Atypical squamous cells: improvement in cytohistological correlation by the 2001 Bethesda System

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Summary

Purpose of investigation: To evaluate the advantages of the 2001 Bethesda System over the 1991 Bethesda System in the management of atypical squamous cells.

Methods: The cytology files of the 8,748 patients were reviewed for diagnosis of atypical squamous cells of undetermined significance (ASCUS) at Zeynep Kamil Hospital. Seventy-two of the 259 smear specimens with the diagnosis of ASCUS were reviewed and reclassified according to Bethesda 2001.

Results: Of the 8,748 specimens, 259 (2.96%) were diagnosed as ASCUS. In re-evaluation of the 72 specimens according to the 2001 Bethesda system, the number of cervical smears with a diagnosis of atypical squamous cells (ASC) decreased to 21 in number. Of the 21 cervical smears with an ASC category, eight patients (38.1%) had high-grade intraepithelial squamous lesions (HSIL) and six (28.6%) had low-grade intraepithelial squamous lesions (LSIL) in histopathological specimens. The detection rates of squamous abnormalities ($\chi^2 = 24.79$, $p < 0.0001$) and high-grade squamous abnormalities ($\chi^2 = 8.31$, $p = 0.0039$) were significantly higher according to Bethesda 2001.

Conclusion: The 2001 Bethesda System seems to reduce the number of cervical smear diagnoses of ASC, without causing any impairment in the diagnosis of HSIL thus decreasing the number of unneccesary interventions like cervical biopsy and decreasing the cost, inconvenience, anxiety and discomfort.

Key words: Atypical squamous cells; 1991 Bethesda system; 2001 Bethesda system.

Introduction

The category of ASCUS (atypical squamous cells of undetermined significance) was created by the Bethesda System in 1988 to denote squamous cellular changes that were more marked than those attributable to reactive or inflammatory changes but were not quantitatively or qualitatively diagnostic of a preneoplastic or neoplastic condition [1]. In 1991, the Bethesda System was revised and it was recommended that the category of ASCUS be qualified as "favor reactive", as "favor dysplasia" (favor squamous intraepithelial lesions [SIL]), and as "ASCUS not otherwise specified (NOS)" (if neither of these categories seemed to fit), to facilitate subsequent patient management [2]. ASCUS was reported to constitute approximately 4.4% of Papanicolaou (Pap) test diagnoses [3]. The clinical follow-up of ASCUS was variable for repeated cytological evaluation or colposcopy with cervical biopsy, especially when ASCUS was persistent [4]. Thus, a large number of cervical biopsy specimens were unnecessarily obtained because of ASCUS diagnosis. The reported dysplasia rates in biopsy specimens varied widely from 15% to more than 60% [5-9]. However the majority of the lesions were low grade; only 15-30% of cases showing cervical intraepithelial neoplasia (CIN) were high grade [10-12]. Increasing the accuracy of the test will decrease economic costs, unnecessary interventions like frequent cytologic sampling, colposcopy, biopsy or human papillomavirus (HPV) typing and decrease inconvenience, anxiety and discomfort.

In the last revision, the 2001 Bethesda system separated atypical squamous cells (ASC) into two categories; of undetermined significance (ASC-US) and cannot exclude high grade intraepithelial lesions (ASC-H) and eliminated "favor reactive" smears [13]. With this background, we planned to determine the advantages and disadvantages of the 2001 Bethesda System over the 1991 Bethesda System in management of atypical squamous cells.

Materials and Methods

Between January 2000 and June 2001, 259 of 8,748 (2.96%) cervical smear specimens collected from patients who had been admitted to the gynecological outpatient clinics of Zeynep Kamil Women and Children's Disease Education and Research Hospital were diagnosed as having ASCUS. The patients were informed about the meaning of ASCUS cytology and to the study protocol of our hospital. ASCUS "favor dysplasia" cases and ASCUS NOS cases had underwent immediate colposcopy, ASCUS "favor reactive" cases were followed either by repeated testing or colposcopy. Repeat Pap tests were performed at 4-month intervals until patients had two negative squamous intraepithelial lesion results before returning to a routine screening protocol. If repeat cytology revealed ASCUS or a more

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serious result, the patient was referred for colposcopy. There were 259 Pap tests with a diagnosis of ASCUS for 226 patients. Nine of these 226 patients did not accept care in our hospital. Of the remaining 217 patients, 178 had repeat Pap smears and 39 underwent immediate colposcopy-guided biopsies. Of these 178 cases, 141 had normal, four had HSIL or LSIL and 33 had ASCUS as the cytological diagnosis in repeated tests. Colposcopy guided biopsy was also performed in these 33 cases; thus a total of 72 patients with ASCUS smear diagnoses and with cervical biopsy results constituted the study group.

Colposcopic evaluation, cervical biopsies and endocervical curettage were performed in 72 patients with smear diagnoses of ASCUS and these results formed the basis of the study. The cervical smears, which had been reported according to the 1991 Bethesda system, were revieved by two pathologists who did not have knowledge of biopsy results and reclassified according to the 2001 Bethesda system as ASC category. If both the pathologists were in agreement on a cervical smear finding, the result was accepted. If any discrepancy existed between the smear results, that smear was re-evaluated and the ultimate cytology result determined.

All cervicovaginal smears were collected with a cervical cytobrush. Two slides per case were prepared. All smears were immediately fixed in a 95% solution of alcohol and sent to the cytopathology laboratory. The term "clinically significant lesion" was used to denote any lesion that was malignant or premalignant including cervical intraepithelial neoplasia (CIN) 1, CIN 2, CIN 3. Any histopathological result other than malignant or premalignant was defined as a negative pathological finding. The histopathologic diagnosis of CIN I was classified as low-grade squamous intraepithelial lesion (LSIL) and CIN II and CIN III as high grade squamous intraepithelial lesion (HSIL).

The GraphPad Prisma V.3 package program was used for the statistical analysis in this study. In the evaluation of the data, chi-square (χ^2) analysis was used. The significance level was set at $p < 0.05$.

Results

Cervical colposcopy guided biopsy and endocervical curettage were performed in 72 patients with smear diagnoses of ASCUS and these cervical smears which had been reported according to the 1991 Bethesda system were re-evaluated according to the 2001 Bethesda System. The mean age of the patients with ASCUS was 42.06 ± 10.23 years (22-73 years). Of these 72 Pap tests with ASCUS cytological findings, 41 (56.9%) were ASCUS "favor reactive", 28 (38.9%) were ASCUS "favor dysplasia" and three (4.2%) were ASCUS NOS. The distribution of smear findings with ASCUS according to the 1991 Bethesda System and with ASC category according to the 2001 Bethesda System, and their association with cervical tissue biopsy findings are presented in Table 1. After reevaluation according to the 2001 Bethesda System, the number of cervical smears with a diagnosis of atypical squamous cells decreased to 21 in the ASC category 13 (61.9%) ASC-US and 8 (38.1%) ASC-H). Fifty-one (70.8%) of the cervical smears with a diagnosis of ASCUS were reevaluated as negative and eliminated according to the 2001 Bethesda system. Of these eliminated smears with ASCUS cytological diagnoses, 41 (80.4%) were in the ASCUS "favor reactive" category, and ten (19.6%) were in the ASCUS "favor dysplasia" category.

Table 1. — *The distribution of smear findings with ASCUS according to the 1991 and 2001 Bethesda Systems and their association with cervical tissue biopsy findings.*

Bethesda 1991	Bethesda 2001	Histopathological results		
		Negative n	LSIL n	CIN 2 more n
ASCUS-reactive n = 41	Negative, n = 41	40	1	–
ASCUS-dysplastic n = 28	Negative, n = 10	10	–	–
	ASC-US, n = 11	4	6	1
	ASC-H, n = 7	1	–	6
ASCUS NOS n = 3	ASC-US, n = 2	2	–	–
	ASC-H, n = 1	–	–	1

Only one ASCUS "favor reactive" case with a histopathological diagnosis of LSIL was reevaluated as negative cytology according to the 2001 Bethesda system and no high-grade squamous lesions were reevaluated as negative according to the 2001 Bethesda system.

According to the 1991 Bethesda System, 15 of the 72 (20.8%) cervical smears in the ASCUS category had squamous cells abnormalities in their histopathological specimens. Eight patients (11.1%) had high-grade squamous lesions and seven (9.7%) showed LSIL in the histopathological specimens.

According to the 2001 Bethesda System, 14 of the 21 (66.7%) cervical smears in the ASC category had squamous cell abnormalities in the histopathological specimens. Eight patients (38.1%) had high-grade squamous lesions and six (28.6%) showed LSIL in the histopathological specimens. Moreover, only one (7.7%) high-grade squamous lesion and six (46.1%) LSILs were detected on histopathological findings of 13 cases with ASC-US. Of the eight cases with ASC-H Pap test results, seven (87.5%) had high-grade squamous lesions and one (12.5%) had negative histopathological findings.

The detection rates of squamous abnormalities ($\chi^2 = 24.79$, $p < 0.0001$) and high-grade squamous abnormalities ($\chi^2 = 8.31$, $p = 0.0039$) were significantly higher in the Bethesda 2001 system when compared with the 1991 Bethesda system. In addition, biopsy-proven negative histopathologic results were detected less often in the ASC category according to the 2001 Bethesda system (33.3%) than in the ASCUS category of the 1991 Bethesda system (79.2%) ($\chi^2 = 15.391$, $p < 0.0001$).

Discussion

The 2001 Bethesda System differs from the previous two sytems in reporting equivocal results of squamous and glandular cells [13]. This new subclassification better reflects the current understanding of the significance of atypical squamous cells on cervical samples and subsequent follow-up cervical tissue findings [14, 15]. In the absence of an obvious inflammatory reaction, cellular evidence of squamous atypia or atypical squamous metaplasia may antedate the occurrence of a squamous

intraepithelial lesion by months or even years. The Bethesda II Criteria Committee [16] reported that ASCUS encompassed those cellular abnormalities more prominent than those attributed to reactive, inflammatory changes but did not have the diagnostic criteria for a squamous intraepithelial lesion. Pathologists were encouraged to qualify ASCUS with respect to whether a reactive process or SIL was favored. As studies accrued on its usage in clinical practice, a higher percentage of follow-up cervical biopsies were negative after a diagnosis of a ASCUS "favor reactive" process as compared to ASCUS "favor dysplasia" [16, 17]. Finally, in the 2001 Bethesda Workshop, the category of ASCUS "favor reactive" was eliminated. Atypical squamous cells are qualified as of "undetermined significance" (ASC-US) or "cannot exclude HSIL" (ASC-H).

In our study, the number of smears with atypical squamous cells decreased from 72 to 21 by reevaluating them according to the 2001 Bethesda System. Therefore, 70.8% of the cervical smears with a diagnosis of ASCUS were eliminated. Among these eliminated negative smears according to the new classification, LSIL was detected in only one case (2%) and no other clinically significant lesion was missed. Forty-one (80.4%) of the 51 eliminated cases according to the 2001 Bethesda System were in the ASCUS "favor reactive" category in Bethesda 1991 while ten (19.6%) were in the ASCUS "favor dysplasia" category, i.e., a great majority of these smears which were re-evaluated as negative according to the 2001 Bethesda system were in the ASCUS "favor reactive" category and no ASCUS NOS case was eliminated according to the latest Bethesda System. Fourteen of the 21 (66.7%) ASC cytology results had squamous dysplasia on histological examination. Six cases (28.6.%) had a histological diagnosis as LSIL, whereas the remaining eight cases (38.1%) had a histological diagnosis of CIN 2 or more. Thus the detection rate of both low-grade and high-grade squamous lesions were significantly higher in the ASC category when compared with the 1991 Bethesda system. No high-grade squamous lesion was missed by the new system. Also in seven of eight (87.5%) in the ASC-H category, clinically significant lesions were detected. This rate was 7.7% for the category of ASC-US.

Studies have reported a 5-17% chance of biopsy-confirmed CIN 2 or CIN 3 after a Pap test with ASC, and a 24-94% chance of biopsy-confirmed CIN 2, 3 after a diagnosis of ASC-H [14-17]. In our study, the rate of biopsy-confirmed CIN2 or CIN3 after a Pap test was 7.7% for the ASC-US category and 87.5% after a diagnosis of ASC-H. These rates were in accordance with the literature.

In 2002, the American Society for Colposcopy and Cervical Pathology published guidelines for the management of patients with abnormal Pap test results [18]. Patients with a cytologic interpretation of ASC should undergo reflex human papilloma virus DNA testing or repeated Pap testing whereas patients with a cytologic interpretation of ASC-H should undergo colposcopic evaluation and cervical biopsy if indicated. Our data from the present study support these recommendations since the rate of biopsy-confirmed SIL after a Pap test was 87.5% for the category of ASC-H and all of them were high grade. The elimination of reactive smears from the ASC category seems to increase the detection rate of squamous abnormalities in the histopathological evaluation of patients with a diagnosis of ASC on smears.

In conclusion, the new 2001 Bethesda System seems to reduce the number of cervical smear diagnoses of ASC, without causing any impairment in the diagnosis of high-grade squamous lesions, thus decreasing the number of unneccesary interventions like cervical biopsy as well as cost, inconvenience, anxiety and discomfort. An ASC-US diagnosis reveals in most cases low-grade squamous lesions or negative histopathological findings but an ASC-H diagnosis deserves a detailed investigation for high-grade squamous lesions. The awareness of clinicians regarding the importance of ASC-H diagnoses must be sustained.

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