

Frequency of infectious agents for vaginitis in patients with a cytological diagnosis of atypical squamous cells of undetermined significance

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Summary

Aim: To evaluate the presence of infectious agents for vaginitis in patients with ASCUS. **Methods:** 33,388 patients who underwent cervical-vaginal cytology from 08/1993 to 05/2002 were included in the study, and 1,104 (3.4%) presented positive ASCUS. The appraised infectious agents were Coccobacilli, *Candida sp*, *Trichomonas vaginalis*, and clue cells (*Gardnerella vaginalis*). **Results:** In the group with ASCUS a larger frequency of Coccobacilli (22.37%) and *Trichomonas vaginalis* (5.25%) was found when compared with the group with negative ASCUS (17.79% and 3.98%, respectively; $p < 0.05$). Cytolysis occurred more frequently in patients with ASCUS (3.8 vs 6.3%, first phase and 4.5 vs 10%, second phase). **Conclusions:** We believe that some diagnoses of ASCUS can be induced by the presence of infectious agents for vaginitis, mainly cocci and coccoides. ASCUS occurs more frequently in the first phase of the menstrual cycle, therefore in less acid vaginal pH.

Key words: ASCUS; Infectious agents; Vaginitis; Papanicolaou smears.

Introduction

One of the methods for diagnosing preneoplastic lesions of the uterine cervix may begin with abnormal results from cervicovaginal material collected on a slide and stained using the Papanicolaou method. Various cytological classifications for these lesions have been used. The Bethesda system for cervicovaginal cytological diagnosis was introduced in 1988 and had the objective of furnishing uniform terminology. After using this system for a period of time, it was observed that findings of atypical squamous cells of undetermined significance (ASCUS) were the most commonly encountered diagnosis from cervicovaginal cytology [1]. This category relates to cellular squamous abnormalities that are not diagnostic for inflammatory reaction or preneoplasia, or that represent conditions that qualitatively and quantitatively are insufficient to be interpreted as cervical intraepithelial neoplasia (CIN) [2, 3].

The National Cancer Institute of the United States has concluded that a diagnosis of ASCUS is expected in around 5% of the patients, and that greater frequency may indicate misuse of the term [4]. The American College of Obstetrics and Gynecology suggests that patients with at least two consecutive diagnoses of ASCUS, or one diagnosis of ASCUS with the presence of a high-risk factor (such as infection by human papillomavirus, smoking or multiple sexual partners) should undergo complementary assessment [5].

An extensive and diverse spectrum of pathogenic and nonpathogenic organisms can be observed in the vaginal

microflora [6]. Different studies carried out with the objective of establishing the frequencies of the most common infectious agents for vaginitis have shown a variety of results. The prevalence found for *Gardnerella vaginalis* has ranged from 8% to 75%, *Candida albicans* from 2.2% to 30% and *Trichomonas vaginalis* from zero to 34% [6-14].

Previous studies by our group have demonstrated that the frequency of infectious agents for vaginitis may be influenced by the presence of the uterine cervix, or may even vary according to the different decades in which the tests were performed [15, 16]. This leads us to wonder whether the frequency of these agents might be related to a diagnosis of ASCUS.

Another point is the difference in vaginal pH that is found between the first and second phase of the menstrual cycle. The second phase is more acidic than the first phase [17]. The question that arises is whether this greater acidity in the second phase of the menstrual cycle might influence a cytologist's interpretation of a diagnosis of ASCUS.

One of the difficulties in interpreting cytological tests is the presence of inflammation. The few studies available in the literature have demonstrated that infectious agents have a strong influence on cell alterations, thereby producing different interpretations. In a study by our group comparing the cytological findings proposed by two different protocols (Bethesda and Emilia-Romagna), bacterial vaginosis was found more frequently in women whose cytological tests revealed ASCUS [18].

Our aim in the present study was to contribute towards this topic, to lead to a better understanding and comprehension of women with a cytological diagnosis of

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ASCUS. The term ASCUS is controversial and the question needs to be asked as to whether the presence of infectious agents might contribute towards making this interpretation. To address this question, our objective was to analyze the age group, phase of the menstrual cycle and frequency of infectious agents (as seen on Papanicolaou smears) among women with a cytological diagnosis of ASCUS.

Material and Methods

Patients

A retrospective study covering the period between August 1993 and May 2002 was conducted. Evaluations were made on the results from 33,388 patients who underwent routine cervicovaginal cytological tests at the cytopathology service of the Federal University of the Triângulo Mineiro (UFTM). Of these, 1,104 (3.4%) presented ASCUS (i.e. ASCUS-positive) and 32,284 (96.6%) did not present atypia corresponding to a diagnosis of ASCUS (i.e., ASCUS-negative). All the patients were assessed for the presence of coccobacilli, *Candida sp*, *Trichomonas vaginalis*, clue cells (*Gardnerella vaginalis*) and cytolysis.

Samples of endocervical material (brush) and cervical and vaginal material (spatula) were obtained from patients who did not undergo hysterectomy. The material collected was fixed in ethyl alcohol and stained using the Papanicolaou method. The interpretations were performed by doctors specializing in cytopathology, all using the same diagnostic criteria in relation to Doderlein bacilli, cytolytic flora, cocci and coccoids, clue cells, *Trichomonas vaginalis* and *Candida sp*.

Women were excluded from the study if they presented bacterial vaginosis or had had active vaginal bleeding or sexual relations, or had used vaginal douches or medication, within the 48 hours preceding sample collection.

This study was granted approval by the Research Ethics Committee of the Federal University of the Triângulo Mineiro.

Cytological criteria [19, 20, 21]

The diagnosis of ASCUS was based on the Bethesda criteria: karyomegaly with an increased nucleus/cytoplasm ratio; slight irregularity of the nucleus with mild hyperchromasia and chromatin, without granulation; binucleation and mild dyskariosis; keratosis and atypical parakeratosis.

The following criteria were used as cytological diagnostic parameters: clue cells – squamous cells covered with coccobacilli, for which the cytoplasmic margins presented as stains; *Candida sp* – pseudohyphae seen, weakly stained with eosin or sometimes with hematoxylin, and/or small spores (diameters of 2-4 nm), stained pale pink; *Trichomonas vaginalis* – single-cell organism of ovoid or rounded shape (diameter of 8-20 nm), with pale or grayish cytoplasm, possibly with eosinophilic granules at the center of the cytoplasm, and a vesicular nuclear format or half-moon shape that was clearly stained using hematoxylin; Cytolysis – defined as a pale coloration, with a vesicular nucleus and little or no cytoplasm in intermediate cells from the cervicovaginal material; Coccobacilli – bacillary and coccal organisms, diffusely scattered in groups or microcolonies; and Lactobacilli – presence of elongated bacillary structures.

Statistical analysis

The chi-square test was used for statistical analysis, with the significance level set at less than 0.05.

Results

In the group of women with a diagnosis of ASCUS, greater frequencies of Coccobacilli (22.37%) and *Trichomonas vaginalis* (5.25%) were found than in the ASCUS-negative group (17.79% and 3.98%, respectively); $p < 0.05$ (Table 1).

Table 2 shows the patients divided according to the phase of the menstrual cycle, taking the first phase as the first to the 15th day and the second phase as the 16th to the 30th day. The majority of the women with a diagnosis of ASCUS (56.5%) were in the first phase of the menstrual cycle, although this finding did not have statistical significance ($p = 0.53$). Thus, there was a greater frequency of *Trichomonas vaginalis* (6.7%; $p = 0.0358$) among the ASCUS-positive patients presenting the first phase of the cycle than among the ASCUS-negative patients presenting the first phase of the cycle.

There were no differences in the frequencies of coccobacilli and cytolysis when the groups were divided according to the phase of the cycle. Coccobacilli remained a more frequent finding among ASCUS-positive patients in both the first and second phase, and cytolysis in the ASCUS-negative patients in both the first and second phase.

When divided by age (Table 3), the women in the ASCUS-positive group aged between 31 and 40 years presented a greater frequency of *Trichomonas vaginalis* (6.8% vs 4.4%; $p = 0.0437$) than did the same age group in the ASCUS-negative group. There were also higher numbers of lactobacilli (80.4%; $p < 0.0001$) than the 70.5% presented by the ASCUS-negative group.

Table 1. — Total number of patients with positive ASCUS, negative ASCUS and frequency of infectious agents.

	Positive ASCUS (N = 1.104)		Negative ASCUS (N = 32.284)	
	N	%	N	%
Cytolysis*	47	4.25	2,592	8.02
Coccobacilli*	247	22.37	5,744	17.79
Clue cells	196	17.75	5,968	18.48
<i>Candida sp</i>	216	19.56	6,072	18.80
<i>Trichomonas vaginalis</i> **	58	5.25	1,288	3.98
Lactobacilli	789	71.46	23,007	71.26

* $p < 0.0001$, ** $p = 0.0358$.

Table 2. — Distribution of patients in the first phase (1st-15th day) and second phase (16th-30th day) of the menstrual cycle.

	First phase				Second phase			
	Positive ASCUS 56.52% (N = 624)		Negative ASCUS 53.64% (N = 17315)		Positive ASCUS 43.47% (N = 480)		Negative ASCUS 46.35% (N = 14969)	
	N	%	N	%	N	%	N	%
Cytolysis	24	3.8	1,092	6.3 ¹	22	4.5	1,501	10.0 ²
Coccobacilli	138	22.1 ³	3,208	18.5	107	22.2 ⁴	2,538	16.9
Clue cells	102	16.3	3,330	19.2	93	19.3	2,639	17.6
<i>Candida sp</i>	110	17.6	2,904	16.7	105	21.8	3,169	21.1
<i>Trichomonas vaginalis</i>	42	6.7 ⁵	751	4.3	15	3.1	538	3.5
Lactobacilli	452	72.4	12,316	71.1	336	70.0	10,692	71.4

Comparison among positive and negative ASCUS: ¹ $p = 0.0124$, ² $p < 0.0001$, ³ $p = 0.0238$, ⁴ $p = 0.0002$, ⁵ $p = 0.0358$.

Note: some patients had more than one infection.

Table 3. — Distribution of patients by age.

Age (N)	Positive ASCUS (N = 1.104)								Negative ASCUS (N = 32.284)							
	< 20 (109) %		21-30 (348) %		31-40 (337) %		> 41 (251) %		< 20 (3,065) %		21-30 (100,26) %		31-40 (11,691) %		> 41 (7,494) %	
Cytolysis	1	0.91	20	5.7	16	4.7	9	3.6	249	8.1 ¹	862	8.5	954	8.1 ²	529	7.0 ³
Coccobacilli	25	22.9	76	21.8 ⁴	81	24.0 ⁵	61	24.7	607	19.8	1,713	17.0	1,979	16.9	1,449	19.3
Clue cells	26	23.8	59	16.9	62	18.3	41	16.3	640	20.8	1,685	16.8	2,262	19.3	1,384	18.4
<i>Candida sp</i>	20	18.3	76	21.8	67	19.8	50	19.9 ⁶	658	21.4	2,167	21.6	2,164	18.5	1,083	14.4
<i>Trichomonas vaginalis</i>	4	3.7	14	4.0	23	6.8 ⁷	14	5.5	120	3.9	319	3.1	526	4.4	326	4.3
Lactobacilli	70	64.2	253	72.7	271	80.4 ⁸	176	70.1	2,103	68.6	7,367	73.4	8,250	70.5	5,279	70.4

Comparison among women with positive and negative ASCUS: ^{1,2,3}All women in the ASCUS-negative group presented a greater frequency of cytolysis, respectively: $p = 0.0061$ (< 20 years), $p = 0.0233$ (31-40 years), $p = 0.0333$ (> 41 years). ⁴Women in the ASCUS-positive group aged between 21 and 30 years presented a greater frequency of coccobacilli ($p = 0.0210$). ⁵Women in the ASCUS-positive group aged between 31 and 40 years presented a greater frequency of coccobacilli ($p = 0.0006$). ⁶Women in the ASCUS-positive group aged > 40 years presented a greater frequency of *Candida sp.* ($p = 0.0159$). ⁷Women in the ASCUS-positive group aged between 31 and 40 years presented a greater frequency of *Trichomonas vaginalis* ($p = 0.0437$). ⁸In the ASCUS-positive group aged between 31 and 40 there were also higher numbers of lactobacilli ($p < 0.0001$) than in the ASCUS-negative group.

Note: some patients had more than one infection.

In the ASCUS-positive group over the age of 40 years, there was a higher frequency of *Candida sp* (19.9%; $p = 0.0006$) than in the same age group of the ASCUS-negative group (14.4%).

Discussion

The diagnosis of ASCUS is still a term leading to much discussion, given that the interpretation differs among pathologists. One large difficulty in interpreting cytological tests when a diagnosis of ASCUS is given is the inflammation caused by infectious agents such as *Candida sp* and *Trichomonas vaginalis* that lead to the development of vaginitis. This discussion is longstanding. In one study published in 1996, in which 50 cervicovaginal smears were analyzed, 16 were initially interpreted as ASCUS and 34 as normal. After reviewing the Bethesda criteria for ASCUS, and identifying the changes associated with the presence of *Candida sp* (focal hyperchromasia, orangeophilia and perinuclear halos), ten of the 16 cases of ASCUS were reclassified as normal [22]. Thus, it was demonstrated that the infectious agents had a strong influence on cell alterations that produced erroneous interpretations and diagnoses.

Our findings demonstrated that coccobacilli were more frequently found among ASCUS-positive women. This may reinforce the possibility that there might be a relationship between ASCUS and bacterial vaginosis. According to the discussion in a review of the Bethesda criteria (Bethesda System 2001), one modification to the nomenclature would be removal of flora with Coccobacilli, which would be replaced by suggestions of vaginosis [23]. A previous study by our group found greater frequency of bacterial vaginosis in patients with a diagnosis of ASCUS, thus favoring neoplasia [18]. Another interesting finding was the greater presence of *T. vaginalis* in ASCUS-positive women. Findings of *T. vagi-*

nalis in cytological tests have dropped in frequency over recent decades to around 3% [6]. It seems that this finding is much greater among ASCUS-positive women (5%) although, because of the low numbers, it is improbable that the presence of this infectious agent for vaginitis would lead to a cytological interpretation of ASCUS.

These affirmations demonstrate that there is a strong relationship between the presence of infectious agents and the presence of ASCUS. The basis for this is studies that have demonstrated classifications for bacterial vaginosis [24, 25], in which all the classes demonstrated that the presence of coccobacilli was one of the factors involved in bacterial vaginosis. Our suggestion is that the simple presence of coccobacilli may be a factor to be considered in cell alterations, and it may influence the interpretation of the results regarding ASCUS.

Vaginal pH is related to the phase of the menstrual cycle, and it is known that it is higher during the menstruation phase (1-5 days) and proliferative phase (8-12 days) of the cycle than in the secretory phase (18-22 days) [26]. Our results showed that a greater number of women with a diagnosis of ASCUS were in the first phase of the cycle (1st-15th day; 56.5%), versus 43.4% in the second phase (16th-30th day), which leads us to suggest a hypothesis that the lower vaginal pH that occurs in the secretory phase of the cell cycle does not influence the cytological alterations associated with ASCUS. Another fact that reinforces this finding is that cytolysis (an event that is related to lower pH) occurred almost twice as often among the ASCUS-negative women.

In the United States, it has been estimated that 3.5 million colposcycological tests give rise to a diagnosis of some abnormality every year, of which approximately 2.5 million are interpreted as ASCUS [27]. The estimates have suggested that 10-20% of the women with ASCUS have high-grade intraepithelial lesions, and that one in every 1,000 has invasive cancer [23]. Because of this

undetermined nature, diagnoses of ASCUS create great doubt for doctors, since this category may represent some lesion, and delaying the diagnosis could alter the prognosis for the patient. It also creates anxiety for the patients, because the possibility of malignant disease has not been discarded. Our results demonstrated that there was greater frequency of coccobacilli and *T. vaginalis* in the women with a diagnosis of ASCUS; the greatest number of women with *Trichomonas* were in the group aged between 31 and 40 years; and *Candida sp* was most frequent among ASCUS-positive women aged over 40 years, as we had already shown in another study by our group [16]. These results lead us to believe that it is possible that many diagnoses of ASCUS are being induced by the presence of infectious agents for vaginitis, particularly cocci and coccoids, and that ASCUS findings are more frequent in less acidic vaginal pH. Treatment of these alterations is recommended for better interpretation of ASCUS.

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