Significance of Binucleated Cells with Compression in Atypical Squamous Cells of Undetermined Significance

Kiyotada Washiya^a Makoto Motoi^b Takako Kobayashi^b Haruhiko Yoshioka^a
Jun Watanabe^a

^aDepartment of Medical Life Sciences, Hirosaki University Graduate School of Health Sciences, Hirosaki, and

^bDivision of Diagnostic Pathology Center, Fukuyama City Medical Association, Fukuyama, Japan

Key Words

Cytology · Atypical squamous cells of undetermined significance · Nucleus · Binucleated cells with compression

Abstract

Objective: To increase the accuracy of the diagnosis of atypical squamous cells of undetermined significance (ASC-US), ASC-US were divided into high-risk human papillomavirus (HPV HR+) and non-high-risk HPV (HPV HR-) cases to analyze the significance of binucleated cells with compression. **Study Design:** ThinPrep specimens of ASC-US were examined. This study included 21 CIN and HPV HR+ (CIN+), 19 benign and HPV HR- (B-) and 10 benign and HPV HR+ (B+) cases. The number of cells were examined by defining binucleated cells with their nuclei pressing against each other as positive compression, and their relation to the relative light units (RLUs) of the DNA Hybrid capture 2 (HC2) was determined. **Results:** 95.2% of CIN+ and 15.8% of B- cases were compression positive, while 4.8% of CIN+ and 84.2% of B- cases were compression negative, which was significantly different. The average number of cells with positive compression was 5.7 ± 5.3 in CIN+, 2.0 ± 0.7 in B- and 5.5 ± 1.5 in B+ cases, with significant differences between CIN+ and B- and between B- and B+ cases. The number of compression-positive cells increased as HPV HC2 RLUs became higher. **Conclusion:** Positive compression is useful in determining

ASC-US with HPV HR+. The identification of positive compression is highly practical because it can be observed morphologically.

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Introduction

The Bethesda system of uterine cervical cytology for atypical squamous cells of undetermined significance (ASC-US) is effective in detecting lesions in cancer screening. However, the concordance rate of ASC-US is low because cytology is not intended for the estimation of specific lesions; it is a conceptual classification to detect ASC-US. The concordance rate of ASC-US in an internet survey conducted by the US National Cancer Institute was 39.9% [1]. This is comparable with our internet voting results (36.5%) in 129 ASC-US cases [2]. In fact, the diagnosis of ASC-US lacks reproducibility [3]. In Japan, 12.3 million gynecological cytology specimens were tested in 2010. If high-risk human papilloma virus (HPV HR+) tests are conducted at a 5% detection rate of ASC-US, approximately 0.615 million ASC-US cases will be detected at a cost of 2.214 billion yen in medical expenses. Thus, it is important to improve the accuracy of cytology in order to improve the screening efficiency and reduce rising medical expenses.

We focused on the 3-dimensional structures of nuclei in cell smear specimens and reported objective discrimination using 3-dimensional and discriminant analyses of nuclei [4–6]. The nuclei of ASC-US cases in initial cytology were 3-dimensionally analyzed using liquid-based cytology (LBC) specimens prepared by the ThinPrep method. Discriminant analysis was conducted using nuclear analysis parameters (area, number of 3-dimensional focus layers, 3-dimensional alterations in luminance, and roundness) based on Mahalanobis distance. As a result, the nuclei of ASC-US of HPV HR+ and CIN1–2 (CIN+) cases were more often 3-dimensional and showed fewer alterations in fine chromatin than those of non-high-risk HPV (HPV HR–) and benign (B–) cases. The present study demonstrated binucleation of 3-dimensional nuclei pressing against each other in the ASC-US of CIN+ cases. In the present study, the significance of binucleated cells with compression was examined to improve the accuracy of ASC-US analysis.

Patients and Methods

Of 11,209 cases of uterine cervical cytology (April 2010 to March 2011), 217 (1.94%) were diagnosed with ASC-US, and 72 underwent HPV HR tests with consent of self-pay of examination charges. Of the cases initially diagnosed with ASC-US, 21 cases with a histological analysis of CIN1–2 and with HPV HR+ (group 1), 19 cases negative in the follow-up and with HPV HR– (group 2), and 10 cases with a benign histological analysis and with HPV HR+ (group 2) were included (table 1). Hereinafter, the cases of groups 1, 2 and 3 will be referred to as CIN+, B– and B+, respectively.

Samples were collected from the cervix using a broom-type brush and were sufficiently washed off with a ThinPrep storage solution. LBC specimens were prepared using the ThinPrep2000, followed by Papanicolaou staining. Patients diagnosed with ASC-US in initial cytology were microscopically tested by multiple cytotechnologists certified for CYTYC ThinPrep tests. HPV HR tests were conducted with the ThinPrep specimens using the DNA Hybrid capture 2 (HC2) method (13 high-risk genotypes were detected: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68).

Findings suggestive of HPV infection include koilocytosis with little nuclear atypia, squamous epithelial cells with nuclear atypia, dyskeratosis, multinucleated cells, smudge cells, and nuclear enlarged cells with slight hyperchromasia. However, few cytological findings are strongly suggestive of CIN+. In the present study, binucleated cells with their nuclei pressing against each other were defined as positive compression (fig. 1). Visual criteria were employed to eliminate the need of the criteria of nuclear size and the compression rate of binucleation. Cells with separate or overlapping nuclei were determined to be compression negative (fig. 2) in order to examine the presence of compression for each case. Similarly, ASC-US with tri- or more nucleation were examined. In addition, for cases with positive compression, all cells were counted for each specimen. As the total number of cells in ThinPrep specimens was

Table 1. Classification of ASC-US cases

Classification		CIN1	CIN2	Benign
CIN+	HPV HR+ and CIN+	16	5	0
B–	HPV HR– and benign	0	0	19
B+	HPV HR+ and benign	0	0	10
Total		16	5	29

Table 2. Number of compression-positive and -negative cases

		Positive compression	Negative compression	%
CIN+	HPV HR+ and CIN1–2	20*	1*	95.2
B–	HPV HR– and benign	3	16	15.8
B+	HPV HR+ and benign	6	4	60.0

* $p < 0.01$: significantly different, CIN+ compared to B– cases.

in the range of 20,000–100,000, the number of binucleated cells with compression in each specimen was standardized by 100,000.

Subsequently, a Welch test was conducted to examine the presence of a significant difference in the number of cases and cells with positive and negative compression and HC2 relative light units (RLUs).

Results

The number of binucleated cases with positive and negative compression is shown in table 2. A significant difference was noted between the numbers of compression-positive cases: 20 cases of CIN+ and only 3 cases of B–. A significant difference was also noted between the numbers of cases with negative compression: 1 CIN+ case and as many as 16 B– cases. B– cases included 6 with positive compression and 4 with negative compression. Next, the average number of cells/ 1×10^5 with positive compression on ThinPrep specimens was examined (table 3), and a significant difference was observed: 5.7 ± 5.3 for CIN+ and 2.0 ± 0.7 for B– cases. A significant difference was also noted between B+ (5.5 ± 1.5) and B– (2.0 ± 0.7) cases. Thus, the findings of positive compression were useful for discriminating ASC-US.

No significant difference was observed in the number of cases and cells with positive compression due to trinucleation; only 1 case and cell for the B–, 2 cases and cells for the CIN+, and 1 case and 3 cells for the B+ group were seen (table 4). No tri- or more nucleation was noted.

Fig. 1. a–d Binucleated cells with their nuclei pressing against each other were defined as positive compression. Papanicolaou stain. $\times 40$.

Fig. 2. a, b Trinucleated cells with their nuclei pressing against each other were defined as positive compression. **c** Trinucleated cells with their nuclei contacting, but not pressing against each other were defined as negative compression. **d** The arrow indicates cells with nuclei partially overlapping, but not focused, were defined as negative compression. Papanicolaou stain. $\times 40$.

HPV HC2 RLUs were divided into three groups, <1 , ≥ 1 and ≤ 60 , and >60 RLUs, to examine the number of compression-positive cases (table 5). The number of cases with >60 RLUs was 20, with the number of compression-positive cells per specimen being 6.4 ± 5.0 . The num-

ber of cases with ≥ 1 and ≤ 60 RLUs was 12, with the number of compression-positive cells per specimen being as few as 4.4 ± 2.3 . A significant difference was noted between these groups. The number of cases with <1 RLU was 3, with the number of compression-positive cells per

Table 3. Average number of compression-positive cells

		Cases	Cells, $\times 10^5$
CIN+	HPV HR+ and CIN1–2	20/21	$5.7 \pm 5.3^*$
B–	HPV HR– and benign	3/19	2.0 ± 0.7
B+	HPV HR+ and benign	6/10	$5.5 \pm 1.5^*$

* $p < 0.01$: significantly different, CIN+ and B+ compared to B– cases.

Table 4. Average number of trinucleated cells with positive compression

		Cases	Cells, $\times 10^5$
CIN+	HPV HR+ and CIN1–2	2	4
B–	HPV HR– and benign	1	1
B+	HPV HR+ and benign	1	6

Table 5. Average number of compression-positive cells using HPV HC2 RLUs

HPV HC2 RLUs	Cases	Cells, $\times 10^5$
>60	20	$6.4 \pm 5.0^*$
≥ 1 and ≤ 60	12	$4.4 \pm 2.3^*$
<1	3	1.4 ± 1.1

* $p < 0.01$: significantly different, >60 and 1–60 RLUs compared to <1 RLU.

specimen being as few as 1.4 ± 1.1 . A significant difference was noted between ≥ 1 and ≤ 60 and <1 RLUs. Thus, the number of compression-positive cells increased as HPV HC2 RLUs became higher.

Discussion

The number of cases with positive compression due to binucleation was as high as 20 of 21 CIN+ cases (95.2%) and as few as 3 of 19 B– cases (16.8%). The average number of compression-positive cells on ThinPrep specimens was significantly larger for CIN+ (5.7 ± 5.3) than for B– (2.0 ± 0.7) cases. The average number of compression-positive cells was 5.7 per specimen for CIN+ cases, while only a single cell was detected per specimen in 3 of 19 for

B– cases. Thus, high sensitivity was demonstrated. The identification of positive compression is highly practical because it can be observed morphologically with an optical microscope.

Okayama et al. [7] reported that a cytological finding for detecting HPV HR using in situ polymerase chain reaction was 100% positive compression. The detection rate of our HC2 method was 95.2%. However, this difference may be due to differences in the HPV test and sample preparation method. Although Okayama et al. [7] did not show the average number of compression-positive cells per specimen, we presented morphologically understandable images of 1–4 cells with positive compression per specimen from CIN+ cases. Our study provides useful information for a routine method using LBC specimens (ThinPrep), which are the most widely used in the world. ThinPrep specimens are prepared by agitating a sample in a sample storage solution at high speed to be smeared at a diameter of 20 mm onto a glass slide under positive pressure. The instrument stops automatically when a certain number of cells are smeared. Thus, the quality and quantity of samples is the same between gynecological specimens. The compression rate of binucleation counted all cells of each case, and the number of binucleated cells with compression in each specimen was standardized by 100,000.

Subsequently, HPV HC2 RLUs were divided into three groups, <1, ≥ 1 and ≤ 60 , and >60 RLUs, to determine the average number of compression-positive cells. Significant differences were noted: 6.4 ± 5.0 for >60 RLUs, 4.4 ± 2.3 for ≥ 1 and ≤ 60 RLUs, and 1.4 ± 1.1 for <1 RLU. Thus, the number of compression-positive cells increased as HPV HC2 RLUs became higher. The viral load reflects the spread of the CIN lesion [8, 9]. In addition, the viral load has been associated with the progression rate of the CIN lesion [10]. Origoni et al. [11] reported that the prevalence rates of CIN2/CIN3 were 32.2% for >100 RLUs and as low as 4.6% for >1 and <10 RLUs using an HC2 test. Thus, the probability of high-grade squamous intraepithelial lesions becomes higher as the infectious dose of HPV HR increases. Kjaer et al. [12] reported that the risk of developing CIN3 or above within 10 years after a positive result was obtained at least once in a HC2 test and was estimated to be 13.6% in young and >21.2% in middle-aged women using the Denmark databank. HPV causes transient infection in most cases; about 90% of HPV infections disappear within a year. As reported by the American Society of Clinical Oncology [13], patients determined to be positive in an HPV HR test may have a long-term risk of developing a precancerous state.

Initial HPV infection is mostly in an episomal state; circular DNA exists separately from host DNA. However, in this state, DNA chromosomes become unstable, and the host DNA E2 region of HPV DNA is cleaved to be integrated into the host DNA, stabilizing the E6/E7 viral proteins in the nucleus to exert their functions [14, 15]. Although the mechanism by which cells with positive compression increased in the nuclei of CIN+ cases is unclear, we postulate that mitosis is completed with incomplete chromosomal segregation due to HPV infection to regenerate a nuclear envelope around the scattered chromosomes, resulting in multinucleation.

Of the B+ cases, 6 (60%) were compression positive, with the average number of cells being 5.5 ± 1.5 . This may be explained by the nuclear atypia of cells transiently infected with HPV HR, the sensitivity of the HC2 test, or the sampling failure of lesions in tissue. However, in the B- or B+ cases of positive compression, LBC specimens should have been redetermined as ASC-US or low-grade squamous intraepithelial lesions. According to recent large-scale prospective clinical studies [12, 13], HPV HR infection may cause CIN lesions after a long period of time. The number of compression-positive cases with trinucleation was small for all 3 groups, but compressed tri-

nucleation of CIN+ and B+ cases showed larger numbers than B- cases. Thus, the follow-up of cases with positive compression that may progress to squamous intraepithelial lesions should be continued.

According to the 2001 Bethesda system [16], ASC-US is defined as a condition that 'quantitatively or qualitatively fell short of a definitive diagnosis of squamous intraepithelial lesion' and often complicates the determination. We think that cases of binucleated cells with compression that are likely shown in squamous intraepithelial lesions should be checked in detailed examination.

Further investigations need to be conducted to establish more objective diagnostic criteria.

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Disclosure Statement

None of the authors has any conflict of interest.

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