

Cervical cytology ASCUS patients with HPV detection and clinical value

J.X. Cheng^{1,2}, L.L. Yao², H. Xiang³, Y.J. Zhan², P. Zhou², M. Yuan², L.P. Mei², Y. Zhang¹

¹ Department of Gynaecology, Xiangya Hospital of Central South University, Changsha

² Department of Gynaecology, the Affiliated Cancer Hospital of Xinjiang Medical University, Urumqi

³ Department of Gynaecology, the First Affiliated Hospital of Xinjiang Medical University, Urumqi (China)

Summary

Objective: Patients whose cervical cytological exams produced a result of atypical squamous cells of undetermined significance (ASCUS) were asked to undergo human papillomavirus (HPV DNA) genotyping detection to assess the role of HPV infection in ASCUS. **Materials and Methods:** This study included 1,219 patients with ASCUS that were randomly divided into two groups. The first group contained 618 patients. These participants underwent colposcopy with cervical biopsy. The remaining 601 underwent colposcopy and biopsy with HPV DNA detection. **Results:** Out of the 56,000 patients with ASCUS who underwent ThinPrep cytology test (TCT) detection in the authors' hospitals' gynecological outpatient clinics, 1,604 were diagnosed with ASCUS (2.86%). Among the 1,219 patients with ASCUS, the rate of detection of cervical intraepithelial neoplasia (CIN) and cancerization was 22.89% (279/1,219). Among the 601 patients who underwent HPV testing, 182 were positive for high-risk HPV (30.28%). Among HPV-positive samples, the most common high-risk types were HPV16, and HPV58. The most common low-risk types were HPV6 and HPV11. The rate of detection among high-risk patients who were positive for HPV and cervical carcinoma with intraepithelial neoplasia was 70.88% (129/182). The rate of detection for HPV-negative patients with cervical cancer with intraepithelial neoplasia was 11.55% (47/407). The rate of detection of high-risk HPV was higher than among patients who had not undergone HPV detection and among patients who were negative for HPV ($p < 0.05$). **Conclusion:** The results of cervical cytological examination showed that the manner of progression from inflammation to cancer could differ considerably. HPV DNA examination is an effective means of categorizing and managing ASCUS.

Key words: ASCUS; HPV genotyping detection; Colposcopy; Cervical intraepithelial neoplasia (CIN).

Introduction

Cervical cancer ranks second in cancer mortality in women worldwide. Medical intervention of human malignancies [1, 2] is the only way to decrease morbidity and mortality and screening and proper treatment of cervical intraepithelial neoplasia (CIN) is the key to effective prevention and treatment of cervical cancer. Cervical cytology method is considered to be one the most successful medical history of early cancer screening. However, vulnerable subjective factors, especially for cervical cytology results, are not clear for atypical squamous cells of undetermined significance (ASCUS) treatment and therefore poses a clinical dilemma. ASCUS cytology of high-grade squamous intraepithelial lesions (HSIL) or invasive cancer is more common [3, 4]. American Association for International Cancer held a meeting at Bethesda of Maryland in 1988 and proposed the TBS classification of cervical cytology. They clearly put forward the concept about atypical squamous cells of undetermined significance (ASCUS) in 2001. Numerous studies showed that ASCUS occupied the largest proportion of abnormal cervical cytology results, but histological pathology results of abnormal cervical cytology

may be reactive and cervical lesions, even cancer. The biological characteristics reflect the true present situation, that the pathologist are unable to make accurate and repeatable judgment on such specimen [5, 6], so ASCUS management in clinical work becomes important and difficult. Numerous studies have confirmed the development of the majority of cervical intraepithelial neoplasia and cervical cancer with high-risk human papillomavirus (HPV) infection [7-10]. The majority of CIN associated with HPV infection, more than 90% of cervical cancer specimens can detect HPV DNA [11, 12]. It can be used as a first resort HPV DNA detect ASCUS patients monitored, the paper 601 cases of patients with ASCUS under HPV DNA genotyping and multi-point electronic colposcopy biopsy. To investigate the clinical value of detecting HPV DNA ASCUS triage management.

Materials and Methods

From October 2007 to October 2012, a total of 56,000 patients came to a gynecology clinic for treatment. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Cen-

tral South University. Written informed consent was obtained from all participants.

ThinPrep cytology test (TCT)

All the patients were diagnosed by senior, qualified cytological professionals through cervical thin layer solution-based cytological examination (TCT). Patients who had not undergone any medical or surgical treatment for cervical disease were selected for the present study. Women were excluded if they had visible CINs, any history of cervical condyloma, or any recent pregnancy. From these, 601 were selected at random for HPV DNA detection. They also underwent colposcopy and biopsy. The remaining 618 patients underwent colposcopy detection and biopsy but not HPV DNA detection. These 1,219 patients ranged in age from 18 to 65 years. The average age was 34.35 ± 7.15 years (Table 1). Among them, there were 947 ethnic Han Chinese individuals, accounting for 77.69%; there were 250 ethnic Uyghurs, accounting for 20.51%, and 22 women of other nationalities, accounting for 1.8%.

Cervical biopsy under colposcopy

Electronic colposcopy examination was performed and biopsy forceps were used to remove cervical secretions with cotton swabs. Then the cervical mucosa and changes in tubes were observed. Then 3% acetic acid solution was dripped onto cultured cervical cells. The samples were checked for white mucous membranes, mosaics, point-like blood vessels, heterosexual vascular authenticity erosion, grape-like blood vessels, leukoplakia, white spots on the mucosa. Cervical biopsy was performed with colposcopy after one minute. The biopsied tissue was fixed in 10% neutral formalin liquid, embedded in paraffin, and sliced into pieces of the usual size. Then two or three senior pathologists made histopathological diagnoses.

HPV DNA genotyping

For HPV DNA genotyping, a sampling brush was placed into preserving fluid and stored at 4°C. HPV DNA detection was done using rapid flow-through hybridization gene chip technology. This gene chip technology can detect 13 high-risk hypotypes, HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, and 68, five low-risk hypotypes 6, 11, 42, 43, and 44, and three hypotypes that are common in Chinese people (53, 66, and CP8304). The equipment described above was used in accordance with the manufacturers' instructions.

Statistical analysis

Data was entered into a FOXPRO6.0 database and SPSS13.0 software was applied for χ^2 testing with a significance level of $\alpha = 0.05$.

Results

TCT detection

Among the 56,000 women who underwent TCT examination, 1,604 were found to have ASCUS, accounting for 2.86% of the total. These included 1133 ethnically Han

Table 1. — *Age distribution of 1,604 patients with ASCUS.*

| Age (years) | Numbers (n) | Ratio (%) |
|-------------|-------------|-----------|
| < 20 | 24 | 1.5 |
| 21-25 | 180 | 11.22 |
| 26-30 | 280 | 17.46 |
| 31-35 | 320 | 19.95 |
| 36-40 | 380 | 23.79 |
| 41-45 | 220 | 13.72 |
| 46-50 | 150 | 9.35 |
| ≥ 51 | 50 | 3.12 |

Chinese women (70.64%), 439 ethnic Uyghur women (27.36%), and 32 women of other ethnicities (2.00%). They ranged in age from 18 to 65 years. The most numerous age groups were 31-35 and 36-40 years (Table 1).

ASCUS histopathological diagnosis

Among the 1,219 women with ASCUS, all underwent cervical biopsy. The rate of detection of cervical abnormalities was 22.89% (298/1,219); 938 patients had chronic cervicitis CIN I: 32, CIN II: 73 or CIN III: 62, and 14 cervical cancer, accounting for 76.95%, 10.35%, 5.82%, 4.33%, and 1.06% of the total, respectively. Among the 250 Uyghur patients with ASCUS, 176 had chronic cervicitis, CIN I: 32, CIN II: 2 or CIN III: 20, and four had cervical cancer, accounting for 69.12%, 12.80%, 8.40%, 8.00%, and 1.60%, respectively. Among the remaining 22 patients, nine had chronic cervicitis, CIN I: 2, CIN II: 2 or CIN III: 1, or no cervical cancer, accounting for 77.27%, 9.09%, 9.09%, 4.55%, and 0.00%, respectively. No significant differences were found among these three groups (Table 2).

HPV DNA test

The HPV DNA detection results of the 601 patients with ASCUS who had HPV testing were as follows: 194 (32%) patients were positive for HPV and 407 (43%) patients were negative for HPV. Among the 450 ethnic Han Chinese participants, the positive rate was 28.89% (130/450). Among the 141 ethnic Uyghurs, the positive rate was 43.26% (61/141) and among the ten individuals of other ethnicities, the positive rate was 30.00%.

Of the HPV-positive specimens, 182 (93%) showed high-risk subtypes, most of these were 16, 18, and 58. Two patients showed a low-risk hypotype, giving a detection rate of 6.96% (12/194). Most of these were HPV 6, 11, and 42.

Table 2. — *Comparison of patients with ASCUS cervical histopathology results between various nationalities, n (%).*

| Nationality | Quantity | Chronic cervicitis | CIN I | CIN II | CIN III | Cervical cancer |
|--------------------|----------|--------------------|-------------|-----------|-----------|-----------------|
| Han nationality | 947 | 748 (78.99) | 98 (10.35) | 50 (5.82) | 41 (4.33) | 10 (1.06) |
| Uyghur nationality | 250 | 173 (69.20) | 32 (12.80) | 21 (8.40) | 20 (8.00) | 4 (1.06) |
| Other nationality | 22 | 9 (77.27) | 2 (9.09) | 2 (9.09) | 1 (4.55) | 0 (0.00) |
| Total | 1219 | 938 (76.95) | 132 (10.83) | 73 (5.99) | 62 (5.09) | 14 (1.15) |

Table 3. — *The high risk and low risk HPV distributions in different ages of women with ASCUS, n (%).*

| Ages (years) | Screening count | HPV high risk HPV | Low risk HPV |
|--------------|-----------------|-------------------|--------------|
| < 20 | 10 | 0 (0.00) | 0 (0.00) |
| 21-25 | 82 | 16 (19.51) | 3 (3.66) |
| 26-30 | 97 | 25 (25.77) | 1 (1.03) |
| 31-35 | 112 | 30 (26.79) | 3 (2.68) |
| 36-40 | 130 | 39 (30.00) | 3 (2.31) |
| 41-45 | 95 | 46 (48.42) | 1 (1.05) |
| 46-50 | 55 | 22 (40.00) | 2 (3.64) |
| ≥ 51 | 20 | 4 (20.00) | 1 (5.00) |
| Total | 601 | 182 (30.28) | 12 (2.00) |

$\chi^2=114.563$, $p=0.000$.

Table 4. — *HPV detection group undone pathological histology results.*

| | HPV Detection group n (%) | As HPV Detection group n (%) | Total |
|--------------------|---------------------------|------------------------------|-------|
| Chronic cervicitis | 420 (69.88) | 510 (82.52) | 930 |
| CIN I | 85 (14.14) | 51 (8.25) | 136 |
| CIN II | 49 (8.15) | 27 (4.37) | 76 |
| CIN III | 39 (6.49) | 24 (3.88) | 63 |
| Cervical cancer | 8 (1.333) | 6 (0.97) | 14 |
| Total | 601 | 618 | 1219 |

The most common high-risk hypotype was HPV16, which had a detection rate of 46.91% (91/194). The second most common hypotype was HPV58, and it had a detection rate of 20.10% (39/194). HPV18 had a detection rate of 12.37% (24/194). Three patients had unitary type HPV, two had both HPV16 and HPV33, one had both HPV16 and HPV58, one had both HPV33 and HPV39, and one had HPV16, HPV31, and HPV52.

Women of various ages with ASCUS were examined. HPV examination produced positive diagnoses in 182 patients, giving a rate of 30.28% ($p < 0.05$). Among women under 20 years of age, the HPV infection rate was 0, for those aged 41-45 and for 45-50, it was higher (Table 3).

Relationship between high-risk HPV infection and pathological diagnosis

The comparison of between high-risk HPV detection group and HPV detection group undone pathological histology: 601 patients underwent cervical biopsy with colposcopy but did not undergo HPV detection. Pathological results indicated that 510 of patients who underwent HPV detection had chronic cervicitis CIN I: 51, CIN II: 27, or CIN: III and that six of them had cervical cancer, accounting for 82.52%, 8.25%, 4.37%, 3.88%, 0.97% of the total, respectively. Among the 601 patients who underwent cervical biopsy with colposcopy but not HPV detection, pathological results indicated that 420 had chronic cervicitis CIN

Table 5. — *The high-risk HPV positive group and negative pathological histology result.*

| Pathology | High-risk group | Low-risk group | Negative group | Total |
|--------------------|-----------------|----------------|----------------|-------|
| Chronic cervicitis | 53 (29.12) | 7 (58.33) | 360 (88.45) | 420 |
| CIN I | 49 (26.92) | 4 (33.33) | 32 (7.86) | 85 |
| CIN II | 38 (20.88) | 1 (8.33) | 10 (2.46) | 49 |
| CIN III | 34 (18.68) | 0 (0.00) | 5 (1.23) | 39 |
| Cervical cancer | 8 (4.40) | 0 (0.00) | 0 (0.00) | 8 |
| Total | 182 | 12 | 407 | 601 |

$\chi^2=129.317$, $p=0.000$.

Table 6. — *High-risk HPV positive groups and undone HPV detection group histopathology results.*

| Pathology | HPV Detection n (%) | As HPV Detection n (%) | Total |
|--------------------|---------------------|------------------------|-------|
| Chronic cervicitis | 53 (29.12) | 510 (82.52) | 563 |
| CIN I | 49 (26.92) | 51 (8.25) | 100 |
| CIN II | 38 (20.88) | 27 (4.37) | 65 |
| CIN III | 34 (18.68) | 24 (3.88) | 58 |
| Cervical cancer | 8 (4.40) | 6 (0.97) | 14 |
| Total | 182 | 618 | 800 |

$\chi^2=105.733$, $p=0.000$.

I: 85, CIN II: 49, or CIN III: 39, and that eight of them had cervical cancer, accounting for 69.88%, 14.14%, 8.15%, 6.49%, and 1.33% of the total, respectively. After examination, the two groups showed no significance differences (Table 4).

Negative pathological histology in the high-risk-HPV-positive group: If the histopathological results are assumed to be correct, then the rate of detection of high-risk-HPV-positive cervical cancer with intraepithelial neoplasia was 70.88% (129/182), and the rate of detection of low-risk-HPV-positive cervical cancer and intraepithelial neoplasia was 41.67% (5/12), and the rate of detection of patients with intraepithelial neoplasia but not with cervical cancer was 11.55% (47/407). There were statistically significant differences among these three groups ($p < 0.05$) (Table 5). This indicates that, among patients with ASCUS, the rate of detection of high-risk-HPV-positive cervical cancer, intraepithelial neoplasia and cervical cancer was higher than among other individuals.

Histopathology of high-risk-HPV-positive and HPV-negative individuals

If the histopathological results are assumed to be correct, then the rate of detection of high-risk-HPV-positive cervical cancer with intraepithelial neoplasia was 70.88% (129/182), and the rate of detection of cervical cancer with intraepithelial neoplasia but without HPV was 17.48% (108/618). These two groups showed statistically significant differences ($p < 0.05$) (Table 6). This indicates that,

among patients with ASCUS, the rate of detection of high-risk-HPV-positive patients with cervical lesions was higher.

Discussion

Cervical cytology screening is key in controlling and preventing cervical cancer. ASCUS is the most common cervical cytology. The diagnosis is primarily exclusive, but can hint at the presence of dangerous lesions. ASCUS may have a relationship with inflammatory stimuli, poor producers, IUD insertion, old age, gender reassignment surgery, and genital renovation or beautification surgery. It is may also have a relationship with CIN and carcinoma of the cervix [13].

This study involved 1,219 patients with ASCUS. Patients underwent cervical biopsy under colposcopic guidance. Histopathological results showed the rate of detection of CIN and cervical cancer was 22.89% among 938 individuals with chronic cervicitis CIN I: 132, CIN II: 73, CIN III: 62. There were 14 patients with total cervical cancer patients, accounting for 76.95%, 10.83%, 5.99%, 5.09%, 1.15% of the total, respectively. These data suggest that the results of colposcopic biopsy can be chronic cervicitis or CIN. Results can also be early invasive carcinoma if the cytological report is ASCUS. A way of triaging and managing patients with ASCUS would increase the rate of detection of cervical lesions and reduce the number of unnecessary cervical biopsies. This would in turn conserve medical resource and reduce costs.

HPV infection is a basic contributing factor to the development of cervical cancer. It is divided into high-risk HPV and low-risk HPV according to their carcinogenicity. Persistent infection with high-risk HPV is considered a primary cause of cervical cancer and precancerous lesions [14]. The results of the present study showed a significant difference between the rate of high-risk HPV among women of different ages with ASCUS. The rate of high-risk HPV infection was found to be age-related. The rate of HPV infection was considered 0 if the woman was younger than 20 years, but it was considered high if the woman was between the ages of 41 and 50, indicating that high-risk HPV mainly centers on women over 40. It coincides with the peak incidence of cervical cancer among women in their 50s. Most HPV-positive specimens collected in the present work were high-risk HPV16, giving a detection rate of 46.91% (91/194). HPV16-positive patients over 40 years of age with ASCUS should be alerted to the possibility of cervical intraepithelial neoplasia and cervical cancer. It has been reported that around 20% of cytological diagnoses are for ASCUS and patients who also have high-risk HPV infections showed the highest rates of cervical intraepithelial neoplasia and cervical cancer [15]. The data show the detection rate for high-risk HPV-positive cervical cancer with intraepithelial neopla-

sia to be 70.88% (129/182). However, the rate for low-risk HPV-negative cervical cancer with intraepithelial neoplasia was 11.55% (47/107). These groups showed a statistically significant difference ($p < 0.05$). It remains to be discovered whether the detection rate for high-risk HPV-positive cervical cancer with intraepithelial neoplasia is higher than that of HPV-negative patients with ASCUS. This indicates the importance of HPV testing for ASCUS patients and that TCT results may be better for screening cervical intraepithelial neoplasia and cervical cancer compared to other methods.

Currently, for cases of ASCUS that may have cervical lesions, there are three ways of performing further screening: repeating the cytological examination within three to six months, high-risk HPV detection, and direct colposcopic biopsy [16]. The sensitivity of cytological examination is limited [17, 18]. Approximately half of abnormal patients are ASCUS at the first screening. If cytological examination is considered way of triaging ASCUS patients, then it cannot be considered suitable for the elimination of precancerous cervical lesions. Solomon *et al.* [19] tested 3,488 patients with ASCUS by using all three methods of colposcopy, immediate examination, repeat cytological examination, and high-risk HPV detection for the purpose of examining and comparing these methods. Results showed that HPV detection was the best choice for triage management of ASCUS and that it was more sensitive and specific in the diagnosis of both CIN III and cervical cancer than other methods [19]. Cox performed a two-year study of ASCUS patients and found that, of those who first underwent HPV detection for triage, only 55.6% later underwent colposcopy, but 72.3% of CIN III patients later underwent colposcopy. This reduced the number of patients with ASCUS who underwent colposcopy examination [20]. Analysis of the pathological mechanism underlying HPV detection showed the detection rate to be 70.88% for high-risk HPV-positive cervical cancer with intraepithelial neoplasia and 11.55% for low-risk HPV-negative cervical cancer with intraepithelial neoplasia. No cervical cancer was detected in patients who were negative for ASCUS. These results showed that for patients with ASCUS, screening for potential risk of cervical cancer combined with HPV DNA detection can separate individuals with asymptomatic HPV invisible from low-risk HPV-negative women and can improve diagnostic reliability of colposcopic examination. It can also reduce the economic burden on patients by allowing them to avoid unnecessary repeated cytological examinations. HPV DNA detection was here found to be a superior method of detection. It can be used to supplement cytological examination in patients with ASCUS and hence improve the detection rate.

In short, cervical cytological diagnosis of ASCUS patients should be given more attention, and HPV DNA detection is a more reasonable and meaningful triage

approach for patients with ASCUS than cervical cytology testing. It can reduce the number of unnecessary colposcopies performed and therefore lighten the economic burden on patients, conserve medical resources, and reduce the rate of false negative diagnoses of cervical lesions.

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Address reprint requests to:

Y. ZHANG, M.D.

Department of Gynaecology

Xiangya Hospital of Central South University

No. 87 Xiangya Road,

Changsha 410000 (China)

e-mail: yizhangdoc@126.com