ATYPICAL SQUAMOUS CELLS OF UNDETERMINED SIGNIFICANCE: A CYTOHISTOLOGIC STUDY

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Abstract- Although the diagnostic category of atypical squamous cells of undetermined significance (ASCUS) has been known since 1988, its use and appropriate clinical follow up and management still remains controversial. This study was carried out to evaluate the diagnostic value of ASCUS, using cytohistologic correlation. A retrospective review of archival materials in our cytology laboratory files was performed for cervicovaginal smears diagnosed as ASCUS from March 1999 to February 2002. In 8551 cervicovaginal smears examined, ASCUS was reported in 236 (2.76%) cases, with histologic follow up in 98 (41.2%). During the follow up period of patients with ASCUS, 51 patients (52%) had benign/reactive lesions, 43 patients (43.9%) were diagnosed as having a squamous intraepithelial lesion (SIL), 2 (2.1%) specimen were insufficient and finally two cases (2%) proved to be invasive cervical carcinoma on histology. Of cases diagnosed as SIL, 22 (22.4%) were interpreted as flat condyloma, 9 (9.1%) cervical intraepithelial neoplasia (CIN) 1, 2 (2.1%) CIN 1-2, 3 (3.1%) high grade SIL, 6 (6.1%) basal cell abnormality of undetermined significance, and 1 (1%) had features of CIN 1 in presence of severe inflammation. Patients whose smears fall into the category of ASCUS may exhibit a spectrum of findings, ranging from benign/reactive lesions to frequent SIL and rarely to invasive carcinoma. Therefore, patients with a diagnosis of ASCUS justify careful follow-up and investigation.

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Key words: Cervix neoplasms, cervix diseases, atypical squamous cells of undetermined significance, histologic diagnosis

INTRODUCTION

The Bethesda system limits use of the term atypical cells to those cases in which the cytologic findings are of undetermined significance. "Atypia" should not be used as a diagnosis for otherwise defined inflammatory, preneoplastic or neoplastic cellular changes (1).

A variety of diagnostic terms have been used to convey diagnostic uncertainty. In the Bethesda system, the term atypical squamous cells of undetermined significance (ASCUS) has been utilized (2). This term is defined as "cellular abnormality that are more marked than those attributable to reactive changes (inflammation, atrophy, radiation, repair, *etc*) but that quantitatively or qualitatively fall short of a definitive diagnosis of squamous intraepithelial lesion (SIL)". However, the Bethesda system encourages the cytologist to indicate whether he/she thinks that a particular ASCUS case is most likely related to reactive changes or to an intraepithelial lesion (3).

Although the diagnostic category of ASCUS has been known since 1988, its use, as well as its appropriate clinical follow up and management, still remains controversial. Since there is a need to gather further follow up data on patients whose cervical cytologic smears fall under the diagnostic umbrella of ASCUS, we undertook a retrospective, cytohistologic, correlative study to evaluate the significance of a diagnosis of ASCUS on such smears (4).

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MATERIALS AND METHODS

A retrospective review of the archives of the pathology department, Mirza Koochak-Khan hospital, for the period of March 1999 to February 2002, identified 236 patients who had smears diagnosed as ASCUS, with histologic examination in 98 of them within 12 months following the abnormal smear.

The cytologic criteria used to diagnose ASCUS were the ones outlined by the Bethesda II criteria committee and included 1) nuclear enlargement two and a half to three times a normal intermediate squamous cell nucleus with a slight increase in the nuclear/cytoplasmic (n/c) ratio, 2) variation in nuclear size and shape and binucleation observable, 3) mild hyperchromasia possibly present, with the chromatin remaining evenly distributed and without granularity, 4) nuclear outlines usually are smooth and regular with very limited irregularity and 5) cells with some but not all the criteria for human papillomavirus (HPV) infection (4).

We also included in the ASCUS category cases showing 1) atypical squamous metaplasia: squamous cells show less abundant/rounded cytoplasm, which corresponds to the configuration seen in normal metaplastic squamous cells. In immature cells, the increased n/c ratio is more important than nuclear enlargement. Nuclei may be either euchromatic or slightly hyperchromatic,the nuclear envelops are smooth to slightly irregular, and 2) atypical parakeratotic squamous cells: cells shed singly in three-dimensional clusters, characterized by orangeophilic cytoplasm, cellular pleomorphism (caudate or elongated shapes), some with an increased n/c ratio, irregular nuclear contour and dark, often irregularly distributed chromatin, should be categorized as ASCUS or squamous intraepithelial lesion (SIL) depending on the degree of the cellular abnormalities (2).

We qualified each ASCUS smear into one of three groups: 1) favor reactive, 2) favor SIL and 3) not otherwise specified (NOS).

RESULTS

From a total 8551 cervicovaginal cytologic specimens obtained over a three year period, ASCUS was reported in 236 (2.76%) cases, with histologic follow up in 98 (41.2%) within 12 months after the cytologic smear. The ASCUS/SIL ratio obtained by dividing the ASCUS cases by the sum of low grade squamous intraepithelial lesion (LSIL), high grade squamous intraepithelial lesion (HSIL) and carcinoma cases in our study was 3.1. The age of patients ranged from 18 to 79 years with a mean of 43. Table 1 shows proportion of ASCUS patients who received colposcopic biopsy as a follow up method by age group.

Table 2 lists histology results by patient age. Tissue specimens obtained for histology in first follow up visit consisted of 91(93%) cervical biopsies, 4 (4%) endocervical curettages, 2(2%) cervical conizations and 1(1%) hysterectomy. In 17 patients more than one tissue specimen were obtained. Five (5.1%) patients required more than one procedure before significant lesions could be demonstrated (3 CIN 1 cases, 1 CIN 1-2 case and 1 flat condyloma case).

Table 1. Proportion of ASCUS patients who received colposcopic biopsy as a follow up method by age group

	Colposcopic biopsy follow up				
	ASCUS Cases		Biopsy Cases		Percent of ASCUS
Age Group	n	%	n	%	receiving biopsy
≤ 25	14	5.9	6	6.1	42.8
26-35	44	18.6	17	17.3	36.6
36-45	75	31.8	36	36.7	48
46-55	80	33.9	32	32.7	40
≥56	23	9.8	7	7.2	30.4
Total	236	100	98	100	-

Abbreviations: ACUS, atypical squamous cells of undetermined significance.

Table 2. Diagnosis of ASCUS by age group*

Group (yr)	Insufficient specimen	Beingn/ reactive	Atypical†	Flat condyloma	CIN 1	CIN 1-2	HSIL	Invasive SCC of cervix
≤ 35 (n=23)	-	6.1(6)	2.1(2)	10.2(10)	3.1(3)	1(1)	1(1)	-
36-45 (n=36)	1(1)	18.4(18)	2.1(2)	7.1(7)	4.1(4)	1(1)	2.1(2)	1(1)
≥ 46 (n=39)	1(1)	27.5(27)	3(3)	5.1(5)	2.1(2)	-	-	1(1)

Abbreviations: ACUS, atypical squamous cells of undetermined significance; HSIL, high grade squamous intraepithelial lesion; SCC, squamous cell carcinoma.

*Data are given as percent (number).

† Basal cell abnormality of undetermined significance or features of CIN 1 in the presence of severe inflammation.

Of the 98 ASCUS cytologic diagnoses, 51 (52%) were benign/reactive, 7 (7.1%) were atypical, 6 (6.1%) were basal cell abnormality of undetermined significance,1 (1%) had features of CIN 1 in the presence of severe inflammation, 22 (22.4%) were flat condyloma, 9(9.1%) were CIN 1, 2 (2.1%) were CIN 1-2, 3(3.1%) were high grade SIL, 2 (2.1%) were invasive squamous cell carcinoma of cervix and 2 (2.1%) specimen were insufficient.

Overall 43 patients (43.9%) had SIL. The ≤ 35 year olds contained the highest proportion of SIL (15.3%) with 14.3% being CIN 1, CIN 1-2 and flat condyloma and 1% HSIL. No HSIL were found in ≥46 year old group.

Table 3 shows the histologic results by the subcategories of ASCUS. Significant lesions were seen frequently in patients with favor "SIL' 31.6% than those with favor "NOS" 13.3% or "reactive" 1%.

In this study, the smears were satisfactory in 78 cases, satisfactory but limited by poor fixation and drying artifact in 6 cases (2 cases in benign/reactive group and 4 in SIL group), satisfactory but limited by obscuring blood or inflammation in 8 cases (5 cases in benign/ reactive group, 2 cases in SIL group and 1 case in invasive carcinoma group) and satisfactory but limited by absence of endocervical/transiton zone component and absence of LMP in 3 cases (Table 4).

Table 3. Relationship between cytologic and histologic diagnosis of ASCUS*

	Histologic diagnosis							
ASCUS qualification	Insufficient specimen	Benign/ reactive	Atypical	CIN 1	CIN 1-2	Flat condyloma	HSIL	Invasive SCC of cervix
Favor reactive (n=29)	-	28 (28.6)	-	1 (1)	-	-	-	-
NOS(n=27)	-	14 (14.3)	3 (3.1)	3 (3.1)	-	6 (6.1)	1 (1)	-
Favor SIL(n=42)	2 (2.1)	9 (9.2)	4 (4)	5 (5)	2 (2.1)	16 (16.3)	2 (2.1)	2 (2)
Total(n=98)	2 (2.1)	51 (52.1)	7 (7.1)	9 (9.1)	2 (2.1)	22 (22.4)	3(3.1)	2 (2)

Abbreviations: ACUS, atypical squamous cells of undetermined significance; HSIL, high grade squamous intraepithelial lesion; SCC, squamous cell carcinoma.

*Data are given as number (percent).

Table 4. Specimen adequacy of 98 ASCUS cases*

Specimen adequacy	Insufficient specimen(n=2)	Benign/reactive (n=51)	SIL (n=43)	Invasive carcinoma (n=2)
Satisfactory	2	40	35	1
Satisfactory but limited by poor fixation/air drying artifact	-	2	4	-
Satisfactory but limited by obscuring blood/inflammation	-	5	2	1
Satisfactory but limited by absence of EC/TZ component	-	1	2	-
Satisfactory but limited by absence of LMP	-	3	-	-

Abbreviations: ASCUS, atypical squamous cells of undetermined significance; SIL, squamous intraepithelial lesion; EC/TZ, endocervical/transitional zone.

*Data are given as number.

DISCUSSION

Most reports agree that patients whose smears fall into the diagnostic category of ASCUS would have on follow up histologic examinations a significant rate of SIL, with a range from 13.5% to 58% (Table 5).

In our study, while 52.1% of our ASCUS cases had no significant lesion on subsequent follow up, 3.1% turned out to have high grade SIL on subsequent follow up histologic examination and an additional 2% proved to have invasive cervical carcinoma.

Laboratory rates of ASCUS will vary depending on the patient population, the diagnostic criteria utilized and the experience and skill of the microscopists. In a low risk population, the rate of ASCUS should be <5%. For laboratories that serve high risk populations (*e.g,* underscreened populations, patients with sexually transmitted disease or colposcopy clinics), the rate of ASCUS may be higher but should not exceed two or three times the rate of SIL (2). Clearly, there is no consensus on an ideal ASCUS/SIL ratio. In an effort to define common practice, a nationwide laboratory survey found that the median ASCUS/SIL ratio is 2.0 and 80% of laboratories report ratio between 0.64 and 4.23 (12, 13). An ASCUS rate of 2.76% in our study is consistent with those in literature. Our ASCUS/SIL ratios were similar to college of American Pathologists 75th percentile benchmark standards, although one should keep in mind that both volume and type of laboratory cases are significantly associated with the ASCUS/SIL ratio (12). The results of our study underline the importance of continued follow up of ASCUS cases since 5% of patients required more than one procedure before significant lesions could be demonstrated. Others have also stated that there might be a high false negative rate with one time colposcopic follow up (4).

Fourteen of 98 studied specimens were satisfactory but limited by poor fixation, air drying artifact, blood or inflammation. Seven of these had significant lesions in histologic follow up, therefore, more effort is needed to improve the techniques and specimen quality.

Table 6. Review of reports on ASCUS in the literatures

Series	Year	LSIL (%)	HSIL (%)	Others (%)	SIL (%)
Genest *et al*.[5]	1998	11	13	-	24
Vlahos *et al*.[6]	2000	24*	12.9	-	36.9
Lousuebsakul *et al*.[7]	2000	43	4	5.8†	47
Morin *et al*.[3]	2000	16.9	5.3	-	22.2
Lachman and Cavalla-Calvanese[8]	1998	19.5	16.8	-	36.3
Rader *et al*.[9]	1997	19.6	10.8	-	30.4
Sheils and Wilbur[10]	1997	8.5	5	-	13.5
Auger *et al*.[4]	1997	27	10	9‡	42
Williams *et al*.[11]	1997	50	8	-	58
Current study	2002	33.6§	3.1	9.1‖	36.7¶

Abbreviations: ASCUS, atypical squamous cells of undetermined significance; SIL, squamous intraepithelial lesion; LSIL, low grade squamous intraepithelial lesion; HSIL, high grade squamous intraepithelial lesion; BAUS, basal cell abnormality of undetermined significance.

* CIN 1-2.

† atypical 4.8%, carcinoma 1%.

‡ SIL that could not graded 5%, invasive cervical carcinoma 4%.

§consisted of flat condyloma, CIN 1 and CIN 1-2.

‖BAUS 6.1%, features of CIN 1 in presence of severe inflammation 1%, invasive cervical carcinoma 2%.

¶ CIN 1 , CIN 1-2, HSIL , flat condyloma.

In our study, significant histopathologic findings were more frequent among women with ASCUS smears favor SIL than favor NOS or reactive (31.6% versus 13.3% and 1%, respectively).

A recent study has shown the utility of using qualifying statements to further subdivide ASCUS (9). We found that it seems reasonable to continue to identify smears as ASCUS favor SIL and NOS while it is better to eliminate the term ASCUS favor reactive category because follow up reveals that the majority of women with this diagnosis do not have a high-grade dysplasia but have a benign lesion. This latter finding also presented on the web site of the 2001 Bethesda system conference, one of the recommendations is elimination of “favor reactive” for equivocal cytology because the frequency of underlying CIN2 and CIN3 and detection of oncogenic HPV DNA in women with equivocal cytology qualified as favor reactive is low (14).

In conclusion, patients whose smears fall into the category of ASCUS may exhibit a spectrum of findings, ranging from benign/reactive lesions to frequent SIL and rarely to invasive carcinoma, therefore patients with a diagnosis of ASCUS justify careful follow up.

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