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Usefulness of HPV testing in the follow-up of untreated cervical low grade lesions

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Summary. The aim of the present work was to evaluate the usefulness of high-risk human papillomavirus (HR-HPV) testing for the follow-up of women with untreated low grade cervical squamous cell lesions (LSIL). For that, 412 women with a cytological diagnosis of LSIL at entry were monitored by cytology, HR-HPV testing with the Hybrid Capture II assay (HC-II) and colposcopy. Our primary endpoint was clinical progression defined by the presence of a high grade cervical intraepithelial neoplasia (CIN2 and CIN3) at the biopsy. At baseline, histological control revealed 10 CIN2 and 11 CIN3 only in the cohort of women HR-HPV+. In the follow-up, 4 CIN2 and 8 CIN3 were detected, always in the women initially HR-HPV+. Thus, the recurrence of a HR-HPV+ infection clearly selects a population at high-risk for CIN2-3. The semi-quantitative appreciation of the viral load with HC-II could not be used as a good prognostic factor for the follow-up of women with LSIL. HR-HPV testing reduces the number of cytology and colposcopy examinations in the follow-up of women aged >35 years when HPV testing is initially negative. Thus HR-HPV testing should be reserved for the follow-up of this population of women initially HR-HPV+ and proposed 6 to 12 months after the cytological diagnosis of LSIL.

Key words: Hybrid capture, Human papillomavirus, Cervical intraepithelial neoplasia, Follow-up

Introduction

High-risk human papillomaviruses (HR-HPV) have now been conclusively demonstrated to be the causative agents of cervical cancer (Lorincz et al., 1992; Bosch et al., 2002; Zur Hausen, 2002). Indeed, it has been reported that 99.7% of all cervical squamous cell carcinomas contain HR-HPV (Walboomers et al., 1999). In consequence, the usefulness of HR-HPV detection in cytological and/or histological samples has emerged for clinical use and screening. While most authors agree that high-grade squamous intraepithelial lesions (HSIL) should be managed regardless of HPV type as most of them contain HR-HPV, HPV testing is now considered as a useful method for triaging women with a cytological diagnosis of atypical squamous cells of undetermined significance (ASCUS) in their cervical smears and for the follow-up of women treated for HSIL (Manos et al., 1999; Solomon et al., 2001; Paraskevaidis et al., 2004). In the same way, there is an increasing interest in using HR-HPV DNA detection either alone or in addition to the classic cytological examination as a method for primary screening for cervical preneoplastic and neoplastic lesions (Clavel et al., 2001).

Invasive carcinoma is rare in the population of women with low-grade squamous intraepithelial lesions (LSIL). Moreover, a large majority of LSIL spontaneously regresses and do not justify a systematic treatment by laser cryotherapy or cone biopsy. Thus, at the present time, three variants of algorithms are proposed for the follow-up of women with LSIL (Scheungraber et al., 2004): immediate colposcopy, colposcopy after a repeated abnormal cytological result within a 6-month interval, and see-and-treat management. The use of HPV testing in the management of women with LSIL at cytology is also debated. If a cytological interpretation of LSIL, particularly in young women, is too accurate of a marker for HR-HPV testing to be useful (The ALTS, 2000), HR-HPV testing in association with cytology could reduce the number of colposcopies in the follow-up of untreated women. In consequence, the management of women with untreated LSIL requires additional evaluation for clinical and economical reasons. Thus, the aim of the present work was to evaluate the usefulness of HR-HPV testing for the follow-up of women with untreated LSIL.

Materials and methods

Study population

A population of 412 women with LSIL in their smears, with a median age of 30 years (range 18 to 74

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years) was recruited for the study between August 1997 and October 2003. This cohort is a part of a population of 17 003 women who underwent a biennial or triennial routine screening in the Department of Obstetrics and Gynecology of the C.H.U. of REIMS and who had both cytology and HPV testing for primary screening. We excluded subjects on the basis of a recent cytological abnormality and/or an untreated cervical lesion in the past two years, as well as pregnant women and patients with AIDS. All women were informed of the aim of the study and gave their consent.

Cytologic diagnosis

At the first gynecologic examination, two samples were taken from 63 women: first, a cytologic smear with an Ayre's spatula, for classical cytology, then a sample for the Hybrid Capture-II (HC-II) test using a brush provided in the HC-II kit. These samples were suspended in 1 ml of specimen transport medium (STM) for HPV testing (Digene, Gaithersburg, Md). A second group of 349 women had only one cervical scrape with a Cervexbrush (Rovers Medical Devices, Oss, Netherlands) at the first entry. Samples were prepared in PreservCyt medium for liquid-based cytology with the ThinPrep technique (Cytyc Corporation, Boxborough, Mass) and 4 ml of the sample were used for HPV testing. In total we collected 1051 cervical smears and scrapes at baseline and in the follow-up of women. The great majority of these samples (1000 samples - 95.1%) were treated with liquid based cytology. Smears were classified according to the Bethesda system for reporting cervical or vaginal cytological diagnosis. We selected women with adequate smears including metaplastic and/or endocervical cells, according to the criteria of Bethesda, which represented 93% of our total smears. The cytotechnicians and pathologists involved in the study were not informed about the results of the HPV testing. All smears showing cytological abnormalities and biopsy specimens were examined by the same two independent pathologists; when examining the biopsy specimens, the pathologists had no knowledge of cytology results. The results were compared and if the first 2 diagnoses disagreed, a third pathologist reviewed the case with no knowledge of preceding diagnoses. Consensus diagnoses were determined by two-thirds majority when possible and all remaining discrepancies were resolved by conference review. Patients with HSIL were systematically treated by loop electrosurgical excision procedure (LEEP). Data from these LEEP specimens were included in the disease definitions.

HPV testing

When conventional cytology was performed, specimens for HPV DNA testing were suspended in 1 ml of STM transport medium (Digene, Gaitherburg, Md) and stored at -20°C until further processing. When samples were used for liquid based cytology, 4 ml of the sample was centrifuged and the cell pellet was resuspended in 200 µl of Phosphate Buffered Saline for HPV testing. HPV DNA detection was performed by the commercially available HC-II System (Digene). All scrapes were analyzed for the presence of HR-HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. This enzyme-linked immunosorbent amplification of the signal is based on a sandwich hybridization followed by a nonradioactive alkaline phosphatase reaction with chemoluminescence in microplates. The chosen positive threshold of this test was 1.0 pg/ml of HPV DNA as recommended by the manufacturer.

Samples were classified as positive for HR-HPV DNA if the relative light unit (RLU) reading obtained from the luminometer was equal to or greater than the mean of the three positive control values supplied by the HC-II kit. As some authors have reported that increasing HPV DNA levels of HR-HPV types were the principal predictors of CIN (Cox et al., 1995), we used as proposed, the ratio RLU/positive controls values to quantify HR-HPV DNA in our samples. Moreover, we added 2 positive controls such as SiHa cell lines (1 to 2 copies of HPV type 16 per cell) to check the reproducibility of the HC-II sensitivity.

Colposcopic referral

205 women (186 HR-HPV positive and 19 HR-HPV negative women) had an immediate colposcopy control at entry after the cytological diagnosis of LSIL, with biopsies performed in case of suspicion of high-grade cervical intraepithelial neoplasia (CIN2/3). Then, all the women were followed with smears and HR-HPV testing, except those who had a HSIL which required treatment.

According the recommendations of the French Society of Cytology, the first control after the diagnosis of LSIL was within 3 to 9 months (mean = 6 months). Women with smears within normal limits and without HR-HPV infection were recalled 12 to 18 months later for a third visit with cytological examination and HR-HPV testing. Women with smears within normal limits but presenting with a HR-HPV infection were recalled 3 to 12 months later (mean interval = 6 months) for a new cytological examination and HR-HPV testing, followed by colposcopy if a lesion and/or a HR-HPV infection was still detected. Women with cytological lesions (from ASCUS to HSIL) whatever the result of HR-HPV testing had a colposcopy. In all cases, punch biopsy specimens were taken from the areas suspicious for CIN2-3. The primary endpoint of our study was the detection of a histologically proven CIN2-3 at the biopsy and only these lesions were treated. Moreover, at final follow-up, all women with an abnormal smear had systematically a colposcopy and 26 unselected women with normal smears also had a colposcopy for control.

Statistical methods

The statistical methods used were mostly

descriptive. Sensitivity, specificity, positive and negative predictive values were determined by comparing the results of each test to the gold standard of histology. Ninety-five percent confidence interval for these values were assessed using normal distribution, according to the data. Overall occurrence of CIN2/3 was assessed by Kaplan-Meier analysis with the Mantel Cox log-rank score for determining statistical significance.

Results

The total results are summarized in Tables 1 and 2.

At baseline, 10 CIN2 and 11 CIN3 were detected only in the cohort of women HR-HPV+. The median follow-up of the 53 women negative for HR-HPV testing at baseline was 22 months (range 3 to 52 months). At final follow-up, 1 CIN1 was diagnosed in the population of 7 women with abnormal smears who had a colposcopy. The median follow-up of the 338 women positive for HR-HPV testing at baseline was 15 months (range 3 to 84 months). In this latter cohort, 4 CIN2 and 8 CIN3 were detected in the follow-up within 2 years after entry (median = 15 months, range 6 to 24 months). Moreover, 25 CIN1 were diagnosed after colposcopy in the population of 125 women with abnormal smears at final follow-up. At last, colposcopy performed in 26 women with normal smears at final follow-up did not reveal any lesions. The Kaplan-Meier curves comparing the occurrence of CIN2/3 in the whole population (Fig. 1) and in the population without any biopsy which may affect the natural history of the disease (Fig. 2) give similar results and clearly demonstrate that there is a significant difference between the populations with HR-HPV-positive testing compared to population with a negative test ($p < 0.001$).

The regression of HR-HPV infection was observed in 170 cases (50.3%) (median = 9 months, range 3 to 72 months). The cytological regression of the lesions was observed in 259 cases: 46 cases (86.8%) in the population of 53 women initially negative for HR-HPV testing and 213 cases (63%) in the population of 338 women initially positive for HR-HPV testing. The

Table1. Follow-up of 412 women with LSIL at cytology.

BASELINE	HISTOLOGICAL CONTROL AFTER 1st CYTOLOGY	1st CYTOLOGICAL CONTROL	LAST CYTOLOGICAL CONTROL	HISTOLOGICAL CONTROL AT FINAL FOLLOW-UP
53 LSIL HR-HPV-	6 NL	36 NL HR-HPV-	41 NL HR-HPV-	
	6 CIN1	5 NL HR-HPV+	5 NL HR-HPV+	
		5 ASCUS HPV-	4 ASCUS HPV-	
		4 LSIL HR-HPV-	2 LSIL HR-HPV-	4 NL
		3 LSIL HR-HPV+	1 LSIL HR-HPV+	1 CIN1
359 LSIL HR-HPV+	19 NL	125 NL HR-HPV-	153 NL HR-HPV-	
	52 CIN1	63 NL HR-HPV+	60 NL HR-HPV+	
	10 CIN2*	8 ASCUS HR-HPV-	7 ASCUS HR-HPV-	2 NL
	11 CIN3*	24 ASCUS HR-HPV+	24 ASCUS HR-HPV+	16 NL
		8 LSIL HR-HPV-	10 LSIL HR-HPV-	2 NL + 5 CIN1
		94 LSIL HR-HPV+	62 LSIL HR-HPV+	12 CIN1
		16 HSIL HR-HPV+	22 HSIL HR-HPV+	2 NL + 8 CIN1 + 4 CIN2 + 8 CIN3

*: These lesions were treated by cone biopsy

Table 2. Follow-up of 157 women > 34 years with LSIL at cytology.

BASELINE	HISTOLOGICAL CONTROL AFTER 1st CYTOLOGY	1st CYTOLOGICAL CONTROL	LAST CYTOLOGICAL CONTROL	HISTOLOGICAL CONTROL AT FINAL FOLLOW-UP
32 LSIL HR-HPV-	4 NL	26 NL HR-HPV-	26 NL HR-HPV-	
	2 CIN1	2 ASCUS HR-HPV-	2 NL HR-HPV+	
		2 LSIL HR-HPV-	2 ASCUS HR-HPV-	
		2 LSIL HR-HPV+	2 LSIL HR-HPV-	2 NL
125 LSIL HR-HPV+	6 NL	40 NL HR-HPV-	51 NL HR-HPV-	
	19 CIN1	25 NL HR-HPV+	21 NL HR-HPV+	
	5 CIN2*	2 ASCUS HR-HPV-	3 ASCUS HR-HPV-	
	5 CIN3*	5 ASCUS HR-HPV+	9 ASCUS HR-HPV+	10 NL
		3 LSIL HR-HPV-	3 LSIL HR-HPV-	1 CIN1
		34 LSIL HR-HPV+	22 LSIL HR-HPV+	10 CIN1
		6 HSIL HR-HPV+	6 HSIL HR-HPV+	1 CIN3

*: These lesions were treated by cone biopsy

cytological regression was significantly associated with the regression of HR-HPV infection (p<0.00001) and there was a significant correlation between an initial negative HR-HPV testing and the regression of cytological lesions (p<0.001).

If we consider the Bethesda recommendations according the cytology data (ASCUS with positive HR-HPV testing or more), the number of colposcopy examinations were 178 (45.5%) in the population of 391 women with follow-up. The number of colposcopy examinations justified by 2 successive positive HR-HPV tests at 6-12 months of interval, was 159 (40.7%) and was not significantly different (p = 0.25). The number of CIN2/3 detected was the same in both conditions.

The median follow-up of the 32 women aged >35 years negative for HR-HPV testing at baseline was 25 months (range 3 to 74 months). The median follow-up of women positive for HR-HPV testing at baseline was 16 months (range 3 to 84 months).

The regression of HR-HPV infection in the population women >35 years was observed in 57 cases (49.6%) (median = 10 months, range 4 to 67 months). The cytological regression was observed in 100 cases: 28 cases (87.5%) in the population of 32 women initially negative for HR-HPV testing and 72 cases (62.6%) in the population of 115 women initially positive for HR-

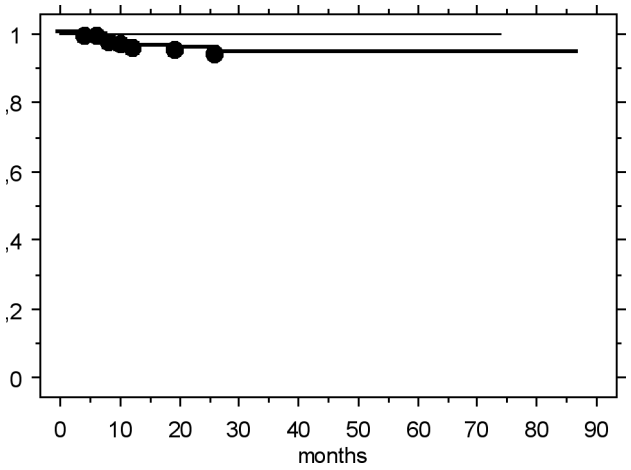

Fig. 1. Kaplan Meier analysis for the occurrence of CIN2/3 in whole population of women with initially LSIL, HR-HPV+ (thick curve) and HR-HPV- (thin curve).

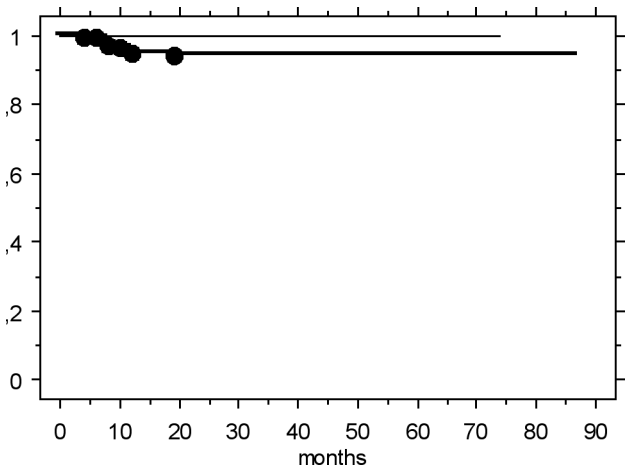

Fig. 2. Kaplan Meier analysis for the occurrence of CIN2/3 in the population of women with initially LSIL, HR-HPV+ (thick curve) and HR-HPV- (thin curve) but without any biopsy.

Table 3. Evaluation of HR-HPV testing and cytology for detecting CIN2/3 in the follow-up of women with LSIL.

	SENSITIVITY	SPECIFICITY	PPV	NPV
Total population				
Cytology	75% 95%CI 70.7-79.3%	59.6% 95%CI 54.1-63.9%	5.5% 95%CI 2.8-7.2%	98.7% 95%CI 69.6-99.4%
HPV testing	100%	14% 95%CI 10.6-17.4%	3.5% 95%CI 1.7-5.3%	100%
HPV testing RLU>10	90.5% 95%CI 88-93.9%	15.3% 95%CI 11.3-18.7%	9.8% 95%CI 6.7-12.9%	94.3% 95%CI 91.5-96.5%
Population of women >35 years				
Cytology	100%	62.3% 95%CI 54.1-69.8%	1.8% 95%CI 0-3.9%	100%
HPV testing	100%	21.9% 95%CI 15.3-28.7%	0.9% 95%CI 0-2.4%	100%
HPV testing RLU>10	100%	16.4% 95%CI 10.6-23.2%	0.8% 95%CI 0-2.1%	100%

PPV: Positive Predictive Value, NPV: Negative Predictive Value; CI: Confidence Interval.

HPV testing.

If we consider the same recommendations for colposcopy as above in this population, the number of colposcopy examinations according to the Bethesda recommendations were 74 (50.3%) in the population of 147 women with follow-up. The number of colposcopy examinations according to the HR-HPV testing (57-38.8%) significantly decreased (p = 0.04). The number of CIN2/3 detected was the same in both conditions.

Tables 3 and 4 summarize the evaluation of cytology and HR-HPV testing, including HR-HPV testing with RLU values >10 for the detection of CIN2/3 in the follow-up of the women with LSIL (total population and population of women without colposcopy at baseline).

Discussion

The present study reports an experience on 412 women with a cytological diagnosis of LSIL at entry. The number of LSIL with HR-HPV infection at baseline was high (87.1% for the total population, and 79.6% after 35 years) and these results are similar to those of the literature (The ALTS, 2000). In 21 cases (10.2%), CIN2/3 were diagnosed at the immediate colposcopy and histological control performed in 205 women. We cannot exclude that more CIN2/3 could be detected if colposcopy was systematically performed in the total population. Indeed, in France, in presence of LSIL at cytology, colposcopy is not systematically performed and the alternative is to propose a cytological follow-up in these women. However, the protocol of follow-up was the same for all the women and the results obtained in the two populations with or without colposcopy at baseline are similar. We have to underline that, at baseline, CIN2/3 were observed only in the HR-HPV+ cohort. In the same way, in agreement with the data of the literature (Gaarenstroom et al., 1994; Zielinski et al., 2001), all the 12 CIN2/3 diagnosed in the follow-up of untreated women developed only in the cohort of HR-HPV+ women and 6 of these women had a normal colposcopy at baseline. Of particular interest are the observations of 3 women who had a regression of the cytological abnormalities at the first control with a recurrence of HR-HPV infection which led to a more intensive cytological control detecting a histologically proven CIN2/3 at the second control. Thus, as we previously reported in women with normal smears (Bory et al., 2002), the recurrence of an HR-HPV+ infection in women with untreated LSIL clearly selects a population at high risk for the apparition of CIN2/3.

The regression of the cytological lesions was frequent in our series (66.2% in the total population, 68% in women aged >35 years) and was significantly associated with the clearance of HR-HPV. Zielinski et al. (2001) and Nobbenhuis et al. (2001) reported that the clearance of HPV infection was observed before the regression of the cytological lesions. However, in our experience, there was a persistence of the cytological lesions (ASCUS/LSIL) in 17 cases (5%) without HR-HPV infection. Recently, Dalstein et al. (2003) have emphasized the usefulness of the viral load appreciated by the HC-II assay as a prognostic factor for progression of cervical intraepithelial neoplasia. We found that the viral load had a limited value for detecting CIN2/3 whatever the age of the population. Indeed, the sensitivity of a viral load >10 RLU for detecting CIN2/3 was of 90.9% with a specificity of 15.3% and a PPV of 9.8%, with a RR of 1.7. There is a loss of sensitivity when compared to the general data obtained with HR-HPV testing, with a faint gain of specificity. These data are analogous to our previous findings on the use of viral load with HC-II as a prognostic factor in normal smears

Table 4. Evaluation of HR-HPV testing and cytology for detecting CIN2/3 in the follow-up of women with SIL and without colposcopy at baseline.

	SENSITIVITY	SPECIFICITY	PPV	NPV
Total population				
Cytology	83.3% 95%CI 53.4-100%	54.2% 95%CI 47.3-61.1%	5.1% 95%CI 0.7-9.5%	99.1% 95% 98.5-100%
HPV testing	100%	16.9% 95%CI 11.8-22.2%	3.5% 95%CI 0.7-6.2%	100%
HPV testing RLU>10	83.3% 95%CI 53.4-100%	17.4% 95%CI 12.2-22.6%	2.9% 95%CI 0.4-5.4%	97.2% 95%CI 91.8-100%
Population of women >35 years				
Cytology	100%	55.5% 95%CI 45.6-64.4%	2.1% 95%CI 0-6.2%	100%
HPV testing	100%	16.7% 95%CI 9.9-24%	1.1% 95%CI 0-3.0%	100%
HPV testing RLU>10	100%	15.7% 95%CI 9.1-22.9%	1.1% 95%CI 0-3.0%	100%

PPV: Positive Predictive Value, NPV: Negative Predictive Value; CI: Confidence Interval.

(Bory et al., 2002). These findings are likely due to the mode of sampling with the use of the ThinPrep sample which cannot allow a standardization of the number of cells collected and to the quality of preservation medium. Thus, the semi-quantitative appreciation of the viral load by HC-II cannot be used as a good prognostic factor for the follow-up of LSIL. Other approaches such as E6/E7 transcripts (Kraus et al., 2004) or $p16^{ink4a}$ (Klaes et al., 2001) detection could likely improve the low specificity and PPV of HR-HPV testing for predicting CIN2/3.

Considering the usefulness of HR-HPV testing for the follow-up of untreated LSIL, we have to underline the significant correlation between an initial negative HR-HPV testing, the regression of the cytological lesions and the absence of CIN2/3 detected in the follow-up of this cohort, as previously reported (Yokoyama et al., 2003). In consequence, a less intensive follow-up could be proposed in such women, with a first control at 12 months with cytology and HR-HPV testing according to the recommendations of the French Society of Cytology. This cohort represents only 12.9% of the total population, but it increases to 20.4% in women aged >35 years and to 24.3% after 45 years. In this population, increased misclassification of LSIL has been noted and has been considered to be responsible for declining detection of squamous intraepithelial lesions at colposcopy (Giles et al., 1989). Thus a negative HR-HPV testing could anticipate the absence of development of lesions in these women. In another way, in most adolescents, both HR-HPV infection and LSIL are transient phenomena and HR-HPV testing is not justified in these particular cases (Mosicki et al., 2001). From an economical point of view, in our experience, the use of HR-HPV testing, even if it has a high sensitivity but a very low specificity, significantly reduced the number of colposcopy examinations at 38.8% when compared to the use of cytology alone (50.3%) in the cohort of women >35 years. These data are similar to those of Guido et al. (2003). Thus, considering all our preliminary results, HR-HPV testing could be reserved only for the follow-up of women aged >35 years initially HR-HPV+. This HR-HPV testing could be proposed 6 to 12 months after the first smear. It will reduce the number of cytology and colposcopy examinations after a negative test. These propositions are in accordance with the guidelines of the American Society for Colposcopy and Cervical Pathology (Cox, 2003).

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