Value of high-risk human papillomavirus testing in the triage of atypical squamous cells of undetermined significance

Kozeta Filipi[1], Anila Xhani[2]

[1]Institute of Public Health, Tirana, Albania;
[2]University Hospital of Obstetrics-Gynecology "Queen Geraldine", Tirana, Albania.

Corresponding author: Kozeta Filipi, MD, Institute of Public Health;
Address: Rr. "Aleksander Moisiu", No. 88, Tirana, Albania;
Telephone: +355672052984; Email: kkozeta@hotmail.com

Abstract

Aim: Atypical squamous cells of undetermined significance cells (ASCUS), occurring in organized cytological screening, may be either high-risk human papillomavirus (HPV) positive or negative. To refine the assessment of women with ASCUS, a high-risk HPV-DNA test is recommended as triage by NIH 1.
Methods: A total of 53 consecutive women (mean age: 30 years; range 21-41 years) with a diagnosis of ASCUS from the primary screening were selected for triage in Tirana, the Albanian capital city, during September 2012 to April 2013. Their cervical smears were collected and evaluated by using conventional cytological examination in combination with a high-risk HPV-DNA test [(*HPV*) *polymerase chain reaction* (*PCR*) *test*).] The women were categorized into four groups: Group A, Cytology + / HPV +; Group B, Cytology–/ HPV +; Group C, Cytology + / HPV–; and Group D, Cytology–/ HPV–. Women within Groups A-C were admitted for colposcopy and cervical biopsy. The women in Group D were considered as a low-risk group for tumor development, and were reexamined after three years.
Results: In women in Group A (N=26), the prevalence of histological verified CIN 2-3 was 42%, in Group B (N=11) it was 63%, and in Group C (n=9) the prevalence was 66%. The prevalence of a high-risk HPV infection decreased with age in women with ASCUS. It was 71% in women <30 years, and 50% in women aged ≥40 years.
Conclusions: In this sample of Albanian women, adding a high-risk HPV test in secondary screening increased the identification of women with CIN 2-3 lesions by 48% in comparison with repeated cytology. The clinical significance of the ASCUS diagnosis varied with age of the women.

Keywords: ASCUS, cervical dysplasia, cervix, cytology, HPV, screening, triage.

Introduction

In Albania, cervical and breast cancer are the most frequent cancers among women of all ages and the leading cause of cancer death among women, according to the World Health Organization (WHO) and another report of 2008 (1). In addition, despite the fact that cervical cancer represents the second most common cancer among women aged 15-44 years, and most cancers are diagnosed at stage III to IV, only 8% of Albanian women that have ever had a routine gynecological exam also had a Pap-smear (2).

There is no organized cervical cancer screening program in Albania. The Pap-smear is offered in selected gynecological-obstetrical centers and private clinics in Tirana, the capital city of Albania (3).

The screening coverage among women of reproductive age in Albania is extremely low, probably the lowest in the region: only 3.2% of women 15 to 44 years old reported having ever been screened with a Pap-smear, with additional differences observed among women in urban areas (4.9%) and those in rural areas (1.8%) (2). Altogether, 2.7% of women 15 to 44 years old reported having had a Pap-smear performed regu-larly every three years (4.3% of women in urban areas compared to 1.5% of women in rural areas) (2).

Studies of organized screening reveal that women, who choose not to participate, represent the most important risk group for tumor development. The second most important risk factor is the relatively *low sensitivity* of a single *Pap test* (4). In Albania the sensitivity of cytology in 2005 was 20% with a specificity of 80% (5). In almost every case of invasive cervical cancer and its pre-malignant progenitors, HPV can be recognized (6-8). Because the HPV test has been repeatedly shown to be more sensitive as compared to the ordinary cytological screening, introducing of HPV test is very important for increasing the quality of medical care in disease diagnosis and treatment. Referring all these women to colposcopy is cumbersome, worrying, and costly; however, the alternative, that is a repeated PAP-test, is not sufficient for the prevention of invasive cancer.

In the present study, a high-risk HPV test was added for secondary screening of women diagnosed with ASCUS in the primary screening. Albania is in the process of developing a national cervical screening program. The aim of this study was to evaluate to what extent the inclusion of the HPV test influenced the capacity to identify CIN 2-3 lesions in the Albanian women and whether such a test affected the sensitivity and specificity in the triage of ASCUS.

Material and methods

A total of 53 consecutive women (mean age: 29.6 years; standard deviation: 4.6 years; range: 21-41 years) who participated in the opportunistic cytological screening program in Tirana from September 2012 to April 2013, and with an ASCUS diagnosis based on the primary screening, were included in this study. These women were reexa-mined about three months later with sampling of two cervical smears, of which one was used for cytology and the other one for a high-risk HPV-DNA analysis. All the HPV-DNA testing was performed in collaboration with "Regina Elena" Institute in Rome.

Women with an abnormal cytology and/ or a positive HPV test were admitted to a colposcopy clinic to undergo colposcopy, a new PAP-smear, a cervical biopsy, and endocervical curettage. High-risk HPV-DNA analysis of the cervical sample was performed through polymerase chain reaction testing. The test identifies 19 HPV types (6, 11, 16, 18, 31, 32, 33, 39, 40, 42, 51, 52, 53, 56, 59, 62, 68, 83, and 84). The cytological slides were stained with the PAP-stain and evaluated according to the following categories: normal, ASCUS, CIN 1, CIN 2, and CIN 3.

The cervical biopsies were fixed in buffered 10% formalin, embedded in paraffin, and sectioned in 4 mm thin sections and stained with hematoxylineosin before light microscopic examination. The biopsies were analyzed by use of the same main categories as the cytological specimens, except for the ASCUS diagnosis.

Based on the test results in the secondary screening, women were categorized into four groups:

- Group A, Cytology+/ HPV+
- Group B, Cytology-/ HPV+
- Group C, Cytology+/ HPV-
- Group D, Cytology-/ HPV-

Women within Groups A-C, as mentioned above, were admitted for colposcopy and cervical biopsy.

Women in Group D were considered as a low-risk group for tumor development and were reexamined after three years.

The study was approved by the Faculty of Technical Medical Sciences, University of Tirana, Albania.

Fisher's exact test was used to compare the groups of women recruited in this study.

Results

A HPV-positive reaction occurred in 37 (70%) of the women (mean age: 29.1 years) and an abnormal cytology in 49 (90%) in the triage of ASCUS (mean age: 30 years). Among women with abnormal cytology, 14 cases showed ASCUS. There were 24 cases of CIN 1 and 11 cases of CIN 2-3. Thus, more women were high-risk HPV positive than with cytological abnormalities (ASCUS-CIN 3). The agreement between the two methods was 0.62 (kappa= 0.135, SE = 0.140, 95% confidence interval: from -0.139 to 0.410). Hence, the strength of agreement between methods is considered quite poor. Abnormal cytology only was seen in 66% of the cases, while a positive HPV test only was found in 70% of the cases.

Of 26 women, 11 (42%) of them had abnormal cytology and a positive high-risk HPV test (Group A: Cytology+/ HPV+) (Table 1). These women in the secondary screening showed CIN 2-3 lesions in a consecutive cervical biopsy. Seven of the 11 women (63%) with normal cytology and a positive high-risk HPV test (Group B: Cytology-/ HPV+) showed CIN 2-3 lesions. Six of the nine women (66%) with abnormal cytology (ASCUS and CIN1) and a normal high-risk HPV test (Group C: Cytology+/ HPV-) showed CIN 2-3.

Thus, the high-risk HPV test improved the detection rate of CIN 2-3 triage of ASCUS by 48%. The difference between high-risk and cytology was not statistically significant (P=0.36) compared to repeated cytology in our study. Group D (N= 7), as a low-risk group for tumor development, did not currently undergo the test, but will be reexamined after three years. In Group C, six out of nine women showed abnormal histological CIN 2-3 lesions.

Table 1. Secondary screening including a combination of cytology and a high-risk HPV testing for detection of CIN 2-3 lesions in women with a primary diagnosis of ASCUS

Group	Number of cases	Number of CIN 2-3	Percentage
Group A, Cytology+ /HPV+	26	11	42
Group B, Cytology-/ HPV+	11	7	63
Group C, Cytology+/HPV-	9	6	66
Group D, Cytology-/HPV-	7	0	0
Total	**53**	**24**	**45**

A pronounced discrepancy was recorded in the association between ASCUS and HPV in relation to age. Among women below 30 years of age with an ASCUS, 71% of them were HPV positive, whereas only 50% of the women more than 40 years of age with an ASCUS were HPV positive (Table 2).

Table 2. Relation between ASCUS in the primary screening and an HPV-positive reaction in the secondary screening in relation to age

Age group (years)	Number of cases	HPV positive
<30	28	20 (71%)
30-39	23	16 (69%)
40-49	2	1 (50%)
Total	**53**	**37 (70%)**

The sensitivity in identifying CIN 2-3 lesions was 75% for the HPV test and 70% for the cytological examination, provided that all abnormal cells (ASCUS CIN 3) were included in the analyses. The specificity for the HPV test was 34% and 37% for the cytological analysis. However, specificity of the HPV test was found to vary and increased with age: it was 44% in women over 40 years of age and 33% in women less than 30 years (Table 2 and Table 3). The relation between high-risk HPV tests and the presence of morphological CIN 2-3 lesions with regard to age was also assessed in our study. The prevalence of CIN 2-3 was 48% in all of the HPV-positive women. However, it varied with age, being highest (1/ 1, 100%) in the group of women between 40 and 49 years of age (Table 3).

Table 3. Relationship between an HPV-positive reaction and the presence of morphological CIN 2-3 lesions in relation to age

Age group (years)	Number of cases	HPV positive	Cytology +	CIN 2-3	Percentage
<30	28	20	19	13	65%
30-39	23	16	15	10	62%
40-49	2	1	1	1	100%
Total	**53**	**37**	**35**	**24**	**65%**

Discussion

Since its introduction, the organized screening program in Albania has been the object of a number of quality analyses and it is now well-established that the most prominent weakness of this screening program is the non-optimal participation rate (5). This has been also shown in other settings (6-8).

A second important problem with the screening program is the limited sensitivity of cytological evaluation (9-15). Therefore, if a primary screening test shows ASCUS, which is the case in nearly half the abnormal tests in the primary screening examination, a second normal smear is not suitable for determining the exclusion of precancerous cervical lesion, called 'triage.' A significant number of cancers occur after a normal smear following ASCUS (16).

A large randomized clinical trial, the ASCUS/ LSIL Triage Study (ALTS), demonstrated the cost-effectiveness of using HPV testing to clarify the risk of an ASCUS Pap result (17).

In Albania, two strategies have been practiced to overcome this problem. One is to refer all these women to colposcopy, which is costly but safe. The other is to take a repeated smear, which is less safe as a number of cancers may appear before the next screening round. A proposed method to secondary cytological screening is adding high-risk HPV tests, as was done in the present investigation. Several reports indicate that the use of high-risk HPV tests improves the chances of identifying women with CIN 2-3 lesions (9-15). In our study there was an increase of 48% in the identification of women with CIN 2-3 lesions.

Our results indicate that the value of HPV testing in the secondary screening program is most evident in the age group 30-49 years, where 64% of the women had CIN 2-3 lesions. However, it should be noted that the inclusion criterion was an initial ASCUS diagnosis and, therefore, only the benefit of adding HPV analysis to the sensitivity of cytology could be calculated.

The mean age of women with abnormal cytology (30 years) and a positive test for high-risk HPV (29 years) as below the mean age of the study population (30 years) indicate that abnormal cytology and a positive HPV test are more frequent in younger women. This finding is consistent with results from other studies. More women are HPV positive than have a cytological abnormality (17-19), and primary HPV screening may reduce the number of deaths from cervical cancer in some settings (20), but the cases that can be prevented by triage are seldom fatal (5). This and several other studies clearly demonstrate that using high-risk HPV-DNA tests as triage in ASCUS markedly increases the efficacy of the screening, both by a more specific identification of women at risk of developing cervical cancer and by the possibility of excluding women who have a low risk for tumor deve-

lopment (normal cytology and a negative HPV test). The method is especially convenient for use in countries with an organized screening program with a well-equipped data system that allows the re-call of women with double negative tests to routine screening. Furthermore, the method is appropriate for women who are referred to colposcopy exhibiting positive tests in the secondary screening. Cytology added little to the triage and could probably be excluded (12,21). HPV triage is especially useful for older women—those over 35 years—because the specificity of the test improves in older women (22).

Conflicts of interest: None declared.

The introduction of HPV test in the ASCUS diagnosis is very important for the Albanian health care system. This test combined with cytology and histology will be the first step in the quality control of the cervical screening programs in Albania.

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