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ORIGINAL ARTICLE

Value of high-risk HPV-DNA testing in the triage of ASCUS

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Abstract

Objective. Atypical squamous cells of undetermined significance (ASCUS) cells, 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 in Sweden. *Methods.* A total of 197 consecutive women (mean age 39 years, range 21–60) with a diagnosis of ASCUS from the primary screening were selected for triage. Their cervical smears were collected and evaluated by using conventional cytological examination in combination with a high-risk HPV-DNA test (hybrid capture 2). 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 re-examined after three years in the next round of the organized screening. *Results.* In women in Group A ($n=58$) the prevalence of histological verified CIN2–3 was 41%, in Group B ($n=41$) 20%, and in Group C ($n=9$) 0%. In Group D ($n=89$), repeated primary screening three years later revealed CIN2–3 in two biopsies from 74 women studied ($<3\%$). The prevalence of a high-risk HPV infection decreased with age in women with ASCUS. It was 74% in women <30 years and 19% in women ≥ 50 years. *Conclusions.* Adding a high-risk HPV test in secondary screening increased the identification of women with CIN2–3 lesions by 33% in comparison with repeat cytology ($p=0.01$). The clinical significance of the ASCUS diagnosis varied with age of the women.

Key words: *Cervix, cytology, screening, squamous carcinoma, HPV, triage, ASCUS, cervical dysplasia, sorting*

Abbreviations: *ASCUS: atypical squamous cells of undetermined significance, CIN: cervical intraepithelial neoplasia, hc2: hybrid capture 2, HPV: human papillomavirus*

Introduction

After the introduction of organized cytological screening in Sweden in the late 1960s, the incidence of cervical carcinoma decreased considerably. The estimated benefit of screening was a reduction in the number of cervical cancer cases by at least 60% (1). Studies of organized screening reveal that women, who choose not to participate, represent the most important risk group for tumor development (2). The second most important risk factor is the relatively low sensitivity of cytological screening. For that reason, around 65% of all cancer cases in Sweden occur among women without a recent screening history and around 25% in women with a normal cytological examination within the recommended screening interval (3–5).

The most important etiologic factor for the development of cervical cancer is a persistent infection with high-risk human papillomavirus (HPV). 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 with ordinary cytological screening, arguments in favor of an HPV-DNA analysis as an adjunct or as a substitute to organized cytological screening are compelling (9–15). However, the method of implementation of HPV tests in different phases of

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organized screening programs is still under discussion.

In the Swedish screening program nearly 2% of all smears are diagnosed as atypical squamous cells of undetermined significance (ASCUS). Referring all these women to colposcopy is cumbersome, worrying, and costly; however, the alternative, to take a repeat PAP-test, is not sufficient for the prevention of invasive cancer (16). For that reason, HPV-DNA tests are now recommended in Sweden as triage of women with ASCUS.

In the present study, a high-risk HPV test was added for secondary screening of women diagnosed with ASCUS in the primary screening. The aim was to evaluate to what extent the inclusion of the HPV test influenced the capacity to identify CIN2–3 lesions in the County of Gävle and whether such a test affected the sensitivity and specificity in the triage of ASCUS.

Material and methods

A total of 197 consecutive women (mean age 39 years, range 21–60) who participated in the organized cytological screening program in the County of Gävle from September 2004 to April 2006, and with an ASCUS diagnosis based on the primary screening, were included. These women were re-examined about three months later with sampling of two cervical smears, of which one was used for cytology and the other for a high-risk HPV-DNA analysis. Those 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. The colposcopist was blinded to the results of the triage, i.e. whether the cytology or the HPV test was positive.

High-risk HPV-DNA analysis of the cervical sample was performed with the hybrid capture 2 method (hc2) (Digene Corp., Silver Spring, MD, USA). The test identifies 13 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). The hc2 technique can detect HPV-DNA concentrations over 1 pg/ml, which is proportional to the light emission of the positive control and corresponds to 5,000 HPV genomes per specimen in the well. The cytological slides were stained with the PAP-stain and evaluated according to the following: normal, ASCUS, CIN1, CIN2, and CIN3.

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

Based on the test results in the secondary screening, 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, as mentioned above, were admitted for colposcopy and cervical biopsy, whereas women in Group D were considered as a low-risk group for tumor development. They were re-examined after three years within the frame of the organized screening. Fisher's exact test was applied to compare the groups.

Results

A HPV-positive reaction occurred in 99 (50%) of the women (mean age 34 years) and an abnormal cytology in 67 (34%) (mean age 35 years) in the triage of ASCUS. Among women with abnormal cytology, 45 cases showed ASCUS. There were 16 cases of CIN1 and six of CIN2–3. Thus, more women were high-risk HPV positive than cytological abnormal (ASCUS–CIN3). The agreement between the two methods was 0.74. Abnormal cytology only was seen in 5% of the cases, while a positive HPV test only was found in 21% of the cases.

Twenty-four of 58 (41%) women with abnormal cytology and a positive high-risk HPV test (Group A: Cytology+/HPV+) in the secondary screening showed CIN2–3 lesions in a consecutive cervical biopsy. Eight of 41 women (20%) with normal cytology and a positive high-risk HPV test (Group B: Cytology −/HPV+) showed CIN2–3 lesions. The rest showed benign histopathology or CIN1 lesions (four cases). None of the nine women with abnormal cytology (ASCUS and CIN1) and a normal high-risk HPV test (Group C: Cytology+/HPV −) disclosed CIN2–3 lesions. Thus the high-risk HPV test improved the detection rate of CIN2–3 triage of ASCUS by 33% ($p = 0.01$) compared to repeat cytology. In Group D ($n = 89$), a repeated primary screening three years later revealed abnormal cytology and histological CIN2–3 lesions in two of 74 (2.7%) women examined (Table I). In Group C, all nine women showed normal cytology after a repeated examination three years later.

The discrimination ability of different kinds of abnormal cytology (ASCUS–CIN3) in combination with a positive HPV test was relatively small. In the high-risk HPV-positive women with ASCUS–CIN1, cytological abnormalities 40% showed CIN2–3 lesions in the cervical biopsies and in women with the cytological diagnosis CIN2–3, 50% exhibited

Table I. Results of the secondary screening process that included a combination of cytology and a high-risk HPV testing for detection of CIN2–3 lesions in women with a primary diagnosis of ASCUS.

Groups	Number of cases	Number of cases with CIN2–3	Percentage (%)
Group A, Cytology+HPV+	58	24	41
Group B, Cytology–HPV+	41	8	20
Group C, Cytology+HPV–	9	0	0
Group D, Cytology −HPV −	89	2[a]	<3
Total	197	34	17

[a]Of the 89 women, 74 could be re-examined in the organized screening three years later and two were found to possess CIN2–3 lesions.

histological CIN2–3 lesions. The difference between the ASCUS–CIN1/HPV+ category and the CIN2–3/HPV+ category was not significant ($p = 0.78$). However, repeated biopsies three months later revealed CIN2–3 lesions in two of the three women with previous normal histology and after that agreement between cytology and histology was obtained in five of six cases.

A pronounced discrepancy was recorded in the association between ASCUS and HPV in relation to age. Of the women below 30 years of age with an ASCUS 73% were HPV positive, whereas only 19% of the women ≥50 years of age with an ASCUS were HPV positive (Table II).

The sensitivity in identifying CIN2–3 lesions was 97% for the HPV test and 73% for the cytological examination, provided that all abnormal cells (ASCUS–CIN3) were included in the analyses. The specificity for the HPV test was 59 and 72% for the cytological analysis. However, specificity of the HPV test was found to vary and increase with age: 83% in women over 50 years of age and 34% in women less than 30 years (Tables II and III).

The relation between high-risk HPV tests and the presence of morphological CIN2–3 lesions with regard to age was also studied. The prevalence of CIN2–3 was 32% in all of the HPV-positive women. However, it varied with age, being highest (14/27, 52%) in the group of women between 30 and 39 years (Table III).

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

Age category (years)	Number of cases	Number of HPV positive
<30	59	43 (73%)
30–39	42	27 (64%)
40–49	54	21 (39%)
≥50	42	8 (19%)
Total	197	99 (50%)

Discussion

Organized cytological screening in Sweden resulted in a marked increase of the number of CIN2–3 diagnoses, an increase in frequency of treatment with cone resections, and accordingly, a pronounced decrease in the proportion of women with cervical cancer. The decrease was around 60% and at present 430 cervical cancer cases are diagnosed each year in Sweden (1). Since its introduction, the organized screening program 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. Around 20% of women between 23 and 60 years have not been tested in the screening program, and 65% of all the cervical cancer cases and more than 80% of all advanced cases occur in these non-participating women (5).

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). In Sweden, 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 repeat 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 CIN2–3 lesions (9–15). In our investigation there was an increase of 33% in the identification of women with CIN2–3 lesions, an increase that was highly significant.

Table III. Relationship between an HPV-positive reaction and the presence of morphological CIN2–3 lesions in relation to age.

Age category (years)	Number of cases	Number of HPV positive	Number of CIN2–3	CIN2–3/HPVpositive	Percentage (%)
<30	59	43	12	12/43	28
30–39	42	27	14	14/27	52
40–49	54	21	5	5/21	24
≥50	42	8	1	1/8	13
Total	197	99	32	32/99	32

Our results indicate that the value of HPV testing in the secondary screening program is most evident in the age group 30–39 years, where 52% of the women had CIN2–3 lesions. However, it should be noted that the inclusion criterion was an initial ASCUS diagnosis and for that reason only the benefit of adding HPV analysis to the sensitivity of cytology could be calculated. The study also showed that in women with abnormal cytology the use of a sub-classification scheme of the abnormal cell (from ASCUS to CIN3) had relatively little effect (from 40 to 50%), a difference that was not significant.

The mean age of women with abnormal cytology and a positive test for high-risk HPV was below the mean age of the study population, indicating 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 development (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 and to only those women are referred to colposcopy who have positive tests in the secondary screening. Cytology added little to the triage and could probably be excluded (12,21).

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