Human papillomavirus (HPV) testing in the management of women with abnormal pap smears. Experience of a colposcopy referral clinic

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Summary

Background and Objectives: Several detailed algorithms for the appropriate use of human papillomavirus (HPV) testing in the management of women with abnormal Pap (Papanicolaou) smears have been launched, but their direct country-to-country adoption is difficult. This necessitates their testing in individual settings, which is ongoing in our colposcopy referral clinic.

Methods: A series of 224 consecutive women attending the clinic with the usual referral indications (ASC-US or higher in Pap) were examined by the conventional diagnostic tools (PAP smear, colposcopy, punch biopsy) and subjected to HPV testing and viral typing for both low-risk (L-R) and high-risk (H-R) types by nested PCR-based techniques. Predictors of the high-grade diagnostic categories were analysed using both univariate- and multivariate modelling, and the performance characteristics (sensitivity, specificity, NPV, PPV) of all tests in detecting high-grade CIN were calculated.

Results: In the PAP test, ASC-US smears were most common (37.9%), followed by low-grade squamous intraepithelial lesions (LSIL) (26.3%) and high-grade SIL (HSIL) (4.9%). Colposcopy was performed for 180 women, of whom 48.3% had a normal transformation zone (TZ), 40.6% had ATZ1 (abnormal TZ grade 1), and 5.6% had ATZ2. In biopsy (n = 71), 49.3% had CIN1, 5.6% CIN2, and 16.9% CIN3. The HPV test was positive in 64 (28.8%) women, more often in those aged < 35 years (p = 0.025). High-grade colposcopy (ATZ2) was significantly associated with HSIL in the Pap test (OR 20.5; 95% CI: 4.34-96.47), and with HPV test positivity (OR 6.37; 95% CI: 1.58-25.73). The most significant predictors of CIN3 were HSIL in the PAP, HPV test positivity, and high-grade colposcopy. HSIL and HPV test (for H-R types), but not colposcopy, retained their significance as independent predictors of CIN3 also in adjusted multivariate models: OR 88.27; 95% CI 4.17-1867.04, and OR 19.46; 95% CI 2.01-187.75, for the HSIL and H-R HPV test, respectively. Changing the cut-off level of the Pap test from ASC-US to HSIL increased the specificity of the test up to 96.4%, with the loss in sensitivity from 87.5% to 43.8%. Colposcopy (ATZ2) had 92% specificity, and NPV competing with that of the Pap test. The sensitivity of HPV test exceeds that of the Pap test at HSIL cut-off level, but the specificity of the PAP test is clearly superior.

Conclusions: Accurate predictors of significant cervical pathology (CIN3) are well defined, but the problem is the different performance of the diagnostic tools in clinical practice. A proficient combination of the tests is likely to result in the most satisfactory clinical practice in the management of women with abnormal Pap tests (MAPS).

Key words: Pap smear; Colposcopy; Biopsy; HPV typing; PCR; Test performance.

Introduction

The causal association of human papillomavirus (HPV) with the development of cervical cancer and its precursors has been convincingly established, the virus being confirmed as the single most important etiological factor of this disease [1-5]. This causal association of an oncogenic virus with the second most frequent malignancy among women worldwide has important implications in the early detection, diagnosis, treatment and follow-up as well as in prevention of this disease [1, 4, 5]. Cervical cancer is the only human malignancy, where organized screening programs based on HPV testing can be targeted to the etiological agent instead of clinical lesions (CIN), detected by screening with the Pap (Papanicolaou) test [6-8]. The feasibility and cost-effectiveness of such screening strategies are currently under intense testing in different settings [9-12].

Apart from organised screening of asymptomatic women, HPV testing has potential implications in the clinical management of women with abnormal Pap smears (MAPS) [13-15]. Such potential areas of HPV testing could include the diagnosis (in cases with equivocal Pap smears), treatment decisions (low- or high-risk lesion) as well as in the post-treatment follow-up of these women (to monitor for residual disease or recurrence) [13-18]. Recently, several professional societies have launched detailed recommendations and algorithms for the appropriate use of HPV testing as an integral part of the management of women with equivocal cytological smears (ASC-US) or those with low-grade squamous intraepithelial lesions (LSIL) [19-21]. Meritorious as many of these algorithms are, they are invariably based on the documentation and accepted practice in the dis-

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tinct (high-resource) diagnostic settings of their origin, and are unlikely to be directly transferable to low-resource settings, e.g. in most of the developing countries [4-6, 22-24].

Even in developed countries, the different infrastructure of the public health organizations makes a direct adoption of the published recommendations for the usage of HPV testing in patient management difficult, if not impossible, in many countries [4-6, 21-23]. This makes extensive (and expensive) testing of these protocols mandatory under field conditions, i.e., in routine clinical practice or as a part of national screening programs [9-12, 25]. The recent literature is crowded with reports of such testing for optional diagnostic tools in patient triage [13-17, 26-28]. Apart from differences in the clinical settings, additional difficulties are due to the rapid progress of molecular technology, providing the end-users with a variety of more and more sophisticated molecular tools for HPV testing [1, 4, 5].

We recently initiated a survey in our clinic to evaluate the applicability and performance of different molecular techniques for HPV testing. In this communication, we report our experience on the use of PCR-based technologies for HPV detection and viral typing in women referred to this colposcopy clinic with the usual indications, i.e., due to an abnormal Pap test, and examined with the conventional diagnostic tools (Pap test, colposcopy and biopsy).

Material and Methods

Study group

The material of the present study consists of a series of 224 consecutive women referred to the Department of Surgery, Section of Gynecology and Obstetrics, University Hospital "Policlinico Tor Vergata" (Rome, Italy) for further examinations and treatment because of a detected abnormality (ASC-US, LSIL or HSIL) in their recent Pap tests. The mean age of the women was 36.8 years (range: 18-71). Only 19 of the women were postmenopausal. All women gave an informed consent to participate in the study. The study protocol was approved by the institutional Ethical Committee.

Methods

PAP test

All women were subjected to a new (confirmatory) Pap smear, taken according to routine procedures, processed according to conventional techniques, and screened and interpreted using the Bethesda 2001 system (TBS 2001) [29].

Colposcopy

Colposcopic examination was performed for 180 women using a Zeiss OPM1F (Karl Zeiss, Jena, Germany). All examinations were done using both acetic acid and iodine application, and the terminology used is the Italian equivalent to the 1990 Colposcopy Nomenclature of the IFCPC [30]. The low-grade abnormalities are called ATZ1 (atypical transformation zone grade 1), and high-grade colposcopic abnormalities are ATZ2, in the Italian modification of the nomenclature.

Biopsy

Directed punch biopsies were taken from all colposcopic abnormalities (n = 71), according to routine procedures. The biopsies were fixed in 10% neutral formalin and processed into HE-sections for light microscopy. On histological examination, the lesions were graded using the cervical intraepithelial neoplasia (CIN) nomenclature and categorized as CIN [1-3]. The morphological evidence for HPV infection was not separately recorded in the CIN lesions [4, 5].

Treatment by LLETZ

Following the histological confirmation of high-grade lesions, the women underwent large loop electrosurgical excision of the transformation zone (LLETZ), according to the technique described before [31]. Altogether, LLETZ treatment was instituted in 18 women.

Sampling for HPV testing

Exe- and endocervical specimens were collected at the Department of Surgery, Section of Gynecology and Obstetrics, and transported to the laboratory of virology in 2 ml of sterile PBS buffer. In the laboratory, the samples were transferred to an Eppendorf tube and centrifuged at 8.000 rpm for 1 min. The supernatant was removed and the pellet stored frozen at -80°C before performing the HPV test.

Polymerase chain reaction (PCR)

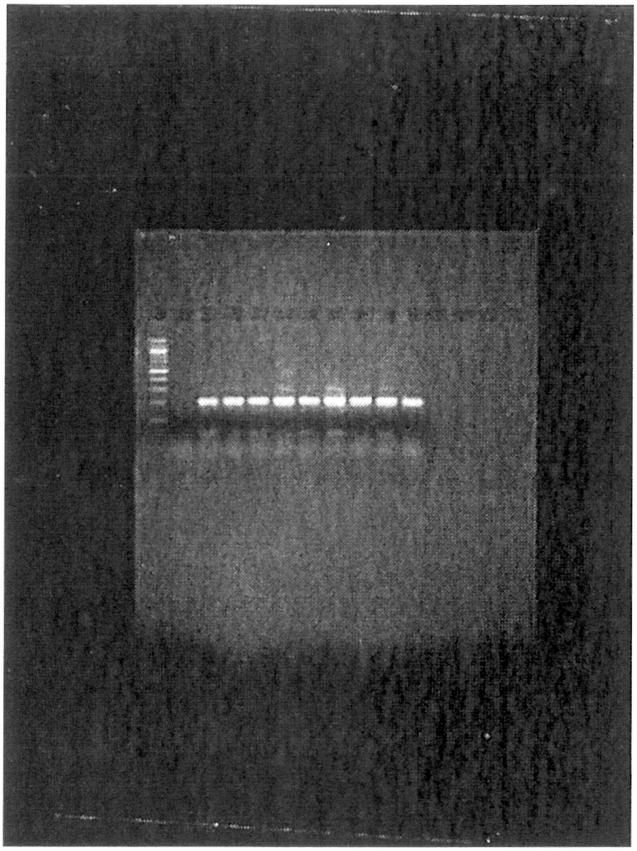
The pellet was resuspended in 200 µl of sterile PBS and the DNA extracted according to the manufacturer's instructions (QIAamp DNA Mini kit, Qiagen, Germany). An aliquot of 5 µl of DNA was first amplified with the b-actin primers (sense: 5'-GGCGGCACCACCATGTACCCT-3', anti-sense: 5'-AGGGGCCGGACTCGTCATACT-3'). The PCR mix contained 200 µM each dNTP, 1.5 mM $MgCl_2$, 1X PCR buffer, 40 pmol sense and anti-sense primer, 1.25 U AmpliTaq Gold (Applied BioSystem, The Netherlands). The PCR conditions were: 94°C, 10 min, for one cycle; 94°C, 30 sec, 60°C, 30 sec, 72°C, 30 sec, for 25 cycles, Finally, 72°C, for 7 min. The amplicons were electrophoresed on a 2% agarose gel, stained with ethidium bromide and visualised uncer UV light (Figure 1A). All samples gave the expected fragment size of 202 bp.

The samples were then screened for the presence of HPV with one round PCR using the degenerated primers MY09/MY11. The PCR conditions for the MY09/MY11 primers were: 94°C for 10 min, one cycle; 94°C, 30 sec, 55°C, 45 sec, 72°C, 30 sec for 40 cycles, followed by an extension step at 72°C for 7 min. The PCR mix contained 40 pmol for each primer, 2 mM $MgCl_2$, 1.25 U AmpliTaqGold. The amplified product was run on 1% agarose gel, stained with ethidium bromide and visualised with UV light. This primer pair amplify a 450 bp fragment of the L1 gene [32] (Figure 1B).

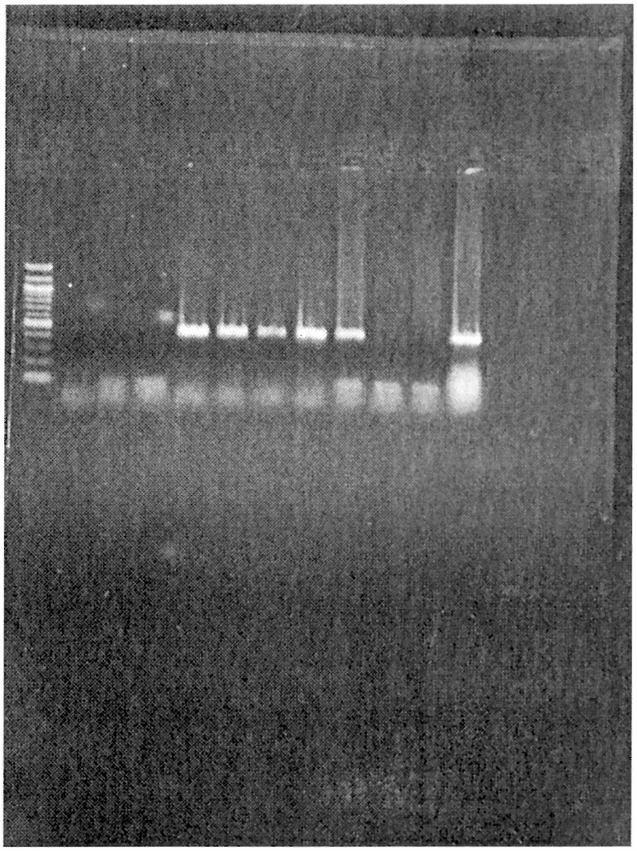
HPV genotyping

Positive samples were subjected to HPV typing using primer sets specific for the E6/E7 region (Amplimedical, Torino, Italy) of the low-risk (L-R) (HPV 6/11) and high-risk (H-R) (HPV 16, 18, 31, 33, 35, 45, 52, 53, 58, 66) HPV types, respectively. The PCR conditions were the following: 94°C for 5 min, 72°C for 10 min (1 cycle), then 40 cycles at 94°C for 1 min, 55° for 1 min, 72°C 1.5 min. Finally, 72°C for 5 min, 80°C for 10 min (1 cycle). The PCR sensitivity was 50 HPV genomes/reaction. The primer sets specific for L-R types (HPV 6/11) amplify a 230 bp fragment, while those for the H-R types amplify a 240 bp (HPV 16, 31, 33, 35, 52, 58) and 270 bp (HPV 18/45), respectively [33]. The PCR products were run on 3% agarose gel, stained with ethidium bromide, and visualised under UV light (Figure 1C).

1A



1B



1C

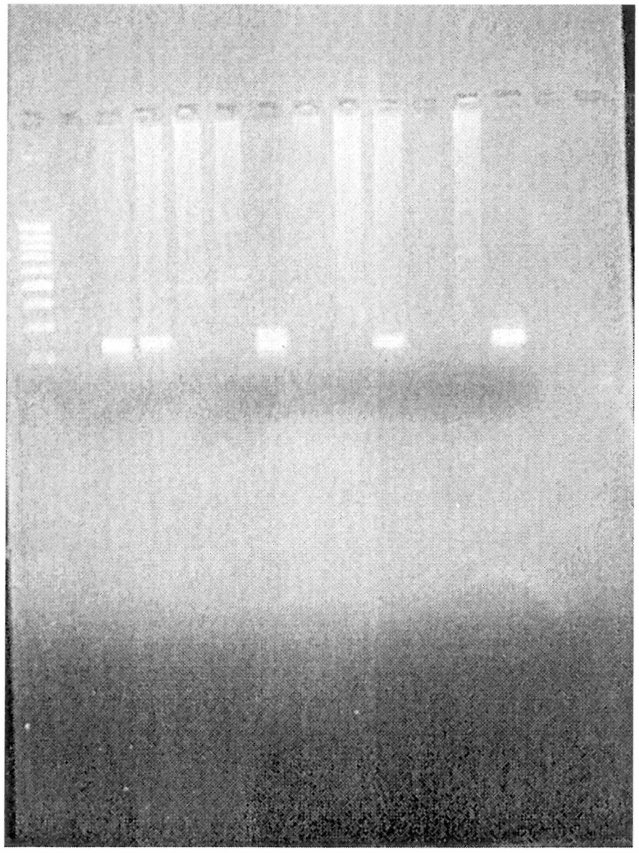


Figure 1A. — Representation of a ß-actin screening of samples included in the study. Lane I: 100 bp ladder; lane 2: negative control; lanes 3-11: ß-actin samples. The PCR amplifies a 202 bp fragment.

Figure 1B. — Samples screened with My09/11. Lane I: 100 bp ladder; lane 2: negative control; lanes 3-11: cervical samples. Lane 12: positive control. This primer set amplifies a 450 bp fragment.

Figure 1C. — HPV typing carried out with primer sets against the E6/E7 region of high- and low-risk types. Lane I: 100 bp ladder; lane 2: negative control for high-risk types; lanes 3-6: samples amplified with primers for high-risk types; lanes 7: positive control for high-risk types (240 bp fragment: types 16, 31, 33, 35, 52, 58; 270 bp fragment: types 18, 45). Lane 8: negative control for low-risk types; lanes 9-12: samples amplified with primers for low-risk types; lane 13: positive control for low-risk types (230 bp fragment: types 6, 11).

Statistical analyses

Statistical analyses were performed using the SPSS, computer program package (SPSS for Windows, version 11.5). Frequency tables were analysed using the Chi-square test, with a likelihood ratio (LR) being used to assess the significance of the correlation between the categorical variables. Odds Ratio (OR) and Cohen kappa (κ) were calculated where appropriate. Differences in the means of continuous variables (age) between the groups were analyzed using ANOVA (analysis of variance) test. The normal distribution of the continuous variables was tested by Kolmogorov-Smirnov's test (with Lilliefors significance correction), and log transformations were made to satisfy the normal distribution requirement of the ANOVA. Binary logistic regression models were used to analyze the power of different variables as predictors of the outcome variables, using the stepwise backward approach and LR (likelihood ratio) statistic for removal testing ($p = 0.10$ probability for stepwise removal, and $p = 0.05$ probability for stepwise entry). Performance characteristics of the diagnostic tests (Pap, colposcopy, PCR) in detecting significant histology were determined using the conventional contingency tables to calculate sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). Confidence intervals (95% CI) were calculated based on the F-distribution (± 1.96 x SE). In all tests the values $p < 0.05$ were regarded as statistically significant.

Results

The results of the PAP test, colposcopy, histological biopsy and HPV test are shown in Table 1. Of the 224 women, 180 were subjected to colposcopy, and of those, directed punch biopsy was taken in 71 women.

Altogether, the HPV test was positive in 64 women (28.8%) and negative in 158 (Table 1). HPV test results were classified into five categories: 1) negative L-R/negative H-R (n = 158), 2) positive H-R/negative L-R (n = 33), 3) positive H-R/positive L-R (n = 8), 4) negative H-R/positive L-R (n = 5), and 5) positive not typed (n = 14). The distribution of the test results was practically identical across the age groups ($p = 0.922$). HPV test positivity was more common in women aged less than 35 years than those beyond that age ($p = 0.025$), and the mean age of HPV-positive women (34.0) was lower than that (38.1) of HPV-negative women ($p = 0.013$).

The factors predicting the results of the three diagnostic tests (PAP smear, colposcopy and biopsy) as outcome variables in univariate analysis are shown in Table 2. The result of the Pap test was most closely related to age when used as a continuous variable in the ANOVA test. The Pap test result was also significantly related to HPV test positivity ($p = 0.001$). On the other hand, there were no significant predictors for the detection of HSIL in the Pap test.

Colposcopic patterns were closely predicted by HSIL in the PAP smear ($p = 0.008$) and positive HPV test ($p = 0.001$), but not the HPV types. When analyzed as a separate variable, high-grade colposcopy (ATZ2) was significantly associated with the Pap test result, HSIL in the Pap test (OR 20.5; 95% CI 4.34-96.47), and also with HPV test positivity (OR 6.37; 95% CI: 1.58-25.73), but not with the HPV types.

The grade of cervical lesion in the biopsy was significantly ($p = 0.0001$) predicted by the Pap test results, and HSIL in the PAP test, as well as HPV test positivity ($p = 0.005$). Finally, the most significant predictors of high-grade CIN (CIN3) were: Pap test result, HSIL in the Pap, HPV test positivity, and high-grade colposcopy (OR 5.11; 95% CI 1.07-24.30).

In the next step, HSIL, high-grade colposcopy and high-grade CIN (as dichotomous dependent variables) were included in the multivariate logistic regression model to find out their independent predictors. Both crude and adjusted ORs were calculated to disclose the potential confounding factors. The results are shown in Table 3.

As in the univariate model, there were no independent predictors of HSIL in the Pap disclosed by this multivariate model. When adjusted for the other predictors, HSIL maintained its independent predictive value of high-grade colposcopy (OR 13.655; 95% CI 2.688-69.371). The significance of the HPV test (±) was however confounded by age, and lost its independent predictive value, despite the high OR 4.295 (95% CI 0.974-18.938). Finally, both HSIL Pap and HPV test results maintained their significance as independent predictors of high-grade CIN, and indeed, the adjusted ORs were markedly higher than the crude (uni-

Table 1. — *Key clinical data and test results of the patients.*

	Number	Percent
Age:		
Mean	36.8	
SD	10.9	
Range	18-71	
PAP smear:		
WNL	62	27.7
ASC-US	85	37.9
LSIL	59	26.3
HSIL	11	4.9
Inflammation	6	2.7
HPV	1	0.4
Biopsy histology:		
Negative	10	14.1
Metaplasia	2	2.8
CIN1	35	49.3
CIN2	4	5.6
CIN3	12	16.9
Chronic cervicitis	4	5.6
Condyloma	4	5.6
Colposcopy:		
Insufficient	3	1.7
NTZ	87	48.3
TA1	73	40.6
TA2	10	5.6
Dystrophy	2	1.1
Ectopion	3	1.7
Condyloma	2	1.1
HPV test results:		
Negative H/L	158	71.2
Pos H/Neg L	35	15.8
Pos H/Pos L	9	4.1
Neg H/Pos L	6	2.7
Positive not typed	14	6.3

Table 2. — *Predictors of diagnostic test results in univariate analysis.*

Dependent Variable	Predictor	Statistics*
Pap test result:		
	Age Group	0.171
	Age < 35 and > 35	0.039
	Age	0.002**
	HPV test (±)	0.001
	HPV type	0.101
HSIL in PAP:		
	Age Group	0.704
	Age < 35 and > 35	OR 0.61 (0.17-2.15) 0.323
	Age	0.387**
	HPV test (±)	OR 2.97 (0.87-10.16) 0.074
	HPV type	0.534
Colposcopy patterns:		
	Age Group	0.387
	Age < 35 and > 35	0.023
	Age	0.000**
	Pap test	0.045
	HSIL PAP	0.008
	HPV test (±)	0.001
	HPV type	0.623
Significant colposcopy (ATZ2):		
	Age Group	0.779
	Age < 35 and > 35	OR 0.71 (0.19-2.63) 0.431
	Age	0.737**
	PAP test	0.006
	HSIL PAP	OR 20.5 (4.34-96.47) 0.0001
	HPV test (+/-)	OR 6.37(1.58-25.73) 0.007
	HPV type	0.073
Grade of cervical lesion:		
	Age Group	0.744
	Age <35 and >35	0.244
	Age	0.362**
	PAP test	0.0001
	HSIL PAP	0.001
	HPV test (+/-)	0.005
	HPV type	0.444
	Colposcopy	0.417
	Significant colposcopy	0.422
High-grade histology (CIN3):		
	Age Group	0.181
	Age < 35 and > 35	OR 1.24 (0.40-3.80) 0.464
	Age	0.327**
	Pap test	0.0001
	HSIL PAP	OR 19.4 (3.46-109.05) 0.0001
	HPV test (+/-)	OR 5.25 (1.49-18.47) 0.007
	HPV type	0.032
	Colposcopy	0.081
	Significant colposcopy	OR 5.11 (1.07-24.30) 0.045

*Chi-square test with LR statistics, if not otherwise indicated; **Age as continuous variable in ANOVA.

variate) ORs, indicating that both predictors were negatively confounded by age.

The performance characteristics of the three diagnostic tests (Pap smear, colposcopy and HPV test) in detecting significant cervical pathology (CIN 3) are depicted in Table 4. Changing the cut-off level of the Pap test from ASC-US to HSIL increased the specificity of the test up to 96.4%, with a loss in sensitivity from 87.5% to 43.8%. The NPV of the Pap test remained high at all cut-off levels. Colposcopy had 92% specificity, when ATZ2 was used as the cut-off value. NPV competed with that of the Pap test. The sensitivity of the HPV test exceeded that of the Pap test at the HSIL cut-off level, as did NPV, but the specificity of the Pap test was clearly superior.

Table 3. — *Factors predicting high-grade diagnostic categories in multivariate logistic regression analysis.*

Dependent Variable	Covariates	Crude OR (95% CI) (95% CI)	*Adjusted OR
HSIL in Pap test:	Age	0.977 (0.927-1.030)	0.968 (0.918-1.020)
	HPV test (±)	2.978 (0.872-10.169)	3.046 (0.891-10.416)
Significant (ATZ2) colposcopy:	Age	0.990 (0.936-1.047)	0.984 (0.925-1.048)
	Pap test HSIL PAP	NC OR 20.53 (4.370-96.472)	NC 13.655 (2.688-69.371)
	HPV test (±)	OR 6.378 (1.581-25.733)	4.295 (0.974-18.938)
	HPV type	NC	NC
High-grade CIN3:	Age	1.028 (0.973-1.087)	1.114 (0.999-1.242)
	Pap test HSIL PAP	NC OR 19.44 (3.467-109.05)	NC 31.980 (3.037-336.772)
	HPV test (±)	OR 5.25 (1.493-18.472)	9.970 (1.519-65.445)
	HPV type	NC	NC
	Colposcopy	NC	NC
	Significant colposcopy	OR 5.11 (1.075-24.303)	1.027 (0.115-9.199)

*Adjusted for the other variables tested in the model; NC, not computable; ATZ2, atypical transformation zone grade 2.

Table 4. — *Performance characteristics of diagnostic tools in detecting significant cervical pathology (CIN3).*

Dependent Test	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
PAP Smear: (ASC-US and more)	87.5 (79.8-95.2)	30.9 (20.1-41.7)	26.9 (16.6-37.2)	89.4 (85.8-93.0)
PAP Smear: (LSIL and more)	87.5 (79.8-95.2)	65.4 (54.3-76.5)	42.4 (30.9-53.9)	94.7 (89.5-99.9)
PAP Smear: (HSIL)	43.8 (32.2-55.4)	96.4 (91.9-100)	77.8 (68.0-87.6)	85.4 (77.1-93.7)
Colposcopy: (ATZ2)	30.8 (19.4-42.2)	92.0 (85.4-98.6)	50.0 (37.7-62.3)	83.6 (74.3-92.9)
HPV Test: (HR-types)*	75.0 (65.0-85.0)	63.6 (52.4-74.8)	37.5 (26.3-48.7)	89.7 (82.4-97.0)

*HPV-positive but non-typed were excluded from the analysis; ATZ2, atypical transformation zone grade 2.

Discussion

A consecutive series of 224 women were included in the study, examined by using the conventional diagnostic tools (Pap test, colposcopy and directed punch biopsy), and supplemented with sampling for HPV testing and typing by nested PCR analysis. These women were not specifically enrolled in a particular design study, and thus not extensively interviewed, e.g., for HPV and CIN risk factors. Thus, our study sample represents a cohort enrolled as a result of systematic random sampling, with no predefined selection criteria and as such is not biased

by accumulation of, e.g., a certain subset of pathology (CIN3) or otherwise skewed by a concentration of HPV infections. This is important because the performance of the HPV test is critically dependent on the prevalence of both HPV infections and CIN lesions in the cohort, as repeatedly emphasized [11, 12, 25].

In the Pap test, the majority of the women had an ASC-US smear (37.9%), while 26.3% had LSIL and only 4.9% showed HSIL. Importantly, however, in 27.7% of the women, the Pap test did not show any abnormality consistent with a cervical lesion. This is not unexpected because we know that Pap test results can fluctuate, and a single Pap test does not necessarily give a representative view of a cervical lesion [1, 4, 5]. It is also well established that cytological abnormalities due to HPV can disappear (sometimes rapidly) because of the substantially high spontaneous regression rate of HPV infections, particularly in young women [1, 4, 5, 34, 35]. In our cohort, almost half (n = 104) of the patients were under 35 years of age, and these younger women were more frequently HPV positive than the older ones (p = 0.025). Of these Pap test negative women, 56.9% were under 35 years of age, and importantly, 32.3% of the cases were HPV-DNA positive. This indicates that those young women had cleared their abnormal Pap test before the first visit to the clinic, but were still HPV-positive when tested with our highly sensitive PCR. By definition, an HPV infection without any clinical signs on Pap test, colposcopy and biopsy, represents a latent HPV infection [1-5]. Indeed, 28.8% of HPV-positive women had completely normal colposcopy, but there were only two cases where biopsy was negative in HPV-positive women. This is consonant with our practice that in women with normal Pap and normal colposcopy, even if HPV-positive, no biopsies are taken. Interesting new data on the events related to clearance of HPV and Pap smear abnormalities have recently been provided by a series of papers [18, 34-37].

Colposcopy was performed in 180 women, of whom 48.3% had normal TZ, 40.6% had ATZ1, and 5.6% had ATZ2. Biopsy was considered indicated in 71 of these women, disclosing 49.3% of CIN1, 5.6% CIN2, and 16.9% of CIN3 lesions (Table 1). However, the undeniable fact is that, out of these 224 women originally referred to the clinic due to an abnormal Pap (ASC-US or higher) test, only 16 cases were detected with significant cervical pathology (CIN2 or higher), and an additional 35 had CIN1 lesions, which have a substantial tendency for spontaneous regression [1-5]. On the other hand, 27.7% had normal Pap tests, and 48.3% had normal TZ on colposcopic examination. When tested for HPV (low- and high-risk types), 71.2% of all women were HPV negative (Table 1). With this high proportion of negative results, we encounter the pertinent question, how to avoid such a high rate of unnecessary referrals for colposcopy [13, 25].

Around this key question a huge amount of work has been done because implementation of any new technology into the algorithms of patient management is essential [9-12, 14-17, 23-25]. Any test to be included in these protocols should significantly increase the performance (specificity, sensitivity, PPV and NPV) of the diagnostic process in order to compensate for the extra costs that are inevitable [4-7, 13, 38]. In other words, we need diagnostic tools that more accurately predict the presence of significant cervical pathology (high-grade CIN). Ideally, such tools should be specific enough to provide the same information, irrespective of the gold standard used, i.e., whether biopsy, Pap test or colposcopy.

With this in mind, we decided to analyze, whether the three available diagnostic tests (Pap, colposcopy, HPV test) could serve as a proxy to each other, i.e., to provide information that accurately predicts the significant abnormality of the other tests (HSIL on Pap, ATZ2 on colposcopy, HPV-positivity). To be clinically meaningful, such information should closely correlate with the true detection of high-grade lesions, established in biopsy as the gold standard [13]. If shown to work, this should have implications in selecting the triage protocols and might modify the practice of colposcopy referrals to the clinic, thus leading to substantial cost savings and better allocation of the available funds [38, 39].

We were unable to establish any significant predictors for HSIL among the tested variables (age and HPV test result). Colposcopy and histology were excluded from this testing because it is not feasible to predict HSIL using colposcopy and punch biopsy. The situation did not change when only the H-R HPV types were included in the analysis. Overall, however, the HPV test (±) proved to be a significant predictor (p = 0.001) of the Pap test result (Table 2), when all abnormalities were taken into account. This failure to establish a link between the H-R HPV types and HSIL must be due to the small number of HSIL cases in the present series (n = 11) because large-scale HPV testing for H-R types using Hybrid Capture II (HCII) has been shown to perform particularly well in detecting HSIL cytology in ROC analysis (sensitivity and specificity approaching 90%) [11, 25].

We then proceeded to look at, whether high-grade (ATZ2) colposcopy could be predicted by the other tests. Indeed, this seems to be the case (Table 2). Both HSIL and HPV-positivity were significant predictors of ATZ2 colposcopy, with very high ORs in univariate analysis. Importantly, when entered in the multivariate model, adjusted for the other predictors tested, HSIL maintained its independent predictive value of high-grade colposcopy (OR 13.655; 95% CI 2.688-69.371). The significance of the HPV test (±) was however confounded by age, and lost its independent predictive value, despite the high OR 4.295 (95% CI 0.974-18.938) (Table 3).

Most importantly, there were excellent predictors of high-grade CIN in the biopsy, namely HSIL, HPV test result and significant colposcopy (Table 2). Apart from the latter (ATZ2), which lost its independent predictive value, both the HSIL and HPV test maintained their significant predictive value in multivariate regression analysis (Table 3). In fact, the ORs of both were significantly higher when adjusted for the other factors, indicating a strong negative confounding (most notably by age). Not unexpectedly, the adjusted ORs became even more

impressive, when only the H-R HPV types were counted as positive test results; OR 88.270 (95% CI 4.173-1867.046), and OR 19.462 (95% CI 2.017-187.755), for the HSIL and HR HPV test, respectively (data not in tables). Thus, high-grade CIN lesions can be accurately predicted by both HSIL in the PAP smear and H-R HPV test. This is just another indication that H-R HPV types are intimately linked with the development of cervical cancer and its precursors [1-5]. Both tests also accurately predict high-grade colposcopy, which, however, because of the inherent sensitivity limitations of the technique, does not independently predict CIN3 in the present analysis. These inherent limitations of colposcopy are however well established, and by no means invalidate the use of this highly valuable diagnostic tool [40-42].

Finally, we calculated the performance characteristics for the three tests by using different cut-off values for the Pap test, ATZ2 as the cut-off for significant colposcopy, and H-R types as HPV test results (Table 4). By changing the cut-off for the Pap test to HSIL, the specificity of the Pap tests exceeded 96%, which is superior to that (92%) of colposcopy. Colposcopy suffers from sensitivity problems, but its PPV is higher than that of the HPV test. The latter, in turn has a high NPV (89.7%), exceeded only by that (94.7%) of the Pap test, when LSIL is used as the cut-off. The performance characteristics of the PCR-based HPV test used in this study deviate from those reported by several studies using the Hybrid Capturl II (HCII) test, which usually has higher sensitivity (around 90-95% in most studies) [8-12, 25, 27, 38, 39]. On the other hand, opinions are unanimous in that the specificity of HCII testing does not usually compete with that of the Pap test [38]. These differences are explained by the fact that HCII assay and PCR techniques do not show even close to 100% concordance with each other [43, 44], sometimes resulting in kappa values that are only substantial (0.6-0.8).

In conclusion, a lot of work remains to be done before the management (triage) of women with abnormal Pap test (MAPS) is performed to satisfaction [13]. Today, still far too many unnecessary referrals for colposcopy are made, resulting in nothing else but increased expenditure because of the substantial proportion of healthy women examined by the Pap test, colposcopy and even biopsy. Accurate predictors of significant cervical pathology (CIN3) are well established, as shown in the present study (HSIL, ATZ2 colposcopy, HR HPV type), but the problem is the different performance of these diagnostic tests in clinical practice. Gaining in specificity usually means loss in sensitivity and vice versa (shown here in the Pap test with different cut-offs), and an ideal test with 100% sensitivity and 100% specificity still awaits. As always, the optimal solution is to be found somewhere in the middle; a proficient combination of two (or perhaps even more) tests will most probably result in the most satisfactory clinical practice in the MAPS. To find out the most optimal combination and usage of the tests, however, a substantial amount of work still needs to be done in different clinical settings.

References

[1] IARC Monographs on the evaluation of carcinogenic risks to humans. Papillomaviruses. Lyon. *IARC,* 1995, *64*, 1.

[2] zur Hausen H.: "Cervical carcinoma and human papillomavirus: on the road to preventing a major human cancer". *J. Natl. Cancer Inst.,* 2001, *93*, 252.

[3] zur Hausen H.: "Papillomaviruses and cancer: from basic studies to clinical application". *Nature Rev. Cancer*, 2002, *2*, 342.

[4] Syrjänen K., Syrjänen S.: "Papillomavirus Infections in Human Pathology". Chichester, J. Wiley & Sons, 2000, 1.

[5] Aubin F., Pretet J.L., Mougin C. (eds): "Papillomavirus Humains. Biologie et Pathologie Tumorale". Editions TEC & DOC, Paris 2003, 1.

[6] Miller A.B., Nazeer S., Fonn S., Brandup-Lukanow A., Rehman R., Cronje H. *et al.*: "Report on consensus conference on cervical cancer screening and management". *Int. J. Cancer,* 2000, *86,* 440.

[7] Franco E., Syrjänen K., de-Wolf C., Patnick J., Ferenczy A., McGoogan E. *et al.*: "New developments in cervical cancer screening and prevention". Geneva, Switzerland, June 17-19 1996. Workshop. *Cancer Epidemiol. Biomarkers Prev.,* 1996, *5,* 853.

[8] Davey D.D., Zarbo R.J.: "Human papillomavirus testing-are you ready for a new era in cervical cancer screening?". *Arch. Pathol. Lab. Med.,* 2003, *127*, 927.

[9] Wright T.C. Jr., Denny L., Kuhn L., Pollack A., Lorincz A.: "HPV DNA testing of self-collected vaginal samples compared with cytologic screening to detect cervical cancer". *JAMA*, 2000, *283,* 81.

[10] Sherlaw-Johnson C., Gallivan S.: "The planning of cervical cancer screening programmes in eastern Europe: is viral testing a suitable alternative to smear testing?". *Health Care Manag Sci.*, 2000, *3,* 323.

[11] Schiffman M., Herrero R., Hildesheim A., Sherman M.E., Bratti M., Wacholder S. *et al.*: "HPV DNA testing in cervical cancer screening: results from women in a high-risk province of Costa Rica". *JAMA*, 2000, *283*, 87.

[12] Lorincz A.T., Richart R.M.: "Human papillomavirus DNA testing as an adjunct to cytology in cervical screening programs". *Arch. Pathol. Lab. Med.*, 2003, *127*, 959.

[13] Syrjänen K.J.: "Management of abnormal PAP smears (MAPS): Implications of terminology used in cytopathology". *J. Lower Genital Tract Dis.,* 2000, *4,* 217.

[14] Strickler H., Shah K. Re: "Comparison of three management strategies for patients with atypical squamous cells of undetermined significance: baseline results from a randomized trial". *J. Natl. Cancer Inst.,* 2001, *93,* 951.

[15] Rebello G., Hallam N., Smart G., Farquharson D., McCafferty J.: "Human papillomavirus testing and the management of women with mildly abnormal cervical smears: an observational study". *BMJ,* 2001, *322*, 893.

[16] Morris K.A., Kavanagh A.M., Gunn J.M.: "Management of women with minor abnormalities of the cervix detected on screening: a qualitative study". *Med. J. Aust.,* 2001, *174*, 126.

[17] Lee N.W., Kim D., Park J.T., Kim A.: "Is the human papillomavirus test in combination with the papanicolaou test useful for management of patients with diagnoses of atypical squamous cells of undetermined significance/low-grade squamous intraepithelial lesions?". *Arch. Pathol. Lab. Med.*, 2001, *125*, 1453.

[18] Costa S., Simone P.D., Venturoli S., Cricca M., Zerbini M.L., Musiani M. *et al.*: "Factors predicting Human papillomavirus (HPV) clearance in cervical intraepithelial neoplasia (CIN) lesions treated by conization". *Gynecol. Oncol.,* 2003, *90,* 358.

[19] Wright T.C. Jr., Cox J.T., Massad L.S., Twiggs L.B., Wilkinson E.J.: "2001 Consensus Guidelines for the management of women with cervical cytological abnormalities". *JAMA*, 2002, *287*, 2120.

[20] Wright T.C. Jr., Cox J.T., Massad L.S., Carlson J., Twiggs L.B., Wilkinson E.J.: "2001 consensus guidelines for the management of women with cervical intraepithelial neoplasia". *Am. J. Obstet. Gynecol.*, 2003, *189*, 295.

[21] Patnick J., Monsonego J., de Wolf C., Verbeek A., Bonte J., Agnantis N. *et al.*: "ESGO consensus document on cervical cancer screening. European Society of Gynaecological Oncology". *Eur. J. Gynaecol. Oncol.,* 2001, *22*, 99.

[22] Jin X.W., Nielsen C., Brainard J., Yen-Lieberman B.: "New advances transform the management of women with abnormal pap tests". *Cleve Clin. J. Med.*, 2003, *70*, 641.

[23] Herbst A.L., Pickett K.E., Follen M., Noller K.L.: "The management of ASCUS cervical cytologic abnormalities and HPV testing: a cautionary note". *Obstet. Gynecol.*, 2001, *98*, 849.
[24] Chirenje Z.: "The management of cervical intra-epithelial neoplasia". *Cent. Afr. J. Med.*, 2000, *46*, 309.
[25] Syrjänen S., Shabalova I.P., Petrovichev N., Kozachenko V.P., Zakharova T., Pajanidi A. *et al.*: "Human papillomavirus testing and conventional PAP smear cytology as optional screening tools of women at different risk for cervical cancer in countries of former Soviet Union". *J. Lower Genital Tract. Dis.*, 2002, *6*, 97.
[26] Lonky N.M., Felix J.C., Naidu Y.M., Wolde-Tsadik G.: "Triage of atypical squamous cells of undetermined significance with hybrid capture II: Colposcopy and histologic human papillomavirus correlation". *Obstet. Gynecol.*, 2003, *101*, 481.
[27] Sherman M.E., Schiffman M., Cox J.T.: "Effects of age and human papilloma viral load on colposcopy triage: Data from the randomized atypical squamous cells of undetermined significance/low-grade squamous intraepithelial lesion triage study (ALTS)". *J. Natl. Cancer Inst.*, 2002, *94*, 102.
[28] Little J.: "Human papillomavirus testing. Effectiveness of testing for high risk HPV for triage of low grade abnormal smears is being assessed in TOMBOLA trial". *Br. Med. J*, 2001, *323*, 109.
[29] Smith J.H.: "Bethesda 2001". *Cytopathology*, 2002, *13*, 4.
[30] Stafl A., Wilbanks G.D.: "An international terminology of colposcopy: report of the Nomenclature Committee of the International Federation of Cervical Pathology and Colposcopy". *Obstet. Gynecol.*, 1991, *77*, 313.
[31] Costa S., De Nuzzo M., Bonavita B., Infante F.E., Marinelli M., Rambelli V., Rubino A.: "Cervical loop excision: Colposcopy limits in pre-surgical assessment of CIN Lesions". *Tumori*, 2001, *87*, 13.
[32] Bauer H.M., Greer C.E., Manos M.: "Determination of genital human papillomavirus infection using consensus PCR". In: C.S. Herrington and J.O.D. McGee (eds.), "Diagnostic Molecular Pathology: A Practical Approach". Oxford, Oxford University Press, 1992, 132.
[33] Fujinaga Y., Shimada M., Okazawa K., Fukushima M., Kato I., Fujinaga K.: "Simultaneous detection and typing of genital human papillomavirus DNA using the polymerase chain reaction". *J. Gen. Virol.*, 1991, *72*, 1039.
[34] Franco E.L., Villa L.L., Sobrinho J.P., Prado J.M., Rousseau M.C., Desy M.: "Epidemiology of acquisition and clearance of cervical human papillomavirus infection in women from a high-risk area for cervical cancer". *J. Infect. Dis.*, 1999, *180*, 1415.
[35] Nobbenhuis M.A., Helmerhorst T.J., van den Brule A.J., Rozendaal L., Voorhorst F.J., Bezemer P.D. *et al.*: "Cytological regression and clearance of high-risk human papillomavirus in women with an abnormal cervical smear". *Lancet*, 2001, *358*, 1782.
[36] Schiffman M., Wheeler C.M., Castle P.E.: "Human papillomavirus DNA remains detectable longer than related cervical cytologic abnormalities". *J. Infect. Dis.*, 2002, *186*, 1169.
[37] Syrjänen S., Shabalova I.P., Petrovichev N., Kozachenko V.P., Zakharova T., Pajanidi A. *et al.*: "Clearance of high-risk Human Papillomavirus (HPV) DNA and PAP smear abnormalities in a cohort of women subjected to HPV screening in the New Independent States (NIS) of the former Soviet Union". *Eur. J. Cancer*, 2003, Submitted.
[38] Cuzick J., Sasieni P., Davies P., Adams J., Normand C., Frater A. *et al.*: "A systematic review of the role of human papillomavirus testing within a cervical screening programme". *Health Technol. Assess.*, 1999, *3*, 1.
[39] van den Akker-van Marle M.E., van Ballegooijen M., van Oortmarssen G.J., Boer R., Habbema J.D.: "Cost-effectiveness of cervical cancer screening: comparison of screening policies". *J. Natl. Cancer Inst.*, 2002, *94*, 193.
[40] Bösze P., Luesley D.M. (eds). "EAGC Course Book on Colposcopy". Budapest, Primed-X Press, 2003, 1.
[41] Branca M., Rossi E., Alderisio M., Migliore G., Morosini P.L., Vecchione A. *et al.*: "Performance of cytology and colposcopy in diagnosis of cervical intraepithelial neoplasia (CIN) in HIV-positive and HIV-negative women". *Cytopathology*, 2001, *12*, 84.
[42] Ferris D.G., Wright T.C., Litaker S., Richart R.M., Lorincz A.T., Sun X.W. *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.
[43] Peyton C.L., Schiffman M., Lorincz A.T., Hunt W.C., 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.
[44] Venturoli S., Cricca M., Bonvicini F., Giosa F., Pulvirenti F.R., Galli C. *et al.*: "Human papillomavirus DNA testing by PCR-ELISA and hybrid capture II from a single cytological specimen: concordance and correlation with cytological results". *J. Clin. Virol.*, 2002, *25*, 177.

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