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Human papillomavirus testing improves the accuracy of colposcopy in detection of cervical intraepithelial neoplasia

J. MONSONEGO*, J. PINTOS†, C. SEMAILLE*, M. BEUMONT*, R. DACHEZ*, L. ZERAT‡, A. BIANCHI* & E. FRANCO†

**Institut Alfred Fournier, Paris, France; †McGill University, Montreal, Canada; and ‡Laboratoire Lavergne-Victor Hugo, Paris, France*

Abstract. Monsonego J, Pintos J, Semaille C, Beumont M, Dachez R, Zerat L, Bianchi A, Franco E. Human papillomavirus testing improves the accuracy of colposcopy in detection of cervical intraepithelial neoplasia. *Int J Gynecol Cancer* 2006;**15:**1–8.

To assess the performance of human papillomavirus (HPV) testing and colposcopy in detection of cervical pathology. A series of 389 women referred for colposcopy due to an abnormal Pap smear had cervical swabs analyzed for oncogenic (high-risk [HR]) HPV types using Hybrid Capture II (HC2) assay. Loop electrical excision procedure cone biopsy (88%) or colposcopic biopsy (11%) was used as the gold standard. Of the atypical squamous cells of undetermined significance (ASCUS) smears, 48% were positive for HR HPV, as compared to 76.3% of low-grade squamous intraepithelial lesions (LSIL) smears. HR HPV was detected in 66.7% and 90% of patients with cervical intraepithelial neoplasia (CIN) 1 and CIN2 (or higher), respectively. The sensitivity of the Pap smear using an ASCUS threshold in detecting high-grade CIN was 94.5% (95% confidence intervals (CI): 91–97%) and that of colposcopy 98.5% (95% CI: 95–99%). The respective specificities were 30% (95% CI: 17–28%) and 35.6% (CI: 29–42%). HC2 test had comparable sensitivity, 90% (95% CI: 85–93%), but higher specificity, 54.3% (95% CI: 47–61%). Combining HC2 test with Pap increased specificity, 66.7% and 41.3% for ASCUS and LSIL cutoff, respectively. The minor-abnormality threshold together with HC2 increased specificity of colposcopy with no changes in sensitivity. High viral load (>100 relative light unit/positive control) was associated with significant disease. HPV DNA testing improves the accuracy of colposcopy in the detection of high-grade CIN in women with ASCUS or LSIL smears.

KEYWORDS: ASCUS, cervical intraepithelial neoplasia, colposcopy, human papillomavirus, LSIL, test performance.

Cervical Papanicolaou (Pap) smears classified as atypical squamous cells of undetermined significance (ASCUS) or low-grade squamous intraepithelial lesions (LSIL) are the most common cytologic abnormalities (4–9%) in women screened for cervical cancer[1]. Among the ASCUS smears, 80% of the cases disclose normal cervix, and only 7–10% reveal underlying high-grade cervical intraepithelial neoplasia (CIN) 3. ASCUS and LSIL categories are nevertheless the most common source of high-grade disease in the screened population[1]. The options generally accepted for the management of ASCUS and LSIL smears are a repeat Pap smear after 6 months or immediate colposcopy[2,3]. However, repeated Pap smear is not highly sensitive in detecting high-grade CIN[4,5], and colposcopy has a limited specificity[6–9].

In many Latin European countries, colposcopy is a relatively inexpensive and widely used diagnostic tool. Although colposcopy can theoretically detect almost all cases of high-grade CIN, the cost benefit of this systematic approach is uncertain for patients with minor cytologic abnormalities because of its low reproducibility and specificity[9–11]. However, accurate colposcopy is mandatory for identifying women with significant cervical lesions and to avoid unnecessary intervention in women without any cervical disease[9–11].

Address correspondence and reprint requests to: Dr Joseph Monsonego, MD, Institut Fournier, 174 rue de Courcelles, 75017 Paris, France. Email: jm@eurogin.com

In the United States, the use of Hybrid Capture II (HC2) for the detection of human papillomavirus (HPV) DNA has been proposed as an option in the primary triage of women with ASCUS Pap smears[2], also prompted by the recent results of the ALTS study[12]. The French guidelines for the management of abnormal Pap smears have just yet recognized HPV DNA testing as an alternative management method, but women with ASCUS or LSIL smears are referred to colposcopy in most cases[3]. Several studies have shown that HC2 has higher sensitivity and higher negative predictive value (NPV) than repeat Pap smear or immediate colposcopy in the detection of CIN, reducing the diagnostic variability of equivocal Pap smears[4,5,8,13,14]. The use of HPV DNA testing could have important implications in reducing health care costs while avoiding the additional tests (repeat Pap, colposcopy, biopsy) and unnecessary treatments[6–8,15,16].

In several studies, a directed cervical-punch biopsy has been used as the gold standard while calculating the sensitivity, specificity, and predictive values of HPV DNA testing. However, this approach suffers from considerable inter- and intraobserver variability even when colposcopy is performed by skilled specialists[8–12]. Studies conducted with the first-generation hybrid capture test (HC1), which has lower sensitivity than the HC2 assay[17], suggested a lower specificity than that suggested by repeat Pap smears[18,19]. The HC2 is the refined version with sensitivity comparable to that of polymerase chain reaction testing[20–22]. This high analytical sensitivity of the HC2 assay may be a disadvantage in the management of LSIL smears[23–25] because of the high prevalence of high-risk (HR) HPV types in women with LSIL smears.

The present study was conducted a) to determine whether HPV testing can improve the performance of colposcopy in the diagnosis of high-grade disease and b) to evaluate the performance of HC2 test in the triage of women with abnormal Pap smears, using loop electrical excision procedure (LEEP) specimens as the gold standard.

Materials and methods

Material

The sample of the study included a series of 389 women referred to the Colposcopy Center, Institute Alfred Fournier, Paris, between April and November 1999 due to an abnormal Pap smear (ASCUS, LSIL, high-grade squamous intraepithelial lesions [HSIL]). The sample also includes some women with normal Pap smears but who had previous or current HPV-related disease (external genital warts or CIN treated during the past 6–18 months). The age of the patients ranged from 16 to 70 years, with a mean of 35.8 years and a median of 34.34. All patients in whom the Pap smear, colposcopy, or biopsy could not be evaluated for technical reasons, as well as those who were immunosuppressed or pregnant, were excluded from the study. All patients filled out a questionnaire including information on the following variables: age, oral contraception, tobacco use, date of last menstruation, history of prior HPV infection, history of prior treatment for CIN, age at first sexual intercourse, number of sexual partners, time with the current sexual partner, and history of sexually transmitted diseases. All women were subjected to colposcopy and HPV testing. The study was approved by the ethical committee of the Institute, and all women gave an informed consent to participate.

Methods

Cytology

All women had a conventional Pap smear taken within 3 months of their enrollment in the study (ie, the referral Pap), performed by community physicians. All the abnormal referral smears were reexamined by the same cytopathologist to minimize the diagnostic variability of equivocal (ASCUS) and LSIL smears. The final diagnosis was made after the second reading, and the results were classified according to the 1998 Bethesda system[26]. In the event of discordant diagnoses, priority was given to the second reading.

HC2 test

The clinical specimen for the HC2 test was obtained using the sampling kit specially designed for this test. Specimens were collected from the cervical transformation zone (TZ) by performing three complete rotations of the cone brush either before colposcopy or just before the cone biopsy. The brush was immediately immersed in the transport medium and conserved at −20°C until analysis. Specimens were processed according to the manufacturer's recommendations[27] for detection of the following HR HPV genotypes: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, and 58. Samples were classified as positive for HPV if the relative light unit (RLU) reading was equal to or greater than the positive control (PC), which corresponds to 1 pg of HPV DNA/mL of test solution (about 5,000 copies of HPV genomes per assay).

Colposcopy

After sampling for HPV DNA testing, colposcopic examination of the cervix, vagina, and vulva was performed in all patients by the same colposcopist (J.M.). Lesions in the TZ were assessed by applying 5% acetic acid and iodine solution, under 8× to ×12 magnification. If colposcopy proved unsatisfactory, further exploration of the endocervix was systematically carried out under 20× magnification using a Koogan speculum. 6 The international (IFCPC) nomenclature was used to classify the colposcopic features: normal, abnormal TZ with minor changes with or without features of HPV infection suggesting low-grade CIN (CIN1), atypical TZ with major changes suggesting CIN2-3, and cancer.

Biopsy procedures

Altogether, 344 patients (88%) underwent LEEP cone biopsy, and 43 (11%) had a directed punch biopsy. LEEP cone biopsy was performed in cases with a) Pap test showing HSIL; b) an atypical TZ regardless of the Pap test result, if the atypical TZ was large (≥25% of TZ area); c) an endocervical lesion and with unsatisfactory colposcopy; or d) a squamocolumnar junction localized more than 3 mm within the endocervix. Patients presenting with a normal Pap smear but with external genital warts or in follow-up after treatment for CIN and with an abnormal colposcopy were assessed using the same diagnostic procedures. Patients with an abnormal smear and satisfactory normal colposcopy (two cases) were not subjected to punch biopsy but subjected to reexamination at 4 months. If both the Pap smear and the colposcopy were normal at this 4-month visit, the cervix was considered healthy and no biopsy was performed.

Histology

All biopsies were read by the same pathologist (R.D.). In the event of discordance with the smear result, the biopsy was reviewed by a second pathologist (L.Z.). Final diagnosis was obtained when the two pathologists reached an agreement. The pathologists were unaware of the HPV DNA status. All cases with squamous metaplasia, reactive or inflammatory changes, and satisfactory colposcopy were considered normal and biopsy was not performed. In the final analysis, these patients were included in the group of patients with a normal cervix.

Statistical analysis

The correlation between variables was tested with χ^2 test. Sensitivity, specificity, and predictive values were calculated for Pap smear, HPV DNA testing, and colposcopy in detecting histologically confirmed high-grade CIN. The 95% confidence intervals (95% CI) were computed based on the binomial distribution.

Results

Table 1 shows the relationship between Pap smear abnormalities, HPV detection, and histology. Of the 52 patients (13%) who had a final cytologic diagnosis within normal limits (WNL), 16 had external genital warts, 14 had been treated for CIN, and 22 had initial abnormal smears reassessed as WNL. Most of the advanced lesions (CIN2-3/cancer) had a cytologic diagnosis of LSIL or HSIL, while only 10% were classified as ASCUS and 5.5% as WNL. All six cases of microinvasive cancer had an HSIL smear. The HPV test positivity in the whole series was 68%. HPV

Table 1. Cytologic abnormalities and HPV DNA detection rates related to biopsy results

	Histologic diagnosis (LEEP cone or punch biopsy)			
	Normal[a] (%)	CIN1 (%)	CIN2-3/cancer[b] (%)	Total (%)
Cytologic diagnosis[c]				
WNL	26 (28.3)	15 (15.6)	11 (5.5)	52 (13.4)
ASCUS	37 (40.2)	15 (15.6)	20 (10.0)	72 (18.5)
LSIL	24 (26.1)	56 (58.3)	85 (42.3)	165 (42.4)
HSIL	5 (5.4)	10 (10.4)	85 (42.3)	100 (25.7)
HPV DNA testing[c]				
Negative	70 (76.1)	32 (33.3)	21 (10.4)	123 (31.6)
Positive	22 (23.9)	64 (66.7)	180 (89.6)	266 (68.4)
Total	92 (100.0)	96 (100.0)	201 (100.0)	389 (100.0)

[a]Including women with no colposcopically visible lesion and those with LEEP cone or punch biopsy classified as negative, squamous metaplasia, or reactive changes.
[b]Including six cases of microinvasive cancer.
[c]$P < 0.0001$ for linear trend.

prevalence increased in parallel with the lesion grade, from 24% in normal cervices to 67% for CIN1 and 90% for CIN2-3/cancer ($P < 0.0001$ for the trend).

The interrelationship between referral cytology, HPV detection, and histology is summarized in Table 2. The prevalence of HPV infection was 47.9% in ASCUS and 76.4% in LSIL smears. Only 8% of the Pap smears classified as ASCUS and tested negative for HPV were diagnosed as CIN2-3. Among those ASCUS smears that were HPV positive, 50% (17 out of 34) had CIN2-3 disclosed in the biopsy. Of the women with LSIL cytology, 15% of those who tested HPV negative had a histologic diagnosis of CIN2-3, as compared with 63% of those who were HPV positive.

Performance characteristics of cytology (with 2 cutoff points), HC2, and colposcopy in the detection of CIN2-3/cancer are shown in Table 3. The highest sensitivity was obtained for cytology using the ASCUS cutoff, which was compromised by the lowest specificity. HC2 assay had acceptable sensitivity and higher specificity than cytology with either cutoff points. HC2 had higher sensitivity, positive predictive value (PPV), and NPV than cytology with LSIL cutoff. The performance of colposcopy was calculated for two thresholds, major and minor changes. Minor changes on colposcopy showed an equal sensitivity but a lower specificity than HC2 test. The combination of both minor and major changes had the highest sensitivity and NPV of all and a specificity higher than ASCUS cytology.

Table 4 presents the performance of HPV DNA testing in detecting high-grade CIN stratified according to Pap smear categories. In women with ASCUS, HPV testing showed a sensitivity of 85% and a specificity of 66.7%. Sensitivity was higher (92.6%) and specificity lower (41.3%) among LSIL cases. PPV was high for HPV testing among ASCUS or LSIL smears.

Table 5 shows the performance of colposcopy in HPV-positive women stratified according to referral Pap test. The highest specificity of colposcopy (80%) was observed (in HPV-positive patients) with the major-change cutoff, following an ASCUS Pap. Using the minor-change cutoff, however, colposcopy detected high-grade CIN with 66.7% and 79% sensitivity, among ASCUS and LSIL categories, respectively. Both figures were markedly higher when the major-change cutoff was used, 87.5% and 98.4%, respectively.

Performance of cytology and HPV testing in different age group is depicted in Table 6. Using ASCUS cutoff, sensitivity of cytology increased with advancing age, while sensitivity of HC2 test decreased. With the LSIL threshold, no clear trend was observed.

The semiquantitative viral load, measured as RLU/PC, was correlated with the severity of squamous intraepithelial lesions in Table 7. Of the CIN2-3/cancer lesions, two thirds (67%) had an RLU/PC greater than 100, in contrast to 40% in CIN1 and 8% in women with normal cervix.

Discussion

This study shows that in patients referred to a colposcopy clinic with an ASCUS or LSIL Pap smear, the HPV test significantly increases the specificity of colposcopy, thus advocating the use of HPV testing in the secondary triage together with colposcopy. One of the major strengths of our study, as compared to other studies[15,28], is that the performance of HC2 HPV test was calculated using LEEP cone biopsy as the gold standard in 88% of these patients. Using this approach, any misclassifications due to low reproducibility of colposcopic impression and the punch biopsy are reduced to a minimum[29–31].

9 **Table 2.** Interrelationship of referral cytology, HPV detection, and histology

		Histologic diagnosis							
		Normal		CIN1		CIN2-3/cancer		Total	
Cytology	HPV testing	*N*	%	*N*	%	*N*	%	*N*	%
WNL	HPV−	23	72	8	25	1	3	32	100
	HPV+	3	15	7	35	10	50	20	100
ASCUS	HPV−	28	76	6	16	3	8	37	100
	HPV+	9	27	8	24	17	50	34	100
LSIL	HPV−	17	44	16	41	6	15	39	100
	HPV+	7	6	40	32	79	63	126	100
HSIL	HPV−	2	13	2	13	11	73	15	100
	HPV+	3	4	8	9	74	87	85	100

See Table 1.

Table 3. Performance of cytology, colposcopy, and HPV DNA testing in identification of biopsy-confirmed high-grade CIN

	Sensitivity		Specificity		PPV		NPV	
Diagnostic method	%	95% CI	%	95% CI	%	95% CI	%	95% CI
Cytology (ASCUS or worse)	94.5	91–97	21.9	17–28	56.5	51–62	78.8	66–88
Cytology (LSIL or worse)	84.6	79–89	49.2	42–56	64.2	58–70	74.8	67–82
HPV DNA testing	89.6	85–93	54.3	47–61	67.7	62–73	82.9	75–89
Colposcopic major changes[a]	84.0	78–88	82.2	76–87	84.0	78–88	82.2	76–87
Colposcopic minor changes[b]	87.5	72–95	43.2	36–51	25.0	18–34	94.1	86–98
Colposcopic minor and major changes	98.0	95–99	35.6	29–42	62.8	57–68	94.1	86–98

[a]Atypical TZ with major changes suggesting CIN2-3/cancer.
[b]Atypical TZ with minor changes suggesting CIN1.

In the present cohort, a total of 201 high-grade CIN/cancers were diagnosed. Consistent with some previous studies[1], more than 50% of the cases had an ASCUS or LSIL diagnosis in the previous Pap smear. Altogether, 11 cases (5.5%) of CIN2-3 were diagnosed among women treated for previous CIN or with genital warts and in whom the smears had been misclassified as WNL. This highlights the importance of a close follow-up of all such women.

The prevalence of HPV infection increases in parallel with the severity of cervical lesions, as has been shown in several previous studies by us and others[4,8,12,14,32,33]. In the present series, 90% of CIN2-3 and 60% of CIN1 lesions were positive for high-risk HPV types. The mean age of our patients was 36 years, an age when the prevalence of cervical HPV infection in general population should be below 10%[14,34–36]. The relatively high prevalence of 24% of HR HPV infections among our patients with normal Pap smears is due to the specific characteristics of this population, ie, 52 women had a previous or current 7 HPV-associated lesions; treated CIN or genital warts, respectively.

In the present series, 48% of the women with ASCUS and 76% of those with LSIL smears harbored HR HPV DNA. These results are in full agreement with those of other studies using the HC2 test, the performance of which is comparable to polymerase chain reaction[21,22]. However, only 85% of women with HSIL were HR HPV positive, which is less than in some previously published studies[4,12,14,20,23]. This underestimation is most likely due to the fact that the HC2 kit used in the present study contained only 11 viral types and not 13 as used in the latest version of the same test. In comparable study conditions, the Kaiser study reported 90% prevalence for the HPV test (11 viral types)[4], while in the ALTS study, this figure was 96% for 13 viral types[12]. Other possible explanation might be the time lapse between the referral cytology and the LEEP excision, resulting in disappearance of the disease (even though this was less than 3 months for all cases), or inadequate samples for the HPV test (with few or no cells from the TZ).

The use of HPV test as a secondary triage for recognizing high-grade disease does not necessarily apply equally well to patients under primary screening. An effective screening test must show optimal sensitivity and reliability in the detection of cancer precursor lesions, whereas a secondary triage requires a test with high specificity to avoid overtreating the patients. In the primary screening, the sensitivity of HPV testing by HC2 in detecting high-grade CIN is estimated to be 12–25% higher than that of conventional Pap test or liquid-based cytology[14,20,37]. In the present study, the gold standard was histologic evaluation of the LEEP specimen, thus distinguishing our study from those evaluating HPV test in colposcopic biopsy because of the poor correlation between biopsy and cone sample[31].

In the management of abnormal Pap smears with ASCUS smear as the cutoff for colposcopy, adding

Table 4. Performance of HPV DNA testing in detecting biopsy-confirmed high-grade CIN/cancer in different Pap smear categories

	Sensitivity		Specificity		PPV		NPV	
Referral Pap	%	95% CI	%	95% CI	%	95% CI	%	95% CI
WNL	90.9	62–98	75.6	61–86	50.0	30–70	96.9	84–99
ASCUS	85.0	64–95	66.7	53–78	50.0	34–66	91.9	79–97
LSIL	92.9	85–97	41.3	31–52	62.7	54–71	84.6	70–93
ASCUS + LSIL	91.4	85–95	51.1	43–60	60.0	52–67	88.2	79–93

See Table 1.

Table 5. Performance of colposcopic examination in detecting biopsy-confirmed high-grade CIN/cancer in different Pap smear categories

		Sensitivity		Specificity		PPV		NPV	
Referral Pap	Colposcopy	%	95% CI	%	95% CI	%	95% CI	%	95% CI
ASCUS	Minor changes	66.7	21–94	54.5	35–73	16.7	5–45	92.3	67–99
	Major changes	87.5	64–97	80.0	38–96	93.3	70–99	66.7	30–90
LSIL	Minor changes	78.9	57–92	33.3	21–48	34.9	22–50	77.8	55–91
	Major changes	98.4	92–100	60.0	36–80	91.3	82–96	90.0	60–98
ASCUS + LSIL	Minor changes	77.3	57–90	40.6	30–53	30.9	20–44	83.9	67–93
	Major changes	96.3	90–99	65.0	43–82	91.7	84–96	81.3	57–94

See Table 1.

HPV test did not improve the sensitivity of Pap test in our study (94.5% compared to 89.6% for HPV testing) (Table 3). However, specificity increased markedly when HPV test was added (54.3% compared to 21.9% for Pap alone), leading to an optimal balance between sensitivity and specificity, 89.6% and 54.3%, respectively. This approach may well have an effect on the cost, lead to fewer visits and therapeutic interventions, and thus a more rational overall management of the patients.

Using HPV test as the criterion for colposcopy referral in patients with minor cytologic abnormalities results in higher PPV and NPV (67.7% and 82.9%, respectively) than performing colposcopy for all patients with ASCUS (or higher) smears (56.5% and 78.8%, respectively) (Table 3). Furthermore, when colposcopy is used selectively only in patients with minor cytologic abnormalities and HR HPV–positive test, the sensitivity for the detection of CIN2-3/cancer is increased by 6% as compared to colposcopy of all patients with an abnormal Pap.

Selective use of HPV test according to the Pap smear categories or colposcopic changes further increased the specificity of colposcopy, with very little effect on sensitivity[38,39]. This is because in most cases, minor colposcopic changes are very common, not related to underlying clinical disease, and are not reproducible. Thus, these minor changes are responsible for the highest rate of false-positive results, and specificity of colposcopy is therefore extremely low[9–11]. In women with ASCUS or LSIL smear, HPV test significantly increases the specificity of colposcopy in cases with these minor changes.

In countries, where colposcopy is expensive and not routinely performed, eg, in the United States and Canada, referring women with minor or equivocal smears and with positive HR HPV test may decrease the number of unnecessary repeat Pap tests, while the number of colposcopies remains the same regardless of whether a repeat Pap or an HPV test is selected[12]. In addition, it is important to note that the proportion of false negatives is relatively low. In our cohort, the false-negative rate for referral of women with HPV-positive ASCUS was 8.1%, and for those with LSIL, 15.4%.

Although HPV DNA testing has been suggested for the management of patients with ASCUS smears[4–8,15,16,20,21], its role in patients with LSIL is more controversial[12,23]. In women with LSIL smears in the present series, 76.4% were HPV positive. Such a high prevalence may limit the clinical value of the test in the primary triage of women with LSIL. However, LSIL is a common finding in Pap tests, and if 25% of LSIL patients could avoid the referral for colposcopy,

Table 6. Performance of cytology and HPV testing for detection of high-grade CIN and cancer in different age groups

Age group	Diagnostic method	Sensitivity	Specificity	PPV	NPV
30 years or less	Cytology (ASCUS or worse)	92.2	26.0	52.2	79.2
	Cytology (LSIL or worse)	81.3	39.7	54.2	70.7
	HPV DNA	93.8	49.3	61.9	90.0
31–40 years	Cytology (ASCUS or worse)	94.1	20.3	63.0	70.6
	Cytology (LSIL or worse)	88.2	54.2	73.5	64.2
	HPV DNA	89.4	55.9	74.5	78.6
>41 years	Cytology (ASCUS or worse)	98.1	18.2	53.1	90.9
	Cytology (LSIL or worse)	82.7	56.4	64.2	77.5
	HPV DNA	84.6	58.9	65.7	80.5

See Table 1.

Table 7. HPV viral load and lesion grade in biopsy

	Histology (LEEP cone or punch biopsy)			
HPV viral load	Normal[a] (%)	CIN1 (%)	CIN2-3/cancer (%)	Total (%)
<1 RLU/PC	70 (76.1)	32 (33.3)	21 (10.4)	123 (31.6)
1–100 RLU/PC	15 (16.3)	26 (27.1)	46 (22.9)	87 (22.4)
>100 RLU/PC	7 (7.6)	38 (39.6)	134 (66.7)	179 (46.0)
Total[b]	92 (100.0)	96 (100.0)	201 (100.0)	389 (100.0)

[a]Includes women with no colposcopically visible lesion and those with LEEP cone or punch biopsy classified as negative, squamous metaplasia, or reactive changes.
[b]$P < 0.001$ for linear trend.

this could be a substantial advantage and lead to cost savings.

In contract to situation in the United States and Canada, in countries where colposcopy is relatively inexpensive and routinely performed (eg, in Latin European countries), HPV testing may have a major practical impact. This is shown by the present study, where the efficacy of HC2 test in predicting cervical disease was clearly demonstrated: over 90% of women with major colposcopic changes and 77.3% of those referred after ASCUS–LSIL smears with only minor changes of the TZ, but positive HPV test, had a high-grade CIN.

The prevalence of HPV infection has been shown to decrease significantly with age, peaking in young women[14,34]. In a primary screening setting, HPV DNA testing would have a very low PPV among young women. However, our results show that this is not the case for the triage of women with abnormal Pap smears, given that the performance of the test did not change dramatically with age. Indeed, the sensitivity of HPV testing increased slightly with advanced age, while the specificity decreased. Semiquantitative measurement of the viral load by HC2 may prove useful in the detection of underlying significant disease. In the present series, two thirds (67%) of our patients with a viral load >100 RLU/PC proved to harbor CIN2-3/cancer lesions, as contrasted to only 10.4% of those with a negative HC2 test (Table 7). Specificity of HPV testing has been reported to increase with a high viral load cutoff by several authors[14,40–42]. Moreover, in order to standardize the viral load measurements, the exact number of epithelial cells collected in the sample needs to be known, but HC2 assay does not yet enable this measurement[22,42].

To conclude, our data clearly demonstrate that in settings where colposcopy is routinely performed (eg, in large colposcopy clinics), HPV testing by HC2 is a useful adjunct diagnostic tool. While increasing the accuracy of colposcopic evaluation, HPV testing may have an important role in the secondary triage of women with minor cytologic abnormalities.

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