HPV mRNA testing in triage of women with ASC-US cytology may reduce the time for CIN2+ diagnosis compared with repeat cytology

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Abstract

Background: In delayed HPV triage women with atypical squamous cells of uncertain significance (ASC-US) cytology are retested after 6-12 months in order to decide whether they should be referred for colposcopy, further follow-up cytology or routine screening in three years. Triage using a specific HPV E6/E7 mRNA test may reduce referrals for colposcopy of women with ASC-US cytology compared to HPV DNA testing. We explored whether HPV mRNA triaging could reduce the time from ASC-US index cytology to biopsy compared with repeat cytology, and whether the positive predictive value (PPV) of the HPV mRNA test for high grade cervical intraepithelial neoplasia (CIN2+) was comparable with the PPV of repeat cytology.

Material and methods: We used repeat cytology and the HPV mRNA test PreTect HPV-Proofer, which detects E6/E7 mRNA from HPV subtypes 16, 18, 31, 33 and 45, in the triage of women with ASC-US. We included all women from the two northernmost counties of Norway with a first ASC-US cytology during the period 2004-2008. Two triage methods were evaluated 1) only repeat cytology (n=964) and 2) both HPV mRNA testing and cytology (n=542). Histologically confirmed CIN2+ was the study endpoint.

Results: Among 1506 women with an ASC-US index cytology, 59 women (3.9%) had biopsy taken, of whom 49 women had CIN2+ (PPV 83.1%). The mean time from index ASC-US cytology until the case was resolved (biopsy or return to screening) was 10.6 months in the repeat cytology group and 7.3 months in the HPV group (P<0.001). Of the 964 women in the group with repeat cytology only, 35 women (3.6%) had biopsy and 30 had CIN2+ (PPV 85.7%). Of the 542 women in the group with both HPV test and cytology, 24 women (4.4%) had biopsy and 19 had CIN2+ (PPV 79.2%).

Conclusion: In triage of women with ASC-US, the HPV mRNA test significantly reduced the time from the first abnormal cytology until biopsy and had predictive values comparable with those of repeat cytology.

Introduction

Cervical cancer is a rare outcome of a common, sexually transmitted infection whose etiologic association is restricted to some human papillomavirus (HPV) types [1]. In many developed countries cervical cancer screening programs are still based on cytology with repeat cytology or HPV-testing in triage. The aim of cervical screening is to detect and thereby initiate treatment for high-grade cervical intraepithelial neoplasia (CIN2+). Screening and subsequent conization can reduce the likelihood that CIN2+ will develop into invasive cancer. If cytological high-grade cervical lesions (HSIL) are detected, additional assessment is performed through colposcopy and biopsy [2,3]. In Norway, women with ASC-US or LSIL (minor cervical lesions) are retested after 6-12 months in order to decide whether they should be referred for colposcopy, further follow-up cytology or routine screening in three years. Women with cytological ASC-US and LSIL have a small, but significantly increased risk of developing cervical cancer compared to women with normal smears [4]. The purpose of triage of minor cervical lesions is to differentiate between women with high versus low risk of CIN2+. In women with ASC-US and LSIL, triage with an HPV DNA test is more sensitive for CIN2+, but less specific than triage with repeat cytology [5,6].

Since 2005, HPV testing has been included in the Norwegian national guidelines in triage of women with minor cervical lesions. If the diagnosis of the index cytology is ASC-US or LSIL, women are referred for repeat cytology and HPV testing in 6-12 months (delayed triage). A positive HPV test combined with ASC-US or LSIL cytology will result in a referral for colposcopy and biopsy. A positive HPV result combined with normal Pap-smear at triage requires further follow-up with a new HPV-test after 12 months, and if the HPV infection persists, the woman is referred for colposcopy. Women with HSIL cytology at triage have a high risk of CIN2+ and are referred for colposcopy, independent of the HPV result. Figure 1 provides a flowchart for follow-up of women with ASC-US and LSIL.

In this study we explored whether HPV mRNA triage could reduce the delay from ASC-US index cytology to biopsy compared with repeat cytology triage and whether the positive predictive value (PPV) of the HPV mRNA test for high grade cervical intraepithelial neoplasia (CIN2+) was comparable with that of repeat cytology.

Material and methods

The national cervical screening program recommends that women aged 25-69 have a Pap-

smear every three years. Each year, our department receives and analyzes 23 000 cervical smears from the population of Troms and Finnmark counties, representing 69 000 women. Of the 59 041 smears from women aged 15-69 that we processed during the period 2004-2008, 2 819 (4.8%) had an ASC-US diagnosis.

Women with a previous abnormal histology and/or a previous abnormal smear (n=1 283) were excluded because they are at higher risk of having CIN2+ compared to women with a first ASC-US result. Triage is performed in order to decide whether they should undergo further follow-up or be referred for screening at three-year intervals.

Two triage methods were evaluated 1) repeat cytology only ("repeat cytology group") and 2) HPV mRNA testing in addition to repeat cytology ("HPV group"). HPV-testing can only be analyzed in liquid based cytology (LBC). In total, 1 506 of 2 819 (53.4%) women with ASC-US index cytology were included in the study. Among these 542 women (36.0%) had follow-up with LBC, making it possible to do HPV mRNA testing. This was not the case for 964 women (64.0%) who had Pap-smear only (Figure 2 and Table 1). Our national guidelines do not recommend HPV-testing in triage of women younger than 25 years. Hence, only a few women in the age group 15-24 years were triaged using the HPV-test.

Routine diagnostic practice at our hospital is to use the HPV E6/E7 mRNA test PreTect HPV-Proofer (detecting E6/E7 mRNA from the HPV types 16, 18, 31, 33 and 45; NorChip AS, Norway) for triage of women with cytological finding ASC-US or LSIL. We extracted cervical cells from LSIL by the ThinPrep® 2000 (Cytyc Corporation, Marlborough, MA, USA) for cytological examination. DNA/RNA was isolated from 5 ml of the leftover material and analyzed with PreTect HPV-Proofer. The mRNA testing was performed according to the recommendations of the national guidelines (Figure 1) and the manufacturer's instructions.

We used our diagnostic database (SymPathy) to gather the cytological and histological diagnoses. Biopsies were evaluated by at least two independent pathologists and we used the the CIN terminology in the histological report [7]. According to national guidelines CIN2 is the threshold for treatment by conization. In contrast, negative histology (normal or CIN1) results in a recommendation of a new Pap-smear and another HPV test within 6-12 months. In our routine diagnostic, biopsies with uncertain diagnosis were analyzed with p16(INK4a) immune-staining (CINtec® Histology, MTM, Heidelberg, Germany) in order to detect occult CIN lesions.

All statistical analyses used SPSS version 18 to perform Chi-square tests, Mann-Whitney tests and survival analysis. A p-value < 0.05 was considered statistically significant.

Results

In the study group 49.5% (745/1506) were 25-44 years and 84.9% (1279/1506) were 25-70 years (Table 1). Of the 964 women in the repeat cytology only group, 79 women (8.2%) were referred for colposcopy, 802 women (83.2%) received a recommendation of routine screening in three years and 83 women (8.6%) were unresolved 35 months after ASC-US index cytology. Of the 542 women in the HPV group, 46 women (8.5%), were referred for colposcopy and biopsy, 496 women (91.5%) received a recommendation of routine screening in three years (returned to screening) and one woman (0.5%) was unresolved at 35 months follow-up ($P<0.001$) (Table 2).

Most patients in the HPV group were resolved within 12 months (Figure 3). While 36.8% (355/964) of the women in the repeat cytology only group were unresolved after 12 months, only 8.1% (44/542) in the HPV-group were unresolved after 12 months ($P<0.001$). The mean time from ASC-US index cytology until resolved was 10.6 months in the repeat cytology only group and 7.3 months in the HPV-group ($P<0.001$).

While 8.3% (125/1506) of all women were referred for colposcopy/biopsy according to the screening algorithm, we received biopsies from only 47.2% (59/125) of the referred women. In the repeat cytology only group (N=964) 35 women (3.6%) had biopsy of whom 30 women had CIN2+ (PPV 85.7%). In the HPV group (N=542) 24 women (4.4%) had biopsy of whom 19 women had CIN2+ (PPV 79.2%) (Table 3).

Discussion

The present study demonstrates that HPV mRNA testing in triage of women with ASC-US cytology significantly reduced the time from index cytology to diagnosis of and treatment for CIN2+ and had the same PPV when compared to repeat cytology triage. The mean time to resolved status was 10.6 months in the repeat cytology only group and 7.3 months in the HPV-group. While 36.8% of the women in the repeat cytology only group were unresolved after 12 months, only 8.1% in the HPV-group were unresolved after 12 months.

In Norway, the prevalence of LSIL or recurrent ASC-US is approximately 4% in the screening population. In our population 4.8% (2819/59041) had ASC-US, and 2.6% (1506/59041) had the index ASC-US cytology as the first abnormal smear. Generally an underlying high-grade histological lesion (CIN2+) requiring treatment is found in 8% of these patients [4,8,9]. In our material only 3.9% of the women with ASC-US index cytology had CIN2+. The prevalence of CIN2+ was higher in patients with recurrent minor cervical lesions than in patients who are diagnosed from their first ASC-US cytology.

Although 8.3% of women were referred for colposcopy, we received biopsies from only 47.2% of these, which is lower than we observed in a previous study (85.5%) [10]. Although omitting random biopsies for women with a negative colposcopy is considered acceptable in clinical practice [11], the updated Norwegian guidelines recommend biopsies even if colposcopy is normal.

Colposcopies are costly procedures that can also cause psychological stress [12]. Histopathologic diagnoses of cervical biopsies are prone to poor inter-observer reproducibility [13,14] and a high referral rate for colposcopy and a high biopsy rate will inevitably result in some degree of over treatment. Furthermore, conization increases the risk for premature birth and late abortions in subsequent pregnancies [15–17].

As only a small proportion of women with ASCUS or LSIL will harbor high-grade disease, a test of high specificity would be desirable in order to avoid colposcopy referrals for large numbers of women. A low positivity rate of the HPV mRNA test can be translated into a low referral rate for colposcopy, which is very appealing for triage situations. Prior to introduction of HPV testing in clinical practice, all patients with persistent ASC-US/LSIL were referred for follow-up after one year. Today, women with repeated ASC-US/LSIL and a negative HPV mRNA result are also referred for follow-up cytology. In contrast, women with a positive HPV result, are referred for colposcopy [10]. Thus, a triage test with high PPV will

reduce the number of unnecessary follow-ups. A reduced rate of referral to colposcopy will reduce the costs to the health care system, reduce overtreatment, reduce the negative impact of cervical treatment on pregnancy outcomes and reduce psychological stress for the women.

In our material the referral rate for colposcopy was lower in the HPV group than in the repeat cytology group (8.5% and 8.2%, respectively). In the ASCUS/LSIL triage study the referral rate was 53% in the HPV group (Hybrid Capture II) and 8% in the repeat cytology group [4,8,9].

Several publications have demonstrated the value of HPV mRNA testing in the triage of women with minor cytological lesion [4,10,18,19], a result that reflects the high positive predictive value (PPV) and the high specificity of the HPV mRNA test. In fact, women with a positive HPV mRNA test and a concurrent cytological HSIL diagnosis could be treated directly without the need for confirmation by biopsy prior to conization. This is also the case for women over age 40 if the HPV result is positive, regardless of the cytology result [10].

In conclusion, HPV mRNA testing in triage of women with ASC-US cytology significantly reduced the time from index cytology to diagnosis and treatment of CIN2+ while having the same PPV as repeat cytology triage, contributing to a more efficient follow-up algorithm.

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Figure 1.

Flow chart showing the guidelines for follow-up of minor cytological lesions.

Figure 2.

Flow chart showing the two study populations.

Figure 3.

Survival curves of time (months) from index ASC-US cytology until resolved.

Table 1. Study population by age and triage method

Age, years	Repeat cytology only[1] N (%)	HPV and repeat cytology[2] N (%)	Total N (%)
15-24	211 (21.9)	16 (3.0)	227 (15.1)
25-34	229 (23.8)	149 (27.5)	378 (25.1)
35-44	199 (20.6)	168 (31.0)	367 (24.4)
44-70	325 (33.7)	209 (38.6)	534 (35.5)
Total	964 (100.0)	542 (100.0)	1 506 (100.0)

[1]2004-2008

[2]2006-2008

Table 2. Outcome of triage by triage method

Recommended action	Repeat cytology only N (%)	HPV and repeat cytology N (%)	Total N (%)
Non-resolved	83 (8.6)	0 (0.0)	83 (5.5)
Colposcopy/biopsy for high grade index cytology	46 (4.8)	27 (5.0)	73 (4.8)
Routine screening in three years	802 (83.2)	496 (91.5)	1 298 (86.2)
Colposcopy/biopsy after triage	33 (3.4)	19 (3.5)	52 (3.5)
Total	964 (100.0)	542 (100.0)	1 506 (100.0)

Chi square = 49.5, P<0.001

Table 3. Histology from biopsies by triage method

Histology	Repeat cytology only[1] N (%)	HPV and repeat cytology[2] N (%)	Total[3] N (%)
Normal	2 (5.7)	2 (8.3)	4 (6.8)
CIN 1	3 (8.6)	3 (12.5)	6 (10.2)
CIN 2	15 (42.9)	9 (37.5)	24 (40.7)
CIN 3	14 (40.0)	10 (41.7)	24 (40.7)
ACIS	1 (2.9)	0 (0.0)	1 (1.7)
Total[4]	35 (100.0)	24 (100.0)	59 (100.0)

1) PPV CIN2+ 85.7% (30/35)

2) PPV CIN2+ 79.2% (19/24)

3) PPV CIN2+ 83.1% (49/59)