samples regarding the application

of HPV detection in patients with cytology report of positive atypical squamous cells, few data are from China. This study analyzed 995 patients who were admitted to the Hospital from June 2007 to December 2008 with ASC in liquid-based cytology test and went through colposcopy-directed cervical biopsy (894 cases of ASC-US; 101 cases of ASC-H). The patients who were not primarily diagnosed with CIN2 or higher-grade lesions were subjected to one-year follow-up to observe the prognosis, to further explore the reasonable approach to ASC management, and to investigate the significance of application of HPV test in performing reasonable diagnosis.

Materials and Methods

Patients who were admitted into outpatient for cervical lesions to the Peking University Third Hospital from June 2007 to December 2008, diagnosed with ASC-US or ASC-H in cervical cytology report, and accepted colposcopy-directed biopsy, were included in this study and recommended for HPV DNA detection. Patients who were positive for both ASC-US and HPV DNA were recommended for colposcopy; HPV DNA negative cases were selected for colposcopy or cytological follow-up based on patients' and clinical needs. ASC-H patients were subjected to colposcopy-directed cervical biopsy and endocervical curettage. Patients who were pregnant, could not be followed up, had history of CIN lesion, or went through hysterectomy, were all excluded from this study. A total of 995 patients were enrolled with an average age of 39.3 years (range 23 - 73), an average number of pregnancies of 2.3 (range 0 - 7), and an average number of births of 1.07 (range 0 - 6).

The first colposcopy-directed multiple cervical biopsies and pathological analysis were carried out in no less than two quadrants of the cervix in ASC-US group and no less than three quadrants in ASC-H group. Patients failed to be diagnosed with

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Table 1. — Follow-up of patients who had not been diagnosed with CIN2 or greater lesions.

Follow-up	ASC-US group First-time HPV				ASC-H group First-time HPV							
	Abnormal	+	Normal	Abnormal	-	Normal	Abnormal	+	Normal	Abnormal	-	Normal
Cytology	92		103	51		55	17		16	5		4
Colposcopy*												
CIN1, warts, inflammation	90			49			17					
≥ CIN2	2**			2**			0			0		
Total	301									248		

* Colposcopy and cervical biopsy were performed when the cytology was abnormal.

** Two cases in HPV DNA positive group and two cases of CIN2 in HPV DNA negative group were diagnosed during follow-up, and all the four cases became HPV DNA positive at the end of follow-up. One case was histologically diagnosed with VaIN2.

CIN2 or higher-grade lesions were cytologically followed-up after six and 12 months and recommended for further HPV detection after 12 months. Patients with recurrent abnormal cytology were subjected to colposcopy-directed cervical biopsy. Follow-up ended when the patient completed two consecutive cytology tests, diagnosed with CIN2 or greater lesions in repeated colposcopy-directed cervical biopsy, or received cone biopsy. This study used the Bethesda System (TBS) 2001 criteria in cervical cytology diagnosis. Liquid-based tests such as SurePath and TriPath (BD Diagnostics) were used in cervical cytology; HR-HPV was detected by hybrid capture 2 high-risk HPV DNA Test system (HC-II, Digene Corporation).

Data were analyzed with SPSS12.0 software, and significant differences were determined when $p < 0.05$ in χ^2 test.

Results

ASC-US slides account for 6.1% (2,815 / 46,180) of the total cell slides in the Hospital during the same period, while ASC-H slides account for 0.2% (112 / 46,180) of the total cell slides. Because of reimbursement issues, a part of the patients were transferred to other hospitals to continue treatment. Therefore, a total of 894 cases of ASC-US and 101 cases of ASC-H received colposcopy and participated in the follow-up. The results of first colposcopy are shown in Table 1.

The ratio of abnormal cytology report during follow-up

A total of 301 of the 767 patients who were not diagnosed with CIN2 or greater diseases completed follow-up in the ASC-US group; during the 12 months of follow-up, the rate of diagnosis with CIN2 was 1.3% (4/301). Among the 54 patients who were without CIN2 or greater diseases in the ASC-H group, 42 completed follow-up; during the 12 months of follow-up, no cases of CIN2 were diagnosed.

The relationship between prognosis and HPV infection

Initially, 71.3% of patients (623/874) in the ASC-US group were HPV DNA positive. During follow-up, among the 301 patients who were not diagnosed with CIN2 or greater diseases in the initial diagnosis and completed follow-up, 195 were first-time diagnosed as HPV positive; the rate of patients turning into HPV negative was 54.9% (107/195).

During the enrollment examination, the rate of HPV DNA positive patients in ASC-H group was 86.7%

(85/98). Among the 42 patients who were not diagnosed with CIN2 or greater diseases during the initial diagnosis but completed follow-up, 33 were first-time diagnosed as HPV positive; during one year follow-up, the rate of patients turning into HPV negative was 51.5% (17/33). The ratios of patients whose HPV DNA turned negative showed no significant difference between ASC-US and ASC-H groups ($p = 0.72$).

Discussion

The recognition and definition of atypical squamous epithelial cells in cervical epithelia exactly reflect the process of modern understanding of cervical cancer and precancerous cervical lesions. A particular category of "atypical cells" was introduced in 1988 [8] and became the most common type of abnormalities. A total of 10% - 17% of the patients in this category have been diagnosed with CIN2/3 [4, 5, 9]; while cervical cancer accounts for 0.1% - 0.3% of the patients in this category [10].

Based on TBS 2001 criteria, patients with atypical cells are divided into two subgroups: ASC-US and ASC-H; ASC-US accounted for 3.6% in the cytology report [11]; while in the literature, ASC-H is rare in cytology report and accounts for only 0.27% - 0.56% of patients [12]. Since most of these patients do not develop CIN2 or greater lesions, the authors require a more sensitive and specific way to perform the subsequent diagnosis in order to decrease the burden on these patients, both psychologically and economically.

The controlled forward-thinking ASC-US/LSIL triage study (ALTS) has been carried out in order to better guide the choice of clinical measures for diagnosis and treatment of ASC-US/LSIL. ALTS has evaluated the follow-up cytology, colposcopy, and HPV triage management, and validated these measures as well [13]. The authors hope that the data of this study can be used to elaborate the clinical significance of these abnormal cytological reports. The outpatient cervical and vaginal cytology specimens in this study are collected from the Department of Gynecology and Obstetrics of the Hospital. In recent years, the different detection rate of ASC-US reflects, to a certain extent, the particularity of a group of patients, especially those without regular periodic physical examinations. Therefore, a reasonable treatment of these patients who are first-time diagnosed with

abnormal cytology, is particularly important in reducing the future incidence of cervical cancer. This study has for the first time shown that among patients who were not diagnosed with CIN2 or greater diseases by colposcopy in the initial examination, the chance to be diagnosed with CIN2 or greater diseases during one year follow-up was 1%. Facing such a minor rate of abnormality, comprehensive colposcopy examination becomes important, especially when the rate of loss to follow-up is high.

Based on recent literatures, the diagnosis rate of CIN2 and higher-grade lesions in ASC-H patients is 36% - 88%, higher than that in ASC-US patients, but lower than that in HSIL patients; therefore, the treatment principle of ASC-H patients also differs from that of the latter two [14-16]. The American Society for Colposcopy and Cervical Pathology (ASCCP) 2006 guidelines recommend colposcopy and multiple biopsies for those cytologically diagnosed ASC-H patients and closer follow-up for patients who are first-time diagnosed with CIN2 or higher-grade lesions but without histological confirmations. In this study, the ratio of first-time diagnosis of CIN2 or higher-grade lesions was 46.5%, but there were no CIN2/3 cases detected during follow-up. This is consistent with the existing reports and may be related to the less number of patients and shorter period of follow-up. All cervical lesions in this study were diagnosed by using this method during the first examination, which suggests that in combination with endocervical curettage, colposcopy is the most effective way for detecting CIN2 or higher-grade lesions.

Using HPV in the screening of cervical lesions has also experienced a process of gradual acceptance, and initially the methods have been recommended only for "those who know their usefulness" [17].

During the continuous investigation of ASC-US, further specification of the morphology has encountered difficulties. Some studies, which are attempting to further define the ASC-US, have shown that the cellular changes are highly subjective; even professional pathologists face the same problem of poor reproducibility [18-21]. For the difficulties in dealing with the large number of ASC-US patients, HR-HPV testing plays a critical triage role for further diagnosis [22, 23]. The relationship between persistent HR-HPV infection and the incidence of cervical cancer have been well-recognized since 1990 and HPV testing has also begun to be used in the screening of cervical cancer and precancerous lesions and in post-treatment follow-up [19]. ALTS and other studies show that triage through detection of HPV DNA is objective, economic, and can almost immediately detect CIN2 or higher-grade lesions; the rate of accurate negative predictions achieves 98%-100% [24]. This study has also shown that the rate of accurate HPV negative predictions in first-time colposcopy was 99%. During follow-up, no CIN2/3 patients had been detected in HPV negative patients.

For cytologically-diagnosed ASC-H patients, physicians should pay more attention to the first comprehensive examination; in addition to colposcopy-directed multiple biopsies, HPV testing has a high value for negative prediction of the disease.

Due to the detection of CIN2 or greater diseases during follow-up, more attention should be paid to patient management after the primary diagnosis [25]. Unfortunately, because the high-rate of patient loss to follow-up is a common problem, appropriate manpower and resources are needed for reducing patient loss during follow-up, and the individualized patient treatment should be performed during the tracking process.

After effective training, even in developing countries such as China, the diagnostic quality in some areas can approach the level of that in developed countries, where new cases have been reduced by 90% because of effective implementation of cervical cancer screening. However, the follow-up strategy in developed countries is difficult to implement by developing countries due to the lack of medical resources [26]. To improve the follow-up rate, various efforts may be required, such as to educate patients with medical knowledge, effective follow-up from the hospital, and financial support from the country to relieve patients' medical and economic burdens.

This study has shown that during one-year follow-up after initial colposcopy, in both ASC-US and ASC-H groups, the detection rate of CIN2 and greater lesions was very low. Although ASCCP has preferred HPV DNA triage management for ASC-US patient, due to the high-rate of loss to follow-up in developing countries, the authors recommend that once diagnosed with abnormal cytology, the patient needs to undergo an immediate colposcopy with cervical biopsy, which will timely and effectively detect cervical cancer, as well as precancerous lesions.

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