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ORIGINAL ARTICLE

A comparison of the human papillomavirus test and Papanicolaou smear as a second screening method for women with minor cytological abnormalities

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Background. Of the estimated one million Papanicolaou (pap) smears performed annually in Sweden, about 4% show any degree of abnormality. Approximately, 1% of these cases contain moderate or severe atypia (high-grade squamous intraepithelial lesions) and the rest contain low-grade atypia. Recommendations for the management of minor abnormalities vary in various parts of Sweden. Generally, a second Pap smear is obtained 4–6 months after the first one showing low-grade atypia. The aim of this study is to compare the sensitivity of human papilloma virus (HPV)-DNA testing for the detection of cervical intraepithelial neoplasia (CIN) 2–3 with that of a second Pap smear in women, who had low-grade atypia in their first Pap smear.
Methods. Women with low-grade atypia in the Stockholm area, detected at a population-based cytology screening, were enrolled. A repeat Pap smear, HPV test, and colposcopically directed biopsies were obtained. For the detection of HPV, Hybrid Capture II (HC II) was used.
Results. The HPV-DNA test was positive in 66% of the 177 participating women. The sensitivity of the second Pap smear and HPV-DNA test to detect CIN 2–3 was 61 (95% CI = 45–74) and 82% (95% CI = 67–91), respectively. The positive and negative predictive values of HPV testing were 27 (95% CI = 18–35) and 89% (95% CI = 80–97), respectively.
Conclusions. In Sweden, a second Pap smear is often obtained for the follow-up of women with low-grade atypia. The results of our study show that compared to the second Pap smear, HPV testing with HC II is a more sensitive method for detecting high-grade lesions.

Key words: CIN; HPV; Pap smear; screening

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Since the early sixties (1960s), population-based vaginal cytological screening has been available in most Swedish counties, resulting in a reduction of the incidence of invasive cervical cancer (1,2). Women between 23 and 50 years of age are requested to have a Papanicolaou (Pap) smear every third year and those between 50 and 60 years, every fifth year. Of the estimated one million

Abbreviations:
µl: micro litre; ASCUS: atypical squamous cells of undetermined significance; CIN: cervical intraepithelial neoplasia; DNA: deoxyribonucleic acid; HC II: Hybrid Capture II; HPV: human papillomavirus; HSIL: high-grade squamous intraepithelial lesions; LSIL: low-grade squamous intraepithelial lesions; *P*: probability value; Pap smear: Papanicolaou smear.

Pap smears performed annually in Sweden, about 4% show signs of atypia. Less than 1% of these cases represent high-grade squamous intraepithelial lesions (HSIL) and the rest, low-grade atypia. Only 10–40% of lesions with low-grade atypia may persist or progress to precancerous lesions (3–6).

Clinical recommendations for the management of minor abnormalities vary (7–9).

In most parts of Sweden, a second Pap smear is indicated when the screening test reveals low-grade atypia. However, in the county of Stockholm, all women with any degree of atypia are referred for a colposcopy confirmation. Women with low-grade atypia form a large proportion of those further examined, thereby resulting in significant psychological stress, overtreatment, and increased health care costs (10,11).

Genital papillomavirus infections (HPV) are prevalent, but usually transient among women younger than 40 years (12). The importance of persistent HPV for the progression of cervical neoplasia has been documented: HPV DNA has been found in more than 98% of clinically analyzed biopsies of cervical cancer (13–15).

The aim of this study is to compare the outcome of HPV-DNA testing with that of the Pap smear as second screening method among women with low-grade atypia.

Patients and methods

Women with low-grade atypia, detected at a population-based screening, were examined with colposcopy after 4–6 months at the gynecologic departments of three university hospitals of Stockholm. We consecutively enrolled 177 of these women with minor abnormalities.

Each woman underwent a Pap smear and an HPV test. A Zeiss OMPI colposcope was used for magnification. The ectocervix and distal part of the endocervix were stained with 5% acetic acid. Punch biopsies were obtained from acetowhite areas. Acetic acid causes neoplastic epithelium to turn white and even has the same effect on undifferentiated (metaplasia) and inflammatory epithelium, although less distinct. If no acetowhite area was observed, a biopsy was taken close to the squamocolumnar junction, at 12 o'clock (noon). The samples were evaluated by the local pathologist and were classified according to the cervical intraepithelial neoplasia (CIN) classification (World Health Organization). The most severe grade of atypia found in each biopsy determined the diagnosis, with which the results of the HPV test and Pap smear were compared.

A sample of cells from the ectocervix and endocervix was obtained by means of the cervical brush and was spread on a slide for Papanicolaou staining, after which the brush was inserted into a transport medium provided by the manufacturer of Hybrid Capture II (HC II) (Digene Corpopation). The specimens were subsequently tested at the Department of Virology, Karolinska University Hospital, Stockholm, Sweden, with an HC II assay, performed according to the manufacturer's protocol. DNA from cervical material was denatured and hybridized with a cocktail of 13 RNA probes to oncogenic HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. Hybrids were captured with alkaline-phosphatase-conjugated antibodies specific to HPV-DNA-RNA hybrids. A dioxetane-based chemiluminescent substrate was added, and the resultant relative light units (RLUs) were measured in a luminometer. Specimens with RLUs equal to or above the mean RLUs of triplicate positive control specimens containing 1 pg of HPV DNA/μl (about 5000 copies of the HPV genome) were designated as positive (16,17).

Statistical analysis

χ^2 statistics were calculated in order to test the significance of the data with evident ordering. All statistical tests were two-sided and were considered statistically significant at $P < 0.05$. Logistic regression was used in order to assess the significance of the differences in paired data, such as the comparison of the sensitivities of cytology and HPV testing in the same patients.

The study was approved by the Local Ethics Committee of the Karolinska University Hospital, Stockholm, Sweden.

Results

In total, 177 women with cytological signs of low-grade atypia were enrolled. Of these, 52 had atypical squamous cells of undetermined significance and 125 had low-grade squamous intraepithelial lesions (LSIL). The mean age was 34 years (median: 31; range: 23–60).

The results of the repeat Pap smears, histology of colposcopically directed biopsies, and HPV-DNA testing have been summarized in Tables 1 and 2.

According to histopathology, 76 (43%) of 177 women had various degrees of CIN (Table 1). Of the 93 women with a normal second Pap smear, 15 (16%) had CIN 2–3. Of the 84 women with an atypical second Pap smear, 23 (28%) had CIN 2–3 (Table 2).

Totally, 116 (66%) women were HPV-positive. Of these 31 (27%) had CIN 2–3 (Table 1).

Of the 48 HPV-positive women with a normal second Pap smear, 11 (23%) had CIN 2–3; of the 68 HPV-positive women with signs of atypia in the second Pap smear, 20 (29%) showed CIN 2–3 according to histopathology (Table 2).

The Pap smear had a sensitivity and specificity for the detection of CIN 2–3 of 61 (95% CI = 45–74) and 34% (95% CI = 21–50), respectively, compared to 82 (95% CI = 67–91) and 39% (95% CI = 31–47) for HPV-DNA testing.

The positive and the negative predictive values of the HPV test were 27 (95% CI = 18–35) and 89% (95% CI = 80–97), respectively.

Among women of 30 years or younger, 33 (42%) without any signs of CIN were HPV-positive, compared to 23 (23%) among women older than 30 years.

Table. 1. Results of the histopathological analysis of biopsies and HPV in women with cytological atypia interpreted as LSIL and ASCUS. ASCUS, atypical squamous cells of undetermined significance; HPV, human papilloma virus; LSIL, low-grade squamous intraepithelial lesions.

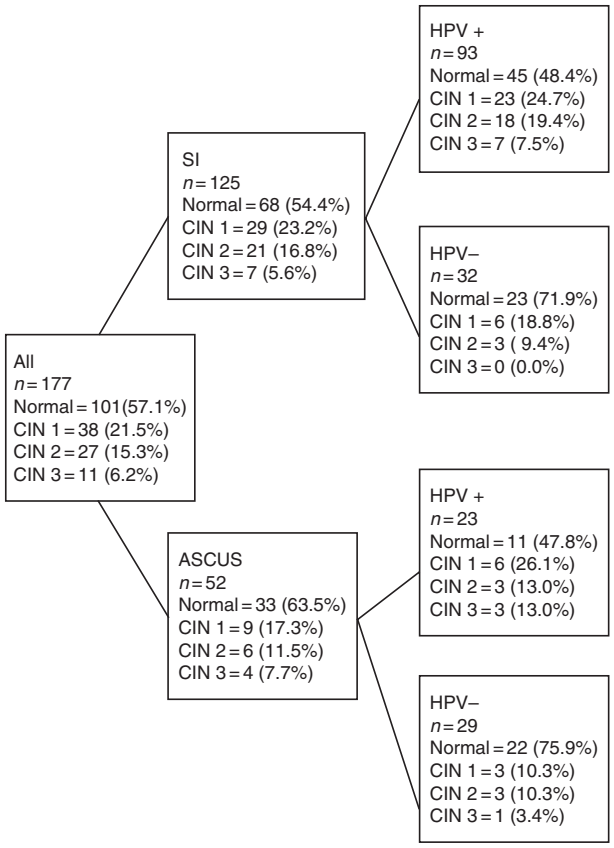
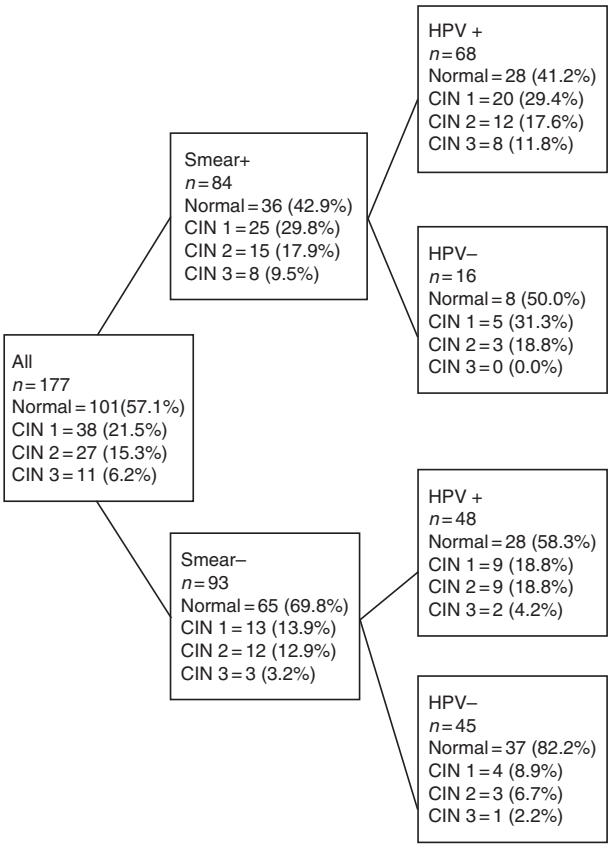


Table. 2. Results of the histopathological analysis of biopsies and HPV in Pap smear-negative and Pap smear-positive women with primary diagnostic cytological atypia interpreted as LSIL and ASCUS. ASCUS, atypical squamous cells of undetermined significance; HPV, human papilloma virus; LSIL, low-grade squamous intraepithelial lesions.



Discussion

The management of women with low-grade atypia differs between the regions of Sweden, partly because of the variation in available medical resources.

In several studies, the Pap smear has been shown to have a high false-negative rate (18). A review of evidence-based data revealed that as many as 50% of precancerous cervical lesions may be missed by a single Pap test (19). Another drawback of cervical cytology is its high false-positive rate (19), which is primarily restricted to the diagnosis of minor abnormalities, and may be attributed to the difficulty in distinguishing between inflammatory reactive atypia and LSIL. According to Manos et al., such smears account for 60–80% of all abnormal cytological findings (5).

Almost 60% of our study population of women with low-grade atypia had normal cervical epithelium according to the histology of target biopsies, whereas 21.5% of the women had CIN 2–3. In another study, it was shown that only 30% of women with histologically confirmed high-grade disease had a corresponding atypia in their Pap smears (20). Although most lesions with minor abnormalities regress spontaneously, the risk of progression to invasive cervical cancer is still 10- or 20-fold higher than that of the general female population (21). According to earlier studies, repeated cytologic examination had a maximum sensitivity of 75% (5). However, we observed that the sensitivity of the repeat Pap smear to detect high-grade lesions was only 61% and hence there is a need for an objective and reproducible test, such as HPV-DNA test, in order to identify the women at risk of high-grade lesions.

In our study, among women with low-grade atypia, the sensitivity of HPV testing to detect high-grade lesions was 82%, whereas in a study by Manos et al., it was 90% (5). In the ALTS study, involving more than 3000 women, the sensitivity of HC II for the detection of high-grade lesions was 95.5% (7). The negative predictive value of the HC II test has been found to be more than 90% (5).

Among our cases with negative HPV tests, 11% had high-grade lesions. It has been proposed that such lesions generally undergo spontaneous healing and are thus at a negligible risk of progression to invasive cancer (14,22).

The presence of high-risk HPV DNA identifies both women with disease and those who are at a particular risk of progression to disease (23). Studies have shown that 15–28% of HPV-DNA-positive women with normal cytology develop CIN within 2 years, compared to 1–3% of HPV-DNA-negative women (24).

HPV testing is thus indicated for women with Pap smears showing borderline abnormalities. If the test is positive, the women should be referred for colposcopy and target biopsies, but if negative, it should be repeated 1 year later. If the HPV test is still negative, the women may return to the population-based screening with a 3-year interval, thereby reducing psychological stress and significantly cutting the mean cost. Sampling for HPV-DNA testing may be undertaken by midwives or even by the women themselves (25).

HPV infections are prevalent and often transient among younger women, with a peak of 20–25% at 20–24 years of age. With increasing age, there is a decline in the prevalence of HPV of about 7% at 35 years of age (26). It is likely that HPV-positive women at that age represent a subset of patients that do not manage to clear their infections spontaneously. The persistence of a high-risk HPV infection is associated with the risk of developing CIN. As expected, in our study population, women aged 30 years or younger were more frequently HPV-positive, but had no signs of CIN, compared to the older women.

In conclusion, our study demonstrates that 39% of the high-grade cervical lesions would not have been detected with a Pap smear as the sole second screening test. Instead, if an HPV test is used, 18% of these lesions will be missed. If these two methods are combined, only 11% of CIN 2–3 cases would have been missed.

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References

1. Mählck CG, Jonsson H, Lenner P. Pap smear screening and changes in cervical cancer mortality in Sweden. Int J Gynaecol Obstet 1994; 44: 267–72.
2. Ponten J, Adami HO, Friberg LG, Gustafsson L, Miller AB, Parkin M et al. HPV and cervical cancer. Int J Cancer 1995; 63: 317.
3. Ferris DG, Wright TC Jr, Litaker MS, Richart RM, Lorincz AT, Sun XW et al. Triage of women with ASCUS and LSIL on Pap smear reports: management by repeat Pap smear, HPV DNA testing, or colposcopy? J Fam Pract 1998; 46: 125–34.
4. Cox JT, Lorincz AT, Schiffman MH, Sherman ME, Cullen A, Kurman RJ. Human papillomavirus testing by hybrid capture appears to be useful in triaging women with a cytologic diagnosis of atypical squamous cells of undetermined significance. Am J Obstet Gynecol 1995; 172: 946–54.
5. Manos MM, Kinney WK, Hurley LB, Sherman ME, Shieh-Ngai J, Kurman RJ et al. Identifying women with cervical neoplasia. Using human papillomavirus DNA testing for equivocal Papanicolaou results. JAMA 1999; 218: 1605–10.
6. Flannelly G, Anderson D, Kitchener HC, Mann EM, Campbell M, Fisher P et al. Management of women with mild and moderate cervical dyskaryosis. BMJ 1994; 308: 1399–403.
7. Solomon D, Schiffman M, Tarone R. ASCUS LSIL Triage Study (ALTS) conclusions reaffirmed: response to a November 2001 commentary. Obstet Gynecol 2002; 99: 671–4.
8. Kurman RJ, Henson DE, Herbst AL, Noller KL, Schiffman M. Interim guidelines for management of abnormal cervical cytology. The 1992 National Cancer Institute Workshop. JAMA 1994; 271: 1866–9.
9. Austoker J. Cancer prevention in primary care. Screening for cervical cancer. BMJ 1994; 309: 241–8.
10. Freeman-Wang T, Walker P, Linehan J, Coffey C, Glasser B, Sherr L. Anxiety levels in women attending colposcopy clinics for treatment for cervical intraepithelial neoplasia: a randomised trial of written and video information. BJOG 2001; 108: 482–4.
11. Idestrom M, Milsom I, Andersson-Ellstrom A. Women's experience of coping with a positive Pap smear: a register-based study of women with two consecutive Pap smears reported as CIN 1. Acta Obstet Gynecol Scand 2003; 82: 756–61.
12. Elfgren K, Kalantari M, Moberger B, Hagmar B, Dillner J. A population-based five-year follow-up study of cervical human papillomavirus infection. Am J Obstet Gynecol 2000; 183: 561–7.
13. Schiffman M, Brinton L. The epidemiology of cervical carcinogenesis. Cancer Epidemiol Biomarkers Prev 1995; 76: 1888–901.
14. Nobbenhuis MA, Walboomers JM, Helmerhorst TJ, Rozendaal L, Remmink AJ, Risse EK et al. Relation of human papillomavirus status to cervical lesions and consequences for cervical-cancer screening: a prospective study. Lancet 1999; 354: 20–5.
15. Schiffman MH, Castle P. Epidemiologic studies of a necessary causal risk factor: human papillomavirus infection and cervical neoplasia. J Natl Cancer Inst 2003; 95: E2.
16. Peyton CL, Schiffman M, Lorincz AT, Hunt WC, Mielzynska I, Bratti C et al. Comparison of PCR- and hybrid capture-based human papillomavirus detection systems using multiple cervical specimen collection strategies. J Clin Microbiol 1998; 36: 3248–54.
17. Schiffman M, Harrero R, Hildesheim A, Sherman ME, Bratti M, Wacholder S et al. HPV DNA testing in cervical cancer screening: results from women in high-risk province of Costa Rica. JAMA 2000; 283: 87–93.
18. Macgregor JE, Campbell MK, Mann EM, Swanson KY. Screening for cervical intraepithelial neoplasia in north east Scotland shows fall in incidence and mortality from invasive cancer with concomitant rise in preinvasive disease. BMJ 1994; 308: 1407–11.
19. Sherman ME, Shiffman MH, Lorincz AT. Toward objective quality assurance in cervical cytopathology: correlation of cytopathologic diagnosis with detection of high-risk human papillomavirus types. Am J Clin Pathol 1994; 102: 182–7.
20. Kinney WK, Manos MM, Hurley LB, Ransley JE. Where's the high-grade cervical neoplasia? The importance of minimally

abnormal Papanicolaou diagnoses. Obstet Gynecol 1998; 91: 973–6.
21. Soutter WP, Fletcher A. Invasive cancer of the cervix in women with mild dyskaryosis followed up cytologically. BMJ 1994; 308: 1421–3.
22. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999; 189: 9–12.
23. Schiffman M, Bauer HM, Hoover RN, Glass AG, Cadell DM, Rush BB et al. Epidemiologic evidence showing that human papillomavirus infection causes most cervical intraepithelial neoplasia. J Natl Cancer Inst 1993; 85: 958–64.
24. Davidson M, Schnitzer PG, Bulkow LR, Parkinson AJ, Schloss ML, Fitzgerald MA et al. The prevalence of cervical infection with human papillomaviruses and cervical dysplasia in Alaska Native women. J Infect Dis 1994; 169: 792–800.
25. Sellors JW, Lorincz AT, Mahony JB, Mielzynska I, Lytwyn A, Roth P et al. Comparison of self-collected vaginal, vulvar and urine samples with physician-collected cervical samples for human papillomavirus testing to detect high-grade squamous intraepithelial lesions. CMAJ 2000; 163: 513–8.
26. Forslund O, Antonsson A, Edlund K, van den Brule AJ, Hansson BG, Meijer CJ et al. Population-based type-specific prevalence of high-risk human papillomavirus infection in middle-aged Swedish women. J Med Virol 2002; 66: 535–41.

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