

# Cervical neoplasia after diagnosis and follow-up of women with atypical squamous cells of undetermined significance

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## Summary

**Background:** Although the cytological finding of atypical squamous cells of undetermined significance (ASCUS) occurs in around 5% of women undergoing cytological screening, the clinical evolution is unknown. The objective of this study was to evaluate women with a diagnosis of ASCUS and compare the histological findings and clinical evolution over six and 12 months. **Methods:** 1,244 patients with ASCUS (two diagnostic cytologists) were evaluated in this study with Pap Smears and colposcopy (biopsy if necessary) at the first visit, and thereafter at second and third visits (6 and 12 months after the first visit). Comparisons were made between cytological findings and histological diagnoses at every visit during the study and during evolution. **Results:** At the first visit, 60.3% of the biopsied patients presented histological findings of CIN I, 17.46% CIN II/III and 6.3% invasive neoplasia. At the second visit, 48.3% of the biopsied patients presented CIN I, 24.1% CIN II/III and none invasive neoplasia. At the third visit, 68.9% of the biopsied patients presented CIN I, 13.8% CIN II/III and none invasive carcinoma. If we consider all visits, a total of 213 colposcopy-guided biopsies were performed, representing 17.1% of all the patients included. Of these, 164 (13.2%) presented a histological diagnosis of cervical intraepithelial neoplasia (CIN) and eight (0.6%) presented with invasive carcinoma. **Conclusion:** We conclude that CIN or invasive lesions frequently occur in women with a diagnosis of ASCUS. Immediate review of the thin sections, new cytological tests or colposcopy and rigorous follow-up should be considered when making a diagnosis of ASCUS.

**Key words:** Neoplasms; Squamous cell; Cancer; Uterine/cervical; Papanicolaou smear.

## Introduction

Well-organized public health programs based on population screening through cytological tests have been shown to significantly reduce the incidence of and mortality due to the malignant forms of cancer of the uterine cervix in such populations [1]. However, the interpretation of the cytological findings has undergone modifications as a result of the high rates of false negatives detected. The Bethesda classification has been put forward in attempt to standardize cytological reports [2]. The purpose of this system was to categorize Papanicolaou (Pap) test diagnoses in a way that would be useful for clinicians in triage and treatment.

The cytological diagnosis of atypical squamous cells of undetermined significance (ASCUS) was created to characterize a spectrum of cell abnormalities that are more severe than those attributed to reactive alterations, but quantitatively or qualitatively insufficient to define a cytological diagnosis of squamous intraepithelial lesion (SIL) [3]. ASCUS presents a difficult diagnosis for cytologists and a clinical dilemma for physicians. A cytological finding of ASCUS occurs in around 5% of women undergoing cytological screening [4].

According to Melnikow *et al.* [5], the natural history of ASCUS may result in spontaneous regression, risk of progression to cervical intraepithelial neoplasia (CIN) I, II or III, or invasive carcinoma. Nonetheless, some

studies have shown that patients with ASCUS diagnosed by Pap smear had a SIL. Depending on age, it was found that 25-75% of CIN in females with ASCUS were diagnosed on Pap smears [6, 7]. A study of our group showed that in the 58 cases of ASCUS, 33 (56.9%) were diagnosed with ASCUS on review, in which 15 cases were probably neoplastic and the other 18 reactive; 23 (39.7%) smears were normal and two (3.4%) showed cervical intraepithelial neoplasia (CIN). From 23 unconfirmed ASCUS cases, 18 (87.5%) had normal cytology and colposcopy results in the first return visit [8]. After introduction of the ASCUS classification, the frequency of SIL and invasive carcinoma has diminished [9, 10] due to the more rigorous follow-up of patients. The frequency of CIN findings ranges from 12 to 61% in biopsies of women with ASCUS [9, 11]. In women with cervical cytology presenting slight atypia, more severe lesions are frequently found in colposcopy [12]. Thus some authors have recommended that immediate colposcopy examination should be performed on patients with ASCUS of probable neoplastic nature who were smokers or had a previous history of human papillomavirus (HPV) infection [13]. In the patients with reliable clinical follow-up who presented with ASCUS with probably reactive criteria, cytological follow-up was proposed. HPV tests in conjunction with a diagnosis of ASCUS can be employed to more efficiently select women to whom further follow-up is recommended [14]. Nonetheless, the ideal screening for patients with ASCUS remains undefined, and colposcopic examination continues to be the most appropriate option [15, 16].

Because of the large numbers of cytological diagnoses of ASCUS and its unknown evolution, there has been a lot of discussion about the clinical importance of this diagnosis and the best propedeutic conduct towards such patients. Thus, the objective of the present work was to evaluate a sample of women with a diagnosis of ASCUS in a cytology facility of a university hospital where patients with low socioeconomic levels are treated, and to relate this with the histological findings and clinical evolution over six and 12 months.

## Materials and Methods

Between 1993 and 1999, 66,882 Pap smears were performed and analyzed within the colposcopy facility of the Department of Gynecology and Obstetrics of the Federal University of "Triângulo Mineiro" (UFTM) with Ethical Committee approval. This is a public clinic serving patients of a low socioeconomic level that has a cervical neoplasia prevention program. The cytopathologists reporting on such cytological tests exclusively followed the Bethesda criteria [17] for the diagnosis of ASCUS: 2.5 to 3-fold enlargement of the nuclei of intermediate-sized squamous cells; slight increase in the nucleus/cytoplasm ratio; slight variations in shape and size of the nuclei, with slight hyperchromasia but with evenly distributed nuclear chromatin and lightly defined outlines. Grouping of the ASCUS cases according to whether they had reactive or neoplastic criteria were not performed since this was a retrospective study prior to 2001, the year when such grouping by the Bethesda system was first done.

Out of the total of 66,882 tests performed during this period, 1,244 (1.86%) were reported to be ASCUS cases. All patients with this diagnosis were immediately sent for colposcopy examination, and colposcycological checkups were performed six and 12 months later. Biopsy was performed when necessary, and the patients whose histological diagnosis demonstrated the presence of a treatable lesion received the appropriate therapy. Patients who underwent treatment were removed from the study. From these data, the cytological and histological findings at the outset and at six and 12 months were correlated.

With regard to clinical characteristics (Table 1), we observed that 423 (34%) of the diagnoses occurred between the ages of 20 and 30 years, 734 women (59%) were married, 286 (23%) were in menopause, 386 (31%) had a history of smoking and 87 (7%) were pregnant.

## Results

The frequency of patient returns for the second and third visits, and also the variation in Pap smear results from these checkups is shown in Table 2. It was observed that there was a high rate of non-returns for the six-month visit. After excluding the patients with positive histological diagnoses who received appropriate treatment for their carcinoma and were removed from the study for calculation purposes, the number of patients who did not return for the second and third visits reached 295 (25.9%) and 620 (56.6%), respectively. Among the patients who returned for the second visit, 11.3% presented with CIN I, 2.6% CIN II/III and one patient (0.1%) presented with an invasive lesion. At the third visit, 9% presented with CIN I, 2.5% CIN II/III and there were no cases of invasive neoplasia.

Table 1. — *Clinical characteristics of patients with ASCUS in pap smears (n = 1,244).*

Clinical characteristics	No.	%
<i>Age</i>		
< 20	63	5
20-30	423	34
31-40	261	21
41-50	274	22
51-60	162	13
> 60	61	5
<i>Race</i>		
White	833	67
Non-white	411	33
<i>Married</i>		
Yes	821	59
No	373	26
Widow	50	4
<i>Parity</i>		
0	547	44
1-3	597	48
> 4	100	8
<i>Hormone use</i>		
OCS	187	15
HRT	87	7
<i>Age of first intercourse</i>		
< 15	361	29
15-20	659	53
> 21	224	17

OCS = oral contraceptives; HRT = hormone replacement therapy

Table 2. — *Pap Smear results of the second and third visit, respectively, 6 months and 12 months after ASCUS diagnoses.*

Pap smear	2 <sup>nd</sup> visit		3 <sup>rd</sup> visit	
	Total	%	Total	%
Not returned	295	23.7	620	49.8
Returned	843	67.8	476	38.3
Excluded	106	8.5	148	11.9
Total	1244	100	1244	100
Normal	456	54.1	294	61.8
ASCUS	112	13.3	39	8.2
Inflammatory	153	18.1	88	18.5
CIN I	95	11.3	43	9
CIN II/III	22	2.6	12	2.5
AGUS	4	0.5	0	0.0
Invasive carcinoma	1	0.1	0	0.0
Total	843	100	476	100

The number of patients who underwent biopsy during the first colposcopic examination and the two successive six-month visits as well as the histological results from these biopsies are shown in Table 3. At some time during the follow-up, 213 patients (17.1%) had indications for biopsy during colposcopy, and the numbers progressively decreased from the second to the third visit. At the first visit, 60.3% of the biopsied patients presented with histological findings of CIN I, 17.5% CIN II/III and 6.3% invasive neoplasia. At the second visit, 48.3% of the biopsied patients presented with CIN I, 24.1% CIN II/III and none invasive neoplasia. At the third visit, 69% of the biopsied patients presented with CIN I, 13.8% CIN II/III and none invasive carcinoma. Thus, considering the diagnoses of CIN II-III and invasive cervical cancer, the

Table 3. — *Histological results in the first, second and third visits.*

Histology	1 <sup>st</sup> visit		2 <sup>nd</sup> visit		3 <sup>rd</sup> visit	
	Total	%	Total	%	Total	%
No	1118	89.87	785	93.2	447	93.9%
Yes	126	10.13	58	6.8	29	6.1%
Total	1244	100	843	100.0	476	100.0
Normal	20	15.87	16	27.5	5	17.2
CIN I	76	42.86	28	34.5	20	58.6
CIN II/III	22	10.32	14	12.1	4	13.8
Invasive carcinoma	8	6.35	0	0.0	0	0.0
Total	126	100	58	100	29	100

follow-up benefited 2.4% of patients (30/1244) at the first visit, 1.6% of the patients (14/843) at the second visit, and 0.8% of the patients (4/476) at the third visit.

The case with a cytological diagnosis of invasive neoplasia at the first six-month checkup was a false-positive result from the Pap smear since there was no histological confirmation of this, and rather of a high-grade lesion.

## Discussion

Even with the technological advances that have taken place in medicine over the last 100 years, the Pap smear continues to be the most widely utilized method for screening for cancer of the uterine cervix. After widespread adoption of the Pap smear as the screening method in the United States, the incidence of invasive carcinoma of the uterine neck decreased by 36% from 1973 to 1991, accompanied by a 42% reduction in the specific mortality rate for age [1]. The implementation of the Bethesda system has had a positive impact on the quality and consistency of laboratory results [2].

According to Davey *et al.* [17], the frequency of this diagnosis ranges from 1.6 to 9%, probably because the diagnosis of ASCUS may present low inter-observer concordance [18]. At a meeting of the National Cancer Institute in 1992, it was concluded that the diagnosis of ASCUS should not exceed 5% of the total number of Pap smears [2]. Moreover, the authors of the Bethesda system, recognizing the possible overuse of the term, recommended that its frequency should not exceed two to three times the rate for CIN I [2, 19]. Gerber *et al.* [20] found a rate of 5% in a sample of approximately 30,000 patients. Slawson *et al.* [21] detected a rate of 6%, Kennedy *et al.* [4] 4.5%, and Roche *et al.* [22] 2.3%. In our sample we found a diagnostic rate of 1.86% for ASCUS, thus testifying to the reliability of the results obtained in this study. A prospective study performed in our institute found that the ASCUS rate did not exceed 1.8 times the total for CIN I, thus demonstrating the quality of the cytological reports issued.

The most significant finding from the present study was the large number of histological lesions. A total of 213 colposcopy-guided biopsies were performed, representing 17.1% of all the patients included in the study. Of these, 164 (13.2%) had a histological diagnosis of CIN and eight (0.6%) had invasive carcinoma. This number

may be an underestimate because of the large number of patients who did not return for the second and third visits. The diagnoses of 100% of the invasive carcinoma cases and 58.6% of the CIN cases were immediately followed by colposcopic examination. Of the CIN diagnoses ( $n = 164$ ), 25.6% and 14.6% were made at the second and third visits, respectively. These numbers allowed for the conclusion that the ASCUS diagnosis has clinical importance, especially for tracking pre-invasive and invasive neoplastic lesions. Our findings are corroborated by Nygard *et al.* [23] who reported that histologically proven CIN was found in 10-60% of the women with a diagnosis of ASCUS, while CIN II/III was present in 5- 30%. We did not find any references in the literature regarding diagnoses of invasive lesions.

The proportion of women with ASCUS in whom CIN II/III is found through colposcopy is uncertain. Previous publications have reported a large range in CIN diagnosis rates from biopsy (from 25 to 60%), and 15-30% of these neoplasias are CIN II/III [22-25]. In a retrospective study, Alanen *et al.* [26] demonstrated that after following up a diagnosis of ASCUS for two years, 3.9% of the women presented a biopsy compatible with CIN II/III. Morin *et al.* [27] demonstrated the presence of CIN in 22.2% of the biopsies among 360 women with ASCUS, of which 16.9% was CIN I and 5.3% CIN II/III. Williams *et al.* [25] followed up 668 patients with a diagnosis of ASCUS for a two-year period and, among the 41% who had a colposcopy-guided biopsy, 4% presented CIN II/III.

The diminishing findings of CIN and lack of findings of invasive neoplasia at the second and third visit is in accordance with the report by Giudice *et al.* [10]. They found that the incidence of CIN and carcinoma diminished as the follow-up of patients with an ASCUS diagnosis became more rigorous. Perhaps the large number of normal cytological findings at the first checkup explains why some patients did not come to the second checkup. However, this rate of abandonment of follow-up shows that there is a need for reinforcement among patients regarding the importance of such follow-up, because of the risk of occult lesions.

Several authors have defended reduced utilization of the ASCUS interpretation. Such proposals have ranged from limiting the use of ASCUS diagnoses to their complete elimination [28, 29]. We believe that such initiatives are rash because of the quantity and severity of neoplastic alterations found following an initial diagnosis of ASCUS. What is really needed is greater qualification and stratification of this diagnosis. The Bethesda system was itself revised in 2001, with modification of the ASCUS interpretation into the categories of ASC US (atypical squamous cells of undetermined significance) and ASC H (atypical squamous cells for which a high-grade lesion cannot be ruled out) [30]. Another attempt at better qualifying the ASCUS diagnosis was described by Guerrini *et al.* [31] from the morphological criteria recommended in the Emilia Romagna regional screening protocol (1997). In this, ASCUS was divided into four

grades: ASCUS 1 – atypical squamous cells with cytoplasm of intermediate-mature type; ASCUS 2 – metaplastic atypical squamous cells; ASCUS 3 – atypical squamous cells with parakeratosis; ASCUS 4 – reactive atypical cells. Patients with ASCUS 1 and 3 are associated with greater colposcopic findings and have more relation with the presence of CIN in biopsies [8, 31].

## Conclusion

We concluded that CIN or invasive lesions frequently occur in women with a diagnosis of ASCUS. Observation over a single period may be rash. Immediate review of the thin sections, new cytological tests or colposcopy should be considered when making a diagnosis of ASCUS.

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