INTERNATIONAL JOURNAL OF IMMUNOPATHOLOGY AND PHARMACOLOGY Vol. 23, no. 1, 317-326 (2010)

IS HPV-DNA TESTING A USEFUL TOOL IN PREDICTING LOW-GRADE SQUAMOUS INTRAEPITHELIAL LESION OUTCOME? A RETROSPECTIVE LONGITUDINAL STUDY

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Received August 8, 2009 – Accepted December 11, 2009

HPV-DNA testing has entered in clinical practice. Three important questions remain controversial: 1) which is the best HPV-DNA technology? 2) Which age group should be targeted? 3) Is HPV-DNA testing predictive of disease outcome? The answers to these queries represent the endpoints of this study. The population of this retrospective study consisted of 272 women, each one having: baseline cytological diagnosis of Low-grade Squamous Intraepithelial Lesion (LSIL); baseline HPV-DNA reports by Hybrid Capture 2 (HC2) and MY09/11 consensus primers PCR; follow-up duration over 3-years; cytological report of disease status at follow-up time. Firstly, we assessed the concordance and the performances of both HPV-DNA testing, then we correlated respectively HPV-DNA results and age of patients to disease outcome. DNA testing methods agreed in 83.4% of cases (K=0.66). Baseline HPV-DNA result was not significantly associated to disease outcome (p=0.06). Within HPV-DNA positive group, we found no evidence of correlation between age and LSIL prognosis (p=0.89). Confining the analysis to age-stratified HPV-DNA negative women, the differences were statistically significant (p=0.01). In conclusion, HPV-DNA testing gives no information about the real behaviour of cervical abnormalities. These findings suggest the demand for additive markers, reflecting the risk of progression, in prevention strategy and clinical approach.

Atypical Squamous Cells of Undetermined Significance (ASC-US) and Low-grade Squamous Intraepithelial Lesion (LSIL) represent the most common diagnosis among cervical abnormalities (about 5% and 2.9% respectively) (1); they frequently test High Risk (HR) HPV DNA positive. Although women harbouring high risk viruses are at increased risk of developing cancer, epidemiologic data strongly suggest that very few (less than 10%) of these lesions will develop Cervical Intraepithelial Neoplasia Grade II or worse (CIN2+), often being self-limiting within the first 2 years (2). On the other hand, some cases of high-grade abnormalities are detected in the follow-up of the so-called low-grade changes.

Papillomavirus-induced infection is an excellent

Key words: HPV-DNA testing, prognostic markers, low-grade cervical lesions

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0394-6320 (2010)

example of strategic evasion of the immune response deriving from the long co-evolution with the host species. HPV induces a state of exclusively local chronic infection, rarely resulting in systemic sequels or death of the host. The cycle of viral infection is exclusively confined to the epithelial compartment, on the surface of which the mature viral particles are released, away from the vascular and lymphatic drains. The inflammatory signals which activate the APCs (Antigen Presenting Cell) are missing; the down-regulating activity of Papillomavirus on interferon response is prominent, with the consequent inhibition of Langerhans Cell expressing CCR6 (3). CCR6 is a specific macrophage inflammatory protein 3α (MIP-3α/CCL20) receptor, able to act as a potent initiator of the immune response. Rarely, the described HPV-induced immune evasion mechanism facilitates the persistence of the virus in the cell; the persistence of the infection induced by High Risk Papillomavirus (HR-HPV) plays a key role in the initiation of cervical carcinogenesis, in that it favours the physical state of integration of viral genome in the genome of the host-cell and the expression of E6/E7 oncoproteins (2).

Based on these observations, the need for careful observation of minor cervical lesions has emerged, at the same time avoiding over-management and overtreatment of those destined to spontaneous resolution. In this context, the target for early cancer detection should be cervical abnormalities at high potential of persistence and at high risk of evolution. The ASC-US/LSIL Triage Study, a multicenter randomized prospective trial, investigated the optimal management of women with a cytological diagnosis of ASC-US/LSIL by three different approaches: repeated cytology or HPV-DNA triage or/and immediate colposcopy. Due to poor reproducibility of cytological and colposcopic examination, HPV-DNA triage emerged as the best strategy (4).

Consistent evidence from the above-mentioned study underlined that the application of an HPV-DNA test in women with ASC-US cytology significantly reduces the number of colposcopies without loss of sensitivity compared to repeated cytology and direct referral for colposcopy. For LSIL lesions, the situation is more complex. A common important weakness of HPV-DNA triage in LSIL lesions is the low specificity and the low positive predictive value in detecting CIN2+ lesions, due to the high prevalence of transient infections (5). In the ALTS study (where conventional cytology was used and where the mean age of women with LSIL was 24.9 years) HPV positivity has been found in 83% of low-grade abnormalities; this suggested limited utility of DNA testing among these patients (4). Consequently, the point is, how to improve these performances? In other terms, since young women with low-grade cervical abnormalities are less likely to develop CIN2+ lesions, it is important to ascertain whether the application of DNA testing to older women represents the best strategy to obtain a better diagnostic accuracy in managing LSIL patients. The HART study showed a clear age dependency: among women with mild dyskaryosis, 87% of those aged 30-35 yrs were HPV-DNA positive, versus 65.8% of those aged 40-60 yrs; the percentage decreased to 59% in women aged above 60 yrs (6). Given the variability of reported findings, adjunctive studies are needed to consider a possible role for DNA testing in the management of women with LSIL Pap test results.

In this context, sensitivity and specificity of HPV testing are important issues. There are open questions about the most appropriated HPV-DNA detection method (7-8). To be of use, the testing method for oncogenic HPV types must demonstrate sensitivity and specificity for HR-HPV infection, but must also give evidence of robustness. Furthermore, since HPV test findings have a significant influence on clinical management, the test's predictive value must also have been clinically validated in terms of disease outcome.

The use of HPV-DNA testing in adjunct to cytology is currently based on DNA hybridisation techniques (i.e. Hybrid Capture II system) and on consensus primers PCR. Hybrid Capture assay (HC2, Digene Corp.) is the only test currently FDA-approved. The evaluation of its analytical performances confirms the reliability and reproducibility of this technique for widespread use (8). PCR method is considered "the best" in terms of sensitivity in detecting HPV-DNA. Considering the rising importance of HPV testing and the demand for more than one test to achieve high quality molecular biology results, it is necessary to assess and compare the performance of these two methodologies and to

clarify their appropriateness for routine practice.

Based on the described background, the first phase of the study is the evaluation and the comparison of the performances of the two most used molecular technologies in HPV-DNA detection; in the second phase, a population of patients with LSIL cytological diagnosis longitudinally is retrospectively analyzed, in order to correlate disease outcome with HPV-DNA status at baseline for women of different ages.

MATERIALS AND METHODS

Patient selection

The population of this retrospective study, collected from the electronic files of the Cytopathology Departments of two Italian hospitals, was 272 women (mean age 34.3 ± 9.4; median 32; range 18-64), referred spontaneously to gynaecological examination between 2000 and 2005. The inclusion of each patient in the study required:

1. A cytological result of LSIL at baseline;
2. HPV-DNA testing performed at baseline and carried out on residual liquid-based cervical cytological samples;
4. Unsatisfactory colposcopic diagnosis;
3. Conservative management of LSIL lesion;
4. A time interval over 3 years between both baseline cytological result-DNA reports and subsequent cervical smear;
5. A cytological report that assesses cervical disease status at the time of repetition.

Pregnant women, HIV-positive or immunosuppressed patients were excluded from the evaluations.

Written informed consent was obtained from all participants, and patient identification codes were assigned in accordance with confidentiality standards. The study, performed in agreement with the standards of the ethics review boards of all participating hospitals, was approved by the Ethical Committees of "G. d'Annunzio" University, in accordance with the principles outlined in the Declaration of Helsinki of 1975.

Sample collection, slide preparation and cytological examination

For each patient, during pelvic examination, both baseline and succeeding cervico-vaginal samples were collected from ecto-endocervix with Ayre spatula and Cytobrush; material was then directly transferred into 20 ml of PreservCyt (Cytyc Corporation, Boxborough, MA) liquid cytology medium and transported to Cytopathology Departments. ThinPrep technology (Cytyc Corporation, Boxborough, MA) was used to prepare cytological slides, which were next stained with Papanicolaou procedure.

All scrapes were evaluated morphologically in thin layer preparations by two different double blinded cytopathologists. Cytological grading of cervical disease was performed according to the "Bethesda System 2001". Cytological results originally reported by a classification system that predated 2001, were converted into Bethesda System 2001 terminology for this study.

HPV-DNA detection

After baseline morphological evaluation, residual cytological samples were analysed for HR-HPV-DNA detection; all 272 patients had their HPV-DNA report determined by HC2 test; both HC2 and PCR reports were available only for one subset (145) of women, in which the HC2 test was carried out before PCR. HPV DNA technologies were performed on the same cytological sample tested for morphology. The removal of cells from samples to prepare cytological slides with Thin Prep procedure has been reported not to affect the performances of the subsequent HPV-DNA tests (9).

Procedures for collecting and carrying out HPV-DNA testing were performed using only the high-risk probe set (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) and according to the manufacturer's protocol as described elsewhere (10). The HC2 test was performed manually according to the manufacturer's instructions. The results of each specimen were expressed as RLUs, which are proportional to the amount of target HPV-DNA present in the specimen. The recommended cut-off value for a positive result is 1.0 RLU, equivalent 1.0 pg HPV-DNA/ml of sampling buffer (corresponding to ~100,000 HPV-16 genomes/ml or 5,000 HPV copies per reaction, see manufacturer's protocol). Ratio value ≥1.0 was considered as the clinical cut-point for HPV-DNA positive result (11).

Following the HC2 test, a volume of 1 ml from each one of the 145 residual cervical samples was removed to perform PCR procedure by using MY09/11 consensus primers. MY09/11 are degenerate primers designed to amplify a 450 base pair fragment located in the L1 region of the HPV genome. After centrifugation of cervical cells at 3500 rpm for 20 min at room temperature, the pellet was re-suspended in 600 µl of PBS. DNA extraction was performed using QIAamp DNA mini kit (Qiagen Inc., Valencia, CA), according to the manufacturer's procedure. DNA concentration was estimated spectrophotometrically ($\lambda = 260$ nm) and samples were diluted at a final concentration of 10 ng/µl with H_2O. The molecular detection of HPV was achieved by using nested-PCR with HPV L1 consensus primers MY09/11 (Amplimedical Spa Diagnostic Group, Assago, Italy), according to the manufacturer's protocol. Briefly, PCR amplification was performed in a 100-ml volume containing 50 mM KCl,

10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl2, 200 mM of each deoxynucleoside triphosphate, 0.5% Nonidet P-40–Tween 20, 2.5 U of *Taq* DNA polymerase, 25 pmol of each outer primer, 50 pmol of each inner primer, and 5 µl of sample DNA. Amplification was performed for a total of 45 cycles. The first 15 cycles consisted of 1 min at 94°C, 1 min at 45°C, and 1.5 min at 72°C. The annealing temperature was subsequently lowered to 38°C, and an additional 30 cycles were performed with the other parameters unchanged. Ten-microliter portions of the PCR products were loaded on 4% agarose gels (NuSieve; FMC BioProducts, Rockland, Maine) and electrophoresed, the gels were stained with ethidium bromide (10 mg/ml), and DNA was visualized by fluorescence under UV light. (Fig. 1). Positive (DNA from HPV-16 or HPV-18 plasmids) and negative controls were included in all runs. Strict procedures were followed to avoid a false-positive reaction due to contamination.

The primers pooled in PCR master mix has been designed to amplify viral DNA from the same 13 HR-HPV types included in the HC2 assay and to provide qualitative information about presence/absence of HPV in the samples.

Disease outcome

Retrospective analyses of the selected patients was carried out considering the LSIL outcome. Based on cytological diagnosis assessing disease status after time interval (mean 40.16 ± 9.02 months), the 272 women were assigned to three different Prognostic Classes (PC):

PC1: clearance of cytological abnormality, without intervention;

PC2: persistence of low-grade cervical lesions;

PC3: progression of LSIL to high-grade disease cytologically defined as HSIL (High-grade Squamous Intraepithelial Lesion), histologically confirmed as CIN2+.

PC 1-3 were then correlated with HPV-DNA status at baseline with age, using 35 years of age as cut-off (12).

Statistical analyses

In the subset of 145 patients in which both HC2 and PCR were employed, we used Cohen's *K* values and percentage of concordance with 95% confidence intervals as measures of the agreement between the two tests. According to the statistical literature, *K* values of 0.40 indicate poor agreement, values from 0.40 to 0.75 signify fair to good agreement and values of > 0.75 designate excellent agreement. For each test, using standard methods we then evaluated sensitivity, specificity, positive and negative predictive values (PPV and NPV, respectively) with 95%CI, in predicting progression to CIN2+ cervical changes. McNemar test was used to evaluate differences in performances.

Relationship between HPV-DNA result at baseline and LSIL outcome, among age groups, were evaluated by the X^2 test, with continuity correction or Fisher's exact test when appropriate. Univariate analysis between HPV-DNA at baseline and cervical changes outcome was based on the Kaplan-Meier method, by using the log-rank test to determine statistical differences.

Statistical analyses were performed by using SPSS software (SPSS for Windows, Inc, Chicago, IL) version 15.0. The significant level was set at $p < .05$ for all statistical analyses.

RESULTS

Comparison of HC2 and PCR results

Women were classified as having positive or negative HPV-DNA result. Overall, 136 (50%) women tested HPV positive and 136 (50%) tested negative. In the subset of 145 patients in which both HC2 and PCR tests were carried out, we showed HC2 positivity in sixty-two (42.8%) cases, HC2 negativity in eighty-three (57.2%) specimens, PCR positivity in sixty samples (41.4%) and PCR negativity in eighty-five cases (58.6%). A two-way comparison of results obtained by the application of both HPV-DNA techniques is shown in Table I. We found an overall percent agreement of 83.4% (95% CI: 77.3, 89.6; McNemar test=0.17, p=0.04) and Cohen's kappa value of 0.66; 24 samples (17%) gave discordant results: 13 were HC2 positive but PCR negative, 11 were HC2 negative but PCR positive.

The accuracy of both HPV-DNA assays for predicting persistent/progressive cervical lesions are summarized in Table II (Mc Nemar test=3.8; p=0.05).

HPV-DNA result and disease outcome

Cytological reports assessing cervical disease status at the time of repetition showed clearance of abnormalities in 205 (75.4%) women (mean age 34.3 ± 9.4), persistence of LSIL in 58 (21.3%) patients (mean age 34.2 ± 9.5) and progression in 9 (3.3%) women (mean age 30.7 ± 8.2).

Of the patients who had remission of their cytological abnormalities, baseline reflex HPV test was positive in 99 (48.3%) cases and negative in 106 (51.7%) cases; among patients with persistent LSIL,

Table I. *Comparison of results obtained by the application of HPV-DNA technologies.*

PCR results	HC2 results		
	Negative	Positive	All
Negative	72	13	85
Positive	11	49	60
All	83	62	**145**

29 (50%) tested HPV-DNA positive at baseline versus 29 (50%) testing DNA negative. In terms of progressing lesions, baseline reflex HPV test was positive in 8 women (88.9%) and negative in 1 (11.1%) woman (Table III). By statistical analysis, no significant differences were observed (X^2=5.68; p=0.06). Cumulative incidence of cleared versus non-cleared (persistent/progressive) LSIL lesions, after a mean time interval of 37.5 months and according to baseline HPV-DNA result, is represented in Fig. 2. Comparison of results (log-rank test=0.24) showed no statistical differences (p=0.6).

Using a higher test positivity cut-off (>2.5) [28], we obtained the HPV-DNA results shown in Table IV. Also in this case, no significant differences were observed (X^2=0.31; p=0.85). In addition, 7 cases of progressing LSIL were lost.

Age and disease outcome

All patients were assigned to HPV-DNA positive or HPV-DNA negative groups and to PC 1-3 by age category (< 35 years or ≥ 35 years) (Table V). One hundred-sixty patients (58.8%) were aged under 35 yrs, of whom 52.5% showed HPV positivity. One hundred-twelve patients (41.2%) were aged 35 yrs or over, 46.4% of whom tested HPV-DNA positive. Among women aged 18-34 years, 78.8% (126/160) cleared the lesion, 18.1% (29/160) had persistent LSIL and 3.1% (5/160) showed progression to high-grade abnormalities. Among women aged 35-64 yrs, the respective values were 70.5% (79/112), 25.9% (29/112) and 3.6% (4/112). No statistically significant association was found linking the age of patients to LSIL outcome in the HPV-DNA positive group (X^2=0.23; p=0.89); confining the analysis to women who were HPV-DNA negative, the differences were statistically significant (X^2=7,14; p=0.01).

DISCUSSION

The knowledge that HR-HPV infection is a pre-requisite for the pathogenesis of cervical cancer (13) has led to the development of molecular techniques able to improve the quality and the accuracy of the cytological examination of cervical specimens. Many studies have focused their attention on HPV-DNA testing (11-12). Wide ranges of methods are currently available for HPV-DNA detection in cytological specimens; the sensitivity of DNA testing depends upon the type of test used. In this context, assay standardization becomes an obligation (14).

Table II. *Performance indicators of HC2 and PCR in detecting persistent/progressive cervical lesion.*

HPV-DNA testing	HPV-DNA test performance indicators (95%CI)			
	Sensitivity	*Specificity*	*PPV*	*NPV*
HC2	48% (28-67)	58.3% (49.3-67.3)	19.4% (9.3-29.4)	84.3 (76.4-92.3)
PCR	33.3% (15.2-51.5)	56.8% (47.7-65.9)	15% (5.8-24.2)	78.8% (70-87.7)

From a laboratory standpoint, the suitability to carry out different molecular tests from the same sample is imperative and might provide a valid tool to set up the algorithm for the appropriate management of patients. Recently, the World Health Organization launched the request of HPV-DNA test standardized procedures for prospective vaccine effectiveness trials. In this context, PCR and Hybrid Capture II test were considered the two best methods for HPV-DNA detection. Of interest are the PCR versus HC2 data from the large ALTS study, employing a prototype PGMY09/11 consensus primers PCR (15). Most laboratories use PCR assay, which utilize consensus primers directed to L1 gene: MY09/11, PGMY09/11, GP5+/6+, SPF primer set. Amplification with each of these primers provides different sized amplification products, resulting in varying sensitivity for HPV-DNA detection (16). To our knowledge, this is the first study comparing results from the second generation of HC2 and MY09/11 consensus primers PCR. The concordance value we found (83.4%) is in agreement with other recent studies based on HC2 and others except MY09/11 consensus amplification technologies (17). In the past, some authors assessed a lack of sensitivity when using the so-called degenerate primers (i.e. MY09/11). Others showed better performances of these primers, especially in the detection of multiple infections. A possible reason for these contradictory results might be due to the quality of samples. Qu et al., for example, utilized paraffin embedded tissues (18); on these materials, because of the damaged and fragmented DNA, it is possible that MY09/11 PCR could have a lower amplification rate than other consensus primers, since it produces longer PCR products (450 bp). It has been also reported the scarce efficiency of MY09/11 primers in detecting HR-HPV genotypes 16, 18, 31, 35, 39, 45, 51, 52 and 68 (19); these HPV types are powerfully detected by HC2. The high concordance value we obtained in comparing HC2 and MY09/11 PCR, did not confirm the last supposed MY09/11 primers limit. However, in our experience, PCR protocol was more labour intensive for routine application than HC2 technology, which was also less time-consuming.

In the present study, we achieved HC2 and PCR accuracy indicators that differ from those observed by other studies (20). Essentially, the dissimilar results we reached are related to the different target we used as "gold standard". Analytical accuracy of a test merely refers to the ability of detection of an endpoint. In contrast, we wanted to assess the

Table III. *Relation between HPV-DNA status and disease outcome.*

Outcome	***DNA status***		
	Positive (%)	Negative (%)	All
Clearance	99 (48.3%)	106 (51.7%)	205
Persistence	29 (50%)	29 (50%)	58
Progression	8 (88.9%)	1 (11.1%)	9
All	136	136	**272**

Table IV. *Relation between HPV-DNA status and disease outcome, using RLU[a] ratio ≥ 2.5.*

Outcome	***DNA status***		
	Positive (%)	Negative (%)	All
Clearance	33 (16.1%)	172 (83.9%)	205
Persistence	8 (13.8%)	50 (86.2%)	58
Progression	1 (11.1%)	8 (88.2%)	9
All	42	230	**272**

[a]*RLU: Relative Light Unit*

Table V. *Relation between HPV-DNA status, age and LSIL outcome.*

	Women's age				
Outcome	*<35 yrs*		*>= 35 yrs*		All
	HPV-DNA+ (%)	HPV-DNA - (%)	HPV-DNA + (%)	HPV-DNA - (%)	
PC1	60 (37.5%)	66 (41.3%)	39 (34.8%)	40 (35.7%)	205
PC2	19 (11.9%)	10 (6.2%)	10 (8.9%)	19 (17%)	58
PC3	5 (3.1%)	0 (0%)	3 (2.7%)	1 (0.9%)	9
All (%)	160 (58.8%)		112 (41.2%)		**272**

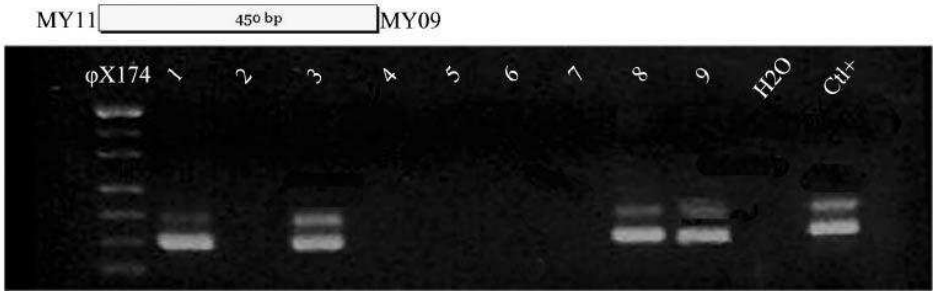

Fig. 1. *Representative agarose ethidium-stained electrophoresis gel of MY09/11 consensus primers PCR. Lane 1: ϕ X174 molecular weight marker. Lanes 1, 3, 8 and 9: HPV-positive PCR products. Lane 2 and lanes 4 through 7: HPV negative PCR products. Line 10: HPV-DNA positive control. Line 11: negative control (DNAsi and RNAsi free water).*

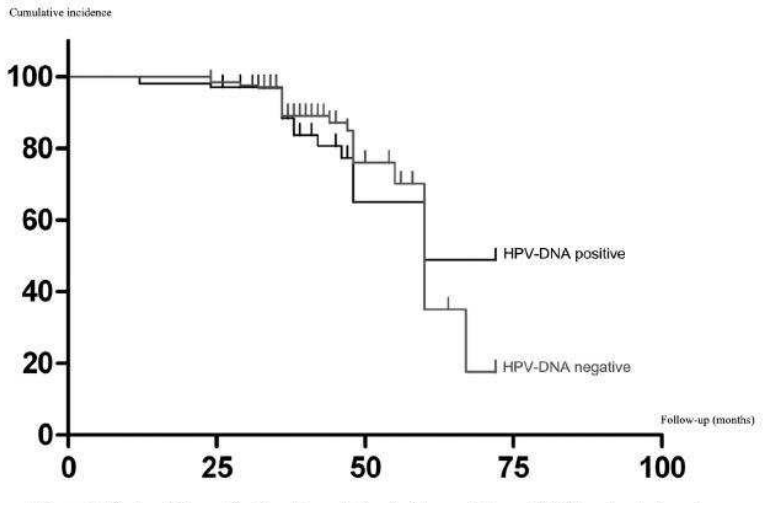

Fig. 2. *Kaplan-Meier estimation of cumulative incidence of cleared (Prognostic Class 1) and not-cleared (Prognostic Classes 2 and 3) low-grade cervical lesions, according to HPV-DNA results (p=0.6).*

clinical accuracy, a parameter that is more related to medical practice of the test: it expresses the ability to detect a relevant phase of disease (19). A test with high analytical performances would not be clinically valid. In our experience, the predictive value of a single baseline HPV DNA testing for the identification of persistent and/or progressing LSIL cervical abnormalities needs improvement.

To date, few studies have investigated LSIL outcome in relation to baseline HPV-DNA status over time. For this reason, we were interested in estimating the predictive value of HPV-DNA test in terms of disease outcome of low-grade cervical changes (20- 21). In this analysis, we established a 3-year mean interval period in consideration of the European screening interval and basing on the observation that regression of the lesions usually occurs within this time (22).

The proportion of LSIL testing DNA positive we found was 50% (61.8% in women < 35 yrs of

age; 38.2 in women ≥ 35 yrs of age), lower than those observed in the ALTS study (22-23). This could depend on national criteria of cytological interpretation or on the fact that conventional cytology was used in ALTS. Our results confirm that HPV positive women were more likely to develop CIN2+ than HPV negative women (eight patients versus one patient), although the difference we obtained did not reach statistically significant values. On the other hand, baseline HPV-DNA positive result underlined LSIL in regression as well as LSIL either persisting or progressing. Investigating age-stratified cervical lesion outcomes in the HPV-DNA positive group, we found that, in women aged 35 or over, the HPV positivity rate was lower than for younger women and that the potential value of HPV DNA testing as an adjunct to cytology in this group was better, but not significant, than for younger women (24). Probably these last data might be explained with the low mean age of women stratified in group ≥ 35 yrs and then with the low proportion of women aged 35-64 (25). Analyzing age-stratified LSIL outcome in HPV-DNA negative patients, we confirm the good negative predictive value of DNA testing, especially in younger women (26).

Among baseline HPV-DNA negative patients, we found one woman (≥ 35 yrs of age) with LSIL progressed to histological confirmed CIN2+ lesion. This finding underlines a substantial problems linked to DNA techniques. Consensus primer MY09/11 PCR target the structural L1 region of the virus; this region is deleted when HPV-DNA is integrated into the host cell genome (26-27). HC2 technology utilizes long single-stranded RNA probe (about 8000 base pairs) which is complementary to the entire HPV-DNA sequence. HPV genome integration causes L1 and E2 gene deletion; subsequently, the RNA probe is unable to recognize its specific target (28). Then HPV-DNA testing might fail in detecting potentially oncogenic infection in which HPV-DNA is integrated into the host genome (2, 29-30).

In conclusion, our data did not totally negate the clear association between HPV infection and neoplastic cervical lesions; furthermore, we did not attempt a risk-benefit analysis. We could only demonstrate that a single positive HPV-DNA test is not sufficient to evaluate the real cancer risk, being incapable of distinguishing low-grade abnormalities planned to regress from those destined to persist and progress. HPV-DNA detection unquestionably permits the identification of the first step of cervical carcinogenesis, but not the tendency to develop high-grade cervical lesions. The question therefore is: which is the best strategy? Too early repetition of HPV-DNA testing would incorrectly characterize many cervical lesions that are destined to resolve spontaneously as persistent; on the other hand, a longer follow-up period might create patient anxiety and a probable loss to follow-up. In our opinion, the best management strategy would be the assessment of the "individual risk of cancer", in order to treat persistent lesions at increased risk of progression towards malignancy, without concerning lesions which would not benefit from the treatment, because of the high probability of regression. Based on these considerations, cytology and HPV-DNA testing urgently needs supplementary markers, predictive of disease outcome, and able to give a predictive value of persistence, progression or regression of cervical lesion (31). There is evidence suggesting that detection of viral oncogene expression both directly and indirectly might constitute a more specific approach for delineating clinically significant infection. HPV oncogene expressions and their deregulation can be monitored through direct detection of viral full-length E6/E7 mRNA transcripts or through detection of the cellular protein p16. For both HPV mRNA and p16 approaches, commercial assays have been introduced and numerous studies have been conducted, however, they need to be validated in routine diagnostic contexts (10, 32). Currently, there are promising data indicating the potential role of HPV-mRNA testing or p16 in cervical cancer screening scenarios (33-35). It is our opinion that their application on HR-HPV DNA positive cases would certainly improve the diagnostic work-up presently in use and would reduce the need for repeated tests. The final advantages would be the correct identification of women with a true risk of developing cervical cancer (36).

The authors declare that they have no competing interests.

ACKNOWLEDGEMENTS

The authors are grateful to Dr.ssa Erica Costantini

and to Dr.ssa Serena Capanna for their excellent assistance.

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