Triaging equivocal cytology of the cervix : identyfying women at risk for high-grade cervical lesions

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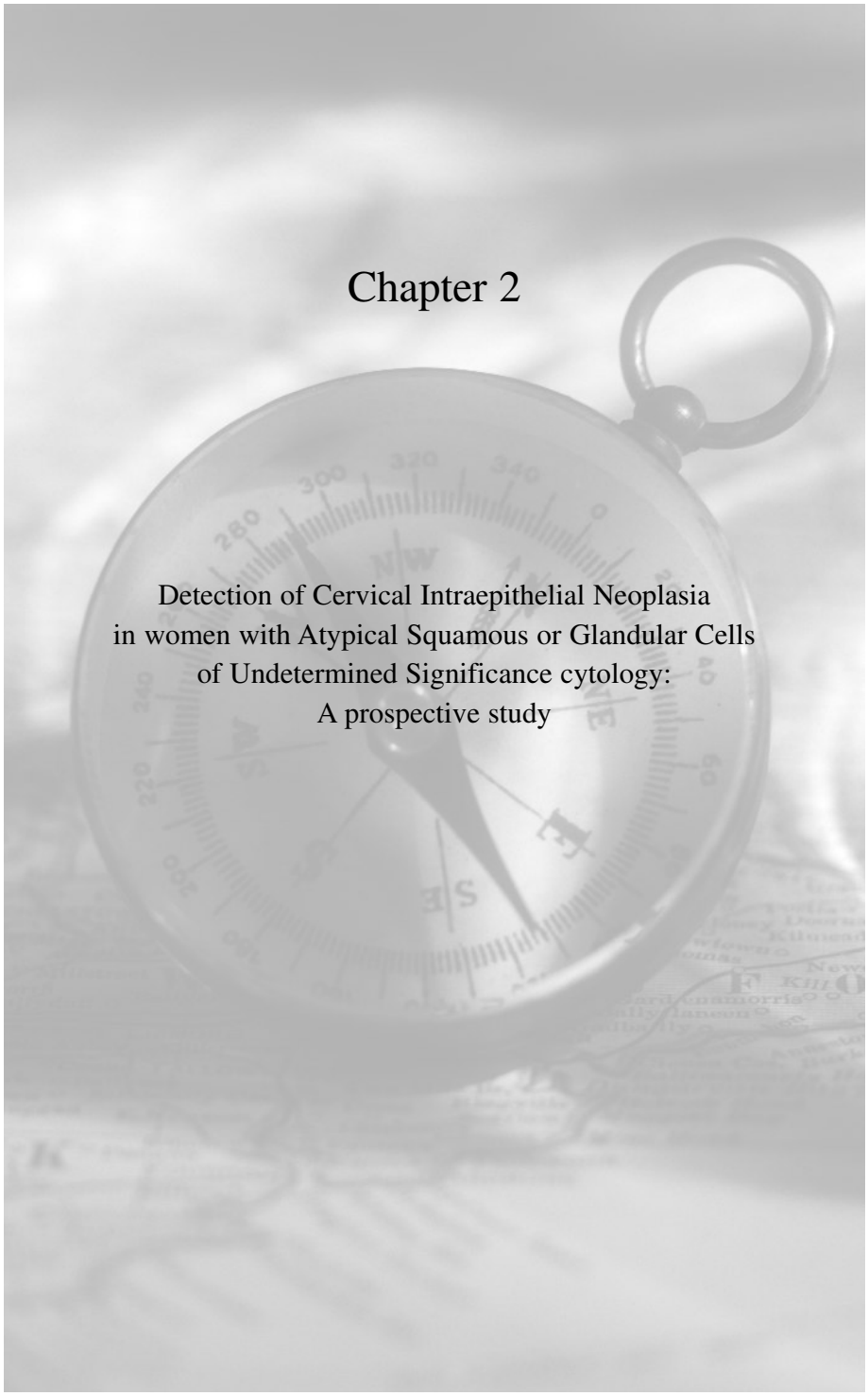

Chapter 2

Detection of Cervical Intraepithelial Neoplasia in women with Atypical Squamous or Glandular Cells of Undetermined Significance cytology: A prospective study

ABSTRACT

Background: (1) To asses the prevalence of histologically confirmed cervical intraepithelial neoplasia (CIN) in patients with cervical smears diagnosed as atypical squamous or glandular cells of undetermined significance (ASCUS/AGUS). (2) To evaluate the role of colposcopy and the presence of human papillomavirus (HPV) in detecting underlying CIN.
STUDY DESIGN: In this prospective cohort 148 women with ASCUS/AGUS were evaluated by colposcopy, histological sampling, and HPV DNA testing.
RESULTS: A histological diagnosis of ≥ CIN II was found in 10/148 women. Women with a histological ≥ CIN II had a higher prevalence of ≥ two abnormal quadrants (90% vs. 38% ≤ CIN I, p=0.002) and of high/ intermediate-risk HPV (90% vs. 42% ≤ CIN I, p=0.005).
CONCLUSION: Our study shows that premalignant lesions of the cervix are present in 7% of the patients with a cytological diagnosis of ASCUS/AGUS. Colposcopy and HPV DNA testing are both important parameters in detecting ≥ CIN II.

INTRODUCTION

In 1988, the Bethesda classification of cervical cytology was introduced and with it, two new categories of cytological diagnosis, namely atypical squamous and atypical glandular cells of undetermined significance (ASCUS/AGUS)[1]. This category corresponds to the Pap II smear as described in the current classification of cervical cytology used in the Netherlands[2]. Recently in the Netherlands stricter criteria of pap II were introduced in order to increase the correlation between cytology and histology[3-6]. These criteria include that borderline nuclear changes in relation to inflammatory epithelial changes or to atrophic cells are no longer classified as Pap II but as Pap I (normal). Pap II still represents mild nuclear changes or borderline nuclear abnormalities, comparable with ASCUS and AGUS of the Bethesda System[1,3]. This resulted in a decrease of frequency of PAP II from 11% in 1997 to 5% in 1999 in the Netherlands[7].
The introduction of the ASCUS/AGUS category has created a management dilemma for clinicians, as a number of studies have shown that 5 - 30% of women with this diagnosis harbour undetected cervical cancer precursors or even, cervical cancer[8-13]. Although the majority of women with ASCUS/AGUS diagnosis will have trivial lesions, some have significant lesions that warrant either closer surveillance or further investigation.
In this prospective study, all women with ASCUS/AGUS cytology underwent colposcopy, histological sampling and HPV DNA testing in order to determine the underlying prevalence of CIN in women with ASCUS/AGUS cytology and to evaluate

the role of colposcopy and HPV DNA testing in detecting underlying CIN or cervical cancer. In addition, women were tested for common sexually transmitted diseases to determine some of the risk factors predictive of underlying CIN in women with ASCUS/AGUS cytology. In this study we were interested in the meaning of ASCUS/AGUS cytology, regardless of the cytological or histological history, of the symptoms, and of social and sexual behaviour of these women.

METHODS

Patients

All patients diagnosed with ASCUS/AGUS (Pap II) on cervical smears were included from April 1997 to March 2000 at the gynaecological outpatients clinic of the Medical centre Haaglanden, The Hague, The Netherlands. Pregnant women and women with HIV were excluded. Pap smears were done because of symptoms or because of a previous ASCUS/AGUS/LSIL (= low squamous intraepithelial lesion) result or just because of wild screening (no indication). After informed consent was obtained all women underwent a gynaecological examination, which included a swab for the detection of sexual transmitted diseases (Chlamydia, Gonorrhoea and Mycoplasma) of the urogenital tract. In addition a questionnaire was administered which included questions concerning age of first sexual intercourse, number of sexual partners, a history of sexually transmitted diseases (STD's), current use of oral contraceptives, and smoking habits. Women in whom a STD was diagnosed, were treated and within twelve weeks following the smear a colposcopic examination was performed, which included biopsy and HPV test. During the study, patients were treated according to current protocols, irrespective of their HPV status. The medical ethical committee of the hospital approved this study.

During the study period 663 women were diagnosed with ASCUS/AGUS. Two hundred and ten women signed informed consent. The missing cytology samples resulted from the exclusion of women who were unable to sign informed consent because of language problems; 85% of the population is foreign. Moreover, some women did not want to participate because the study length was considered too long, and some women were not asked by their gynecologist to participate, especially at the beginning of the project. Despite the missing samples, we did not expect a selection bias because those who participated had similar characteristics to those missing. Of the 210 cases who signed informed consent 62 women did not participate or discontinued the study for various reasons: three women were found to be pregnant, one was HIV positive, in 11 cases there was a delay of more than 12 weeks between the Pap smear and intake, eight because of a normal or dysplastic smear after review, 37 because of not arriving for intake or for colposcopy despite several recalls, and two because of missing HPV test. We excluded one woman because of a carcinoma of the corpus uteri. The mean age on entering was 35 years (range 15-66 years). Thus 148 cases were included in this study.

Cytology

Cervical smears were collected using a Cervex-Brush (Rovers BV, Oss, the Netherlands). All cervical smears were Papanicolaou stained, screened routinely, and independently reviewed to conform eligibility to the study. The initial classifications were made according to the current Dutch cytological classification system (KOPAC-B)[14], a modification of the commonly accepted Papanicolaou procedure used in the Netherlands[2]. These were converted to the Bethesda system for this paper as described in table 1[1,3,4].

Of the 148 smears with Pap II or ASCUS/ AGUS cytology,126 smears showed only atypical squamous cells (ASCUS=P2/P3), two only atypical squamous metaplastic cells (atypical repair=A3), nine only atypical glandular cells (AGUS=C3/C4), one a combination of all three kinds of abnormalities (P3,A3,C3), one a combination of atypical squamous cells and atypical repair (P3.A3), nine a combination of atypical squamous and glandular cells (P2/3,C3/4).

From the 148 cases with ASCUS/AGUS cytology 110 were newly detected, 29 cases showed persistent ASCUS/AGUS cytology, and in nine cases the cytology history showed mild dysplasia.

Table 1. Comparison of the PAP II diagnosis, according to the Dutch KOPAC-B* classification and the Bethesda classification systems

CODE	Bethesda
P2/P3	Atypical squamous cells of undetermined significance (ASCUS)
A3	Atypical squamous metaplastic cells of undetermined significance (= Atypical Repair, ASCUS)
C3/C4	Atypical glandular cells of undetermined significance (AGUS)

* KOPAC-B10 is the Dutch abbreviation of composition of the smear (K), infectious organisms (O), squamous cell abnormality (P), other abnormality (A), endocervical abnormality (C), and adequacy (B).

Histology

The tissue specimens were fixed in buffered formalin 8%, pH = 7.42. After paraffin embedding, sections of 4 µm thickness were cut and processed routinely for haematoxyline eosine (HE) staining. Histological tissues were classified according to CIN classification (WHO). Biopsy specimens revealing koilocytotic atypia were included in the CIN I classification. A histological diagnosis of squamous metaplasia, immature squamous metaplasia, reactive changes, or inflammatory atypia were classified as no CIN. All specimens were reviewed by two of the authors (RV and CW).

Colposcopy

After the application of 3% acetic acid, colposcopy was performed in all patients. Documentation on a standard colposcopy form was made stating the location and appearance of the transformation zone. Colposcopic abnormalities such as acetowhite

changes, punctation, mosaic vascular pattern, and atypical vessels were noted. These characteristics were graded into the following colposcopic categories: no CIN, CIN I, CIN II, CIN III, or cervical cancer. The area considered most abnormal by the colposcopic examination was biopsied. When the colposcopic impression was normal the transformation zone was randomly biopsied. An endocervical curettage was performed if the referral Papanicolaou smear had shown endocervical atypia or if the transformation zone was not visualised.

Detection of HPV and other (non) STD's

Specimens for HPV were collected from the cervix. High/ intermediate-risk types of HPV DNA (16/18/31/33/35/39/45/51/52/56/58/59/68) were detected using the Hybrid Capture II™ technology, which is a signal amplified hybridisation antibody capture microplate assay using chemiluminescence for the quantitative detection of human papillomavirus DNA in cervical specimens (Digene, Beltsville, USA). A specimen ratio of ≥1 was regarded as positive and a cut-off point of 1.0 pg/ml was used as described in detail previously[15].

Specimens for (non) sexual transmitted disease and micro-organisms were collected from the cervix, vagina and urethra. To detect Neisseria Gonorrhoeae (GO) and Chlamydia Trachomatis a Gen-probe Pace 2 (Gen Probe, San Diego, USA) was used, a rapid DNA probe test. Mycoplasma hominis and Gardnerella vaginalis were detected after plating charcoal swabs on specific agar plates[16].

Statistical Analysis

Data were collected and analysed on the Statistical Package for Social Sciences (SPSS/PC+). Chi-square test and logistic regression were used to evaluate the association between pre-malignant lesions and risk factors such as HPV and other STD's. Risk factors were dichotomously entered in the model. Multivariate analysis was not possible due to small numbers. P value of less than 0.05 was considered statistically significant.

RESULTS

Prevalence of underlying CIN in women with ASCUS/AGUS cytology

Of 148 women with ASCUS/AGUS who underwent histological sampling, we found 123 without CIN (83%), 15 with CIN I (10%), seven with CIN II (5%), one with CIN III (1%), and two with squamous cell carcinoma of the cervix stage Ib1 (1%). Overall, 7% (95%CI 3.3-12.1) of the women had a histological diagnosis of CIN II or more.

Colposcopic examination to detect underlying CIN in women with ASCUS/AGUS cytology

We correlated the colposcopic diagnosis with the histological diagnosis (Table 2). A colposcopic diagnosis of CIN II or more for the detection of histologically confirmed CIN II or more, had a sensitivity of 70%, a specificity of 90%, a positive predictive value (PPV) of 33% and a negative predictive value (NPV) of 98%. For the characteristic of more than two quadrants abnormal these values were 90%, 62%, 15%, and 99%, respectively.

Table 2. Colposcopic Characteristics in relation to histology

Histology / Colposcopy	≤ CIN I N=138 no (%)	≥ CIN II N=10 no (%)	p-value
Acetowhite lesion	105 (76.1%)	10 (100%)	0.118
Punctation	30 (21.7)	5 (50%)	0.057
Mosaic	13 (9.4%)	4 (40%)	0.017*
Atypical vessels	5 (3.6%)	3 (30%)	0.011*
≥2 quadrants abnormal	53 (38.4%)	9 (90%)	0.002*
Colposcopic diagnosis ≥ CIN II	14 (10.1%)	7 (70%)	<0.001*

* P-value<0.05, statistically significant

Prevalence of HPV in women with ASCUS/AGUS cytology and in the underlying CIN

Of the patients with ASCUS/AGUS cytology, 67 (45.3%) were positive for high/ intermediate risk HPV.

The high/ intermediate risk HPV test was positive in 36.6% of the women with normal histology, in 86.7% of patients with CIN I, in 85.7% of patients with CIN II, in 100% of patients with CIN III/cervical cancer. The high/ intermediate risk HPV test was negative in one of the seven women with CIN II. The sensitivity of high/ intermediate risk HPV DNA testing to detect histological CIN II or more was much higher than colposcopy at 90% with a lower specificity of 58% (compared to 70% and 90% for colposcopy respectively). The PPV of high/ intermediate risk HPV DNA testing was lower than that of colposcopy at 13% (compared to 33% for colposcopy). The NPV of HPV DNA testing was 99% (compared to 98% for colposcopy) (Table 3).

Table 3. The sensitivity, specificity, PPV* and NPV† of two methods to detect the underlying histological CIN II or more in women with ASCUS/ AGUS cytology

	Method 1 Colposcopic diagnosis of ≥ CIN II	Method 2 A positive high/ intermediate risk HPV DNA test
Sensitivity	70%	90%
Specificity	90%	58%
Positive Predictive Value	33%	13%
Negative Predictive Value	98%	99%

* PPV= positive predictive value. † NPV= negative predictive value

Prevalence of other (non) STD's in women with ASCUS/AGUS cytology

Mycoplasma was detected in 6.1% of the 148 women and 1.4% were positive for Chlamydia trachomatis. No gonorrhoea was cultured. Gardnerella was cultured in 14.9% of the women with ASCUS/AGUS.

Statistical analysis of risk factors predicting CIN in presence of ASCUS/AGUS cytology

Variables that showed a correlation with a histological diagnosis of CIN II or more are listed in table 4. The mean age of women with histological ≤ CIN I and ≥ CIN II was 35 (±11) and 34 (±7) years respectively.

After stratification by HPV infection, we found 67 HPV-positive and 81 HPV-negative women. HPV-positive women were significantly younger than HPV-negative women, mean age of 30 ±9 years and 39 ±10 years respectively (P<0.001). HPV-positive women had more sexual partners (31.8% versus 12.8%, P=0.008), and more often had positive tests for other sexually transmitted diseases (13.4% versus 2.5%, p=0.02). Smoking and current use of oral contraceptives did not show such a correlation.

Table 4. Logistic regression of risk factors for histological ≥ CIN II

Histology	No CIN/ CIN I N=138	≥ CIN II N=10	Odds ratio (95%CI)	Odds ratio adjusted for HPV (95%)
Cigarette smoking	56(41%)	7(70%)	3.4(0.8-13.8)	2.8(0.7-12.0)
Oral contraceptives*	45(33%)	6(60%)	3.1(0.8-11.5)	2.6(0.7-10.2)
Parity ≥ 4	20(14%)	2(20%)	1.5(0.3-7.5)	2.2(0.4-12.7)
First intercourse ≤ 18	82(60%)‡	10(100%)	∞(N/A)¶	∞(N/A)
Sexual partners ≥ 5	26(19%)§	5(50%)	4.2(1.1-15.4)	2.7(0.7-10.7)
History of Candida	46(33%)	7(70%)	4.7(1.2-18.9)	3.8(0.9-16.1)
History of STD†	20(14%)	5(50%)	5.9(1.6-22.2)	5.8(1.4-23.7)
STD test positive	10(7%)	1(10%)	1.4(0.2-12.4)	0.7(0.1-6.9)
HPV test positive	58(42%)	9(90%)	12.4(1.5-101)	

Logistic regression, univariate analysis. * current use. † STD = sexually transmitted disease like Chlamydia, Gonorrhoea and Mycoplasma. ‡ N = 137. § N = 134. ¶ N/A= not available, Relative Risk (RR) = 1.67.

DISCUSSION

In this study we investigated the colposcopic assessment and histological diagnoses of 148 women with ASCUS/AGUS cytology, and correlated these findings with the results of high/ intermediate risk HPV DNA testing. Overall 7% of women with initial cytology of ASCUS/AGUS had CIN II or more confirmed after histological assessment. This is comparable with the 5% of CIN II-III found by Lousuebsakul et al[17] in women with ASCUS cytology and with the 9% found by Williams et al[10]. Other studies however have reported rates of CIN II - III in up to 17 - 18% of women with ASCUS cytology[11,18]. The higher prevalence of ≥ CIN II in the latter studies might be explained by patient selection bias, differences in the follow-up periods, and differences in definition of diagnostic terms. The percentages of ASCUS in these studies[10,11,17,18] were between 2 and 5% in these clinics which is comparable to the prevalence in our hospital (4.4% in 1999).

We found two women with squamous cell cancer of the cervix. In one patient the cervical smear was interpreted as atypical repair and in the other, as ASCUS/AGUS. In both cases the cytological diagnosis was falsely negative. The original slides were reviewed and the atypical cells were again not interpreted as cancer cells. Both women had a one year history of post coital bleeding. HPV DNA testing was positive in both cases of cervical cancer. Macroscopically there was no suspicion of carcinoma, but extensive zones of cervical 'erosion' were seen. In both cases the colposcopic diagnosis was suspicious of cervical cancer and confirmed by histology. Cone biopsy was performed. Both women were staged as Ib1 and a radical hysterectomy with lymphadenectomy was performed. There was no residual cancer in the hysterectomy specimens. For the case of atypical repair the differential diagnosis is invasive cancer. In the other case the histology of the cervix showed a macro-invasive carcinoma with fields of relatively uniform cell types with large hypochromatic nuclei like the atypical cells of the cytology. Neither case had the typical cytological criteria of invasion such as tumour diathesis, necrosis, single atypical cell, and keratinisation and the diagnosis of cancer would not have been made despite careful review of the slide.

To our best knowledge this is the first study to describe the colposcopic impression in cases with ASCUS/AGUS in detail. The colposcopic impression predicted histological ≥ CIN II with a positive predictive value of 33%. A review of the literature revealed that the positive predictive value of the colposcopic impression in cases with CIN II was on average 31% and in cases of CIN III was 86%. These studies included however cytological diagnoses of ≥ Pap III (moderate dysplasia or more)[19]. In our study, the colposcopic finding of two abnormal quadrants had a negative predictive value for histological CIN II or more of 99% and 98% when the colposcopic diagnosis was of CIN II or more. The high negative predictive value of colposcopy suggests a valuable role for colposcopy in the triage of ASCUS/AGUS cytology.

The high/intermediate risk HPV test was positive in 45.3% of the 148 women with ASCUS/AGUS cytology in our gynaecological outpatient clinic. The percentage of positive HPV test was significantly higher in women with ≥CIN II than in women with normal histology or CIN I (90% versus 42%,$p<0.05$). Cox et al showed comparable results of 41.9% positive HPV test in women with ASCUS and 93% underlying ≥ CIN II versus 38% (12). We found a sensitivity of high/ intermediate risk HPV test of 90% and a specificity of 58% to detect underlying ≥ CIN II. Because of only 10 women with ≥ CIN II, it is not possible to estimate the sensitivity accurately. In contrast we could estimate the specificity more exactly, because 138 women showed ≤ CIN I[13].

If we had selected only women with a positive high/ intermediate risk HPV DNA test for colposcopy, only 67 of the 148 women would have been referred, reducing the number of colposcopies by 55%. This would have resulted in one woman with histological CIN II being missed because of a negative HPV DNA test. HPV DNA testing had a very high negative predictive value of 99%, as has been reported by others [13,20,21].

There is now good evidence from molecular, clinical and epidemiological studies that strongly implicates HPV infection of the cervix as the primary cause of CIN and cervical cancer. Known risk factors for progression to CIN III or cervical cancer include HPV type, viral load, cell-mediated immunity, and sexual behaviour[22]. The most consistent risk factors for cervical cancer are the number of sexual partners and the woman's age at first sexual intercourse. In our study we also found a strong association between young age at first sexual intercourse and a histological diagnosis of CIN II or more. Before the role of HPV was known, the number of sexual partners was the major risk factor for cervical cancer. In our study, the number of sexual partners did not increase the risk of developing ≥ CIN II in HPV positive women, as described in other studies [23]. This finding suggests that the number of sexual partners is not an independent risk factor for CIN after adjusting for HPV.

In our study cigarette smoking, current use of oral contraceptives, and the number of sexual partners were not significant for detecting ≥ CIN II after adjusting these variables for HPV, but the confidence intervals are wide because the numbers are small. Additional risk factors as long-term use of oral contraceptives and smoking have been extensively studied in more powerful studies, although an association with CIN and cervical cancer has been less consistently found and varied by region[22]. In most previous studies the HPV status was mostly not known, so they were unable to correct adequately for the effect of HPV or they may not have had sufficient power for detecting high grade disease. This study and others suggest that the other risk factors, such as oral contraceptives, smoking, and sexual behaviour, are probably confounding factors.

The evidence that smoking increases the risk of CIN III (severe dysplasia or carcinoma in situ) has recently been reviewed by Szarewski and Cuzick[24]. They conclude that the increased risk of smoking in most studies was reduced but not eliminated by adjustment for age at first sexual intercourse and the number of sexual partners. In a prospective

intervention study they demonstrated a clear relationship between reduction in smoking and changes in cervical immune cell numbers, like reduction in the number of Langerhans' cells. Heavy smoking was significantly associated (p=0.02) with an increased risk of persistent human papillomavirus infection. They suggest that smoking influences the local immune response to both human papillomavirus and cervical intraepithelial neoplasia[25].

In conclusion this study demonstrates that if the percentage of ASCUS/AGUS is less than 5%, there is a serious risk of high grade lesions in this population, and this risk increases as the percentage of ASCUS/AGUS decreases to 2% or less. Because of this phenomenon we recommend a high risk HPV DNA test of the cervix in women with ASCUS/ AGUS cytology, and if this test is positive a colposcopy with biopsy is needed. If the high risk HPV test is negative the woman can return to the normal screening protocol.

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