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*Brief Communication*

# High risk HPV DNA subtypes and E6/E7 mRNA expression in a cohort of colposcopy patients from Northern Italy with high-grade histologically verified cervical lesions

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**Abstract:** To evaluate the prevalence of HPV DNA genotypes in women diagnosed with cervical intraepithelial neoplasia grade 2 or greater (CIN 2+), together with the detection of mRNA transcripts from HPV 16/18/31/33/45. In 1113 women referred to our colposcopy unit for abnormal cytology, colposcopic assessment was followed by histologic examination for final diagnosis and by presence of HPV DNA and E6/E7 mRNA transcripts. A total of 134 CIN 2+ cases were identified. Out of the 134 women with CIN 2+ cervical lesions, 115 cases (85.8%) tested positive by PCR to HR HPV DNA types, and 19 (14.2%) were HR HPV DNA negative. 68 cases (50.7%) were positive for HPV DNA 16/18/31/33/45 and of them 50 cases were E6/E7 positive, and 18 were E6/E7 negative. 47 cases (35.1%) were positive for high risk types other than 16/18/31/33/45. HPV 16 is the most frequent genotype found in histologically confirmed high grade cervical lesions in our series; HPV 31 is the second most frequent type, contributing significantly to the proportion of women with CIN 2+ lesions in our population and shows a higher prevalence than HPV 18. Out of the 979 women with lesions less than CIN 2, 588 cases tested positive by PCR to high risk HPV DNA types (60.1%), and 98 cases were E6/E7 positive from HPV 16/18/31/33/45 (10.1%). Although HPV DNA and mRNA negative results should be evaluated with caution, since they could represent "false negatives", high risk HPV DNA positivity should be assessed carefully with colposcopy before performing excisional treatments, particularly in adolescents but also in patients who want child, since they may reflect transient situations.

**Keywords:** HPV infection, sexually transmitted disease, cervical cancer, HPV DNA, E6/E7 mRNA transcripts; CIN

## Introduction

Infections with oncogenic genotypes of the human papillomavirus (HPV) are the ultimate cause of cervical cancer [1]. Carcinogenesis is regarded as the consequence of "persistent high risk (HR) HPV infections" that last for more than 6 months [2]. Integration provides the cell with a selective growth advantage [3, 4]. This growth advantage results from an enhanced expression of HPV E6 and E7. High oncogene levels have been attributed to the functional loss of the viral E2 gene product which acts as an intrinsic repressor of E6/E7 expression [5]. Viral gene expression during an initial HPV infection follows a distinct pattern that is strictly related to the differentiation stage of the squamous epithelial host cells [6], which permits major viral gene expression and replication only in terminally differentiated cells of the intermediate and superficial cell layers [7]. If control of the viral genes in the basal cells is lost, the viral oncogenes E6 and E7 become strongly expressed in replication competent basal and parabasal cells [8]. The E6 and E7 genes of HPV are thought to play causative roles, since

E6 promotes the degradation of p53 through its interaction with E6AP, an E3 ubiquitin ligase, whereas E7 binds to the retinoblastoma protein (pRb) and disrupts its complex formation with E2F transcription factors [9]. While HPV DNA testing is one of the most intensively studied alternatives for cytology, it is unlikely to be the optimal strategy in countries with pre-existing cytological screening programmes. Probably the detection of the viral E6/E7 oncogenes from carcinogenic HPV types might serve as a better risk evaluation factor than mere DNA detection [10]. Testing for HPV DNA reveals only the presence of the virus, whereas E6/E7 mRNA expression shows increased transcriptional activity eventually increasing the prognostic value of the test.

The aim of this study was to evaluate the prevalence of HPV DNA genotypes in women diagnosed with cervical intraepithelial neoplasia grade 2 or greater (CIN 2+), together with the detection of mRNA transcripts from HPV 16/18/31/33/45, the most common recognized carcinogenic HPV types associated with cervical cancer.

## Mateials and methods

From January 2008 to July 2009, 2428 consecutive women were referred to the colposcopy unit of our Institute for abnormal cytology. A subgroup of 1113 patients were also assessed for the presence of HPV DNA and mRNA transcripts, in relation to the specialist desire or on request by the woman or her doctor. The other 1315 patients without molecular testing were excluded from the study. Molecular testing was performed on liquid based cytology prior to colposcopic evaluation and treatment. Genotyping was performed using the Linear Array HPV Genotyping Test (Roche Diagnostics). HPV types 16, 18, 31, 33, and 45. E6/E7 mRNA detection was performed using PreTect HPV-Proofer (NorChip AS) in all HPV DNA positive cases irrespective of HPV type (for quality control). Colposcopic assessment was followed by histologic examination for final diagnosis in all cases of high grade cytology or colposcopic impression. Colposcopy was performed by four specialists and histology was reviewed by two pathologists. Patients with vulvar and/or vaginal lesions identified by colposcopy were excluded in all cases where the cervix was defined as normal, as well as HIV positive women and patients already vaccinated against HPV.

*Data analysis*

The frequency of various HPV types was computed with the corresponding 95% Confidence interval (CI) according to the Poisson's approximation. For type specific frequency all genotypes from single and multiple infections were computed individually.

## Results

The mean age of the 1113 women included in our study was 36.7 years (standard deviation +/- 10.6, range 24-70), 27.5% were smokers, 81.9% did not use contraception, 17.4% were taking estro-progestins, 0.7% had an intrauterine contraceptive device and 73.9% were nulliparous.

979 patients were negative for high grade cervical lesions and thus were not treated (CIN 1 or less) irrespective of their viral status: they are being followed with colposcopy and cytology at 6 months intervals.

A total of 134 CIN 2+ cases were identified and all patients were treated with loop electrosurgical excision procedure (LEEP) under local anesthesia. Final diagnosis was CIN 2 or CIN 3. No invasive cancer was found.

Out of the 134 women with CIN 2+ cervical lesions, 115 cases (85.8%) tested positive by PCR to HR HPV DNA types, and 19 (14.2%) were HR HPV DNA negative. Multiple genotypes were observed in 26 patients (2 genotypes in 14 women, 3 in 4, 4 in 6, 5 in 1, 6 in 1).

The distribution of HPV DNA genotypes is presented in Figure 1: 68 cases (50.7%) were positive for HPV DNA 16/18/31/33/45, while 47 cases (35.1%) for high risk types other than 16/18/31/33/45.
50 cases were E6/E7 positive, and 18 were E6/E7 negative. The distribution of these different mRNAs is shown in Table 1.

Out of the 979 women with lesions less than CIN 2, 588 tested positive by PCR to high risk HPV DNA types (60.1%), and 98 were E6/E7 positive from HPV 16/18/31/33/45 (10.1%).

## Discussion

The general results of our study indicate high-risk HPV DNA positivity in 85.8% of histologically

**Table 1.** E6/E7 positivity in CIN 2+ lesions.

| Genotype | mRNA positive No. (%) | mRNA negative No. (%) | Total No. |
|---|---|---|---|
| HPV 16 | 27 (69.2) | 12 (30.8) | 39 |
| HPV 18 | 4 (50) | 4 (50) | 8 |
| HPV 31 | 14 (93.4) | 1 (6.6) | 15 |
| HPV 33 | 3 (75) | 1 (25) | 4 |
| HPV 45 | 2 (100) | --- | 2 |

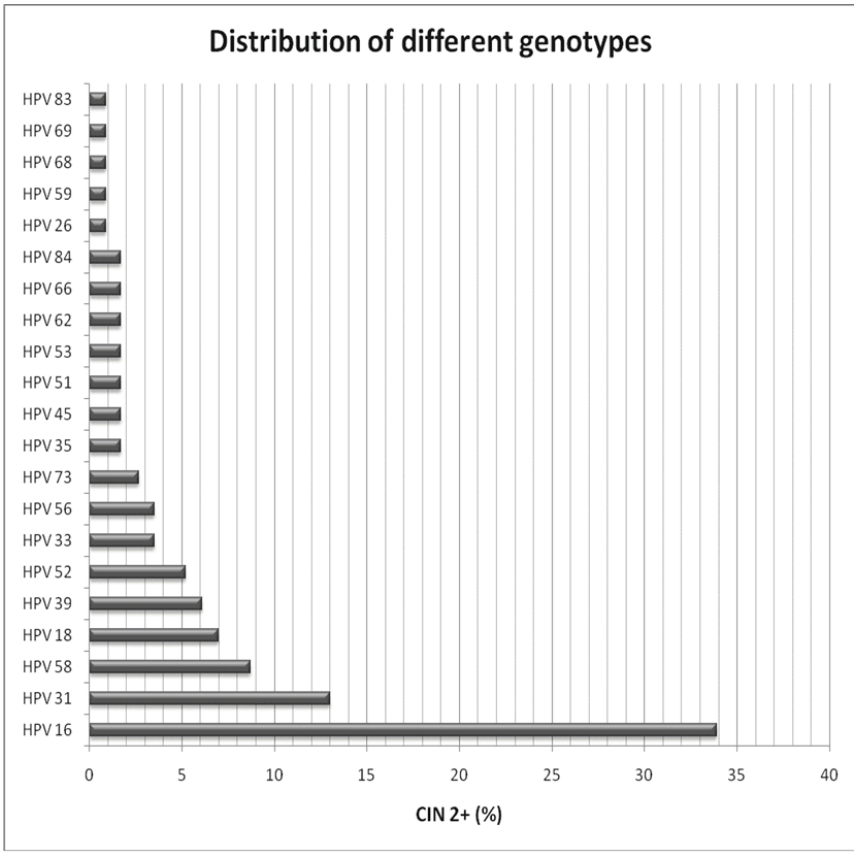


**Figure 1.** Distribution of the different HPV genotypes.

confirmed high grade cervical lesions, and that HPV 16 is the most frequent genotype found. HPV 31 is the second most frequent type, contributing significantly to the proportion of women with CIN 2+ lesions in our population and shows a higher prevalence than HPV 18, in agreement with other studies [11]. HPV 58 is at the third place, while HPV 18 is fourth, HPV 39 fifth and HPV 52 sixth.

Before discussing these results, a potential bias should be considered. The study population includes women referred to a second level clinic after screening procedures, for diagnosis and treatment of abnormal cytology. This is a selected population, representative but not corresponding to all regional screening population. However the Policlinic Hospital of Milan is the main women's hospital in Northen Italy and no specific difference has been observed for women delivering in this Center compared to the population of women delivering in other Northen Italy hospitals, with reference to the age, race, educational level and sexual habits.

On the contrary, among the strengths of this study we have to consider the large sample size that may provide confident information of the HPV genotype distribution in CIN 2+ cases.

The results of this study are in general agreement with those reported in a large study conducted in Italy on 616 patients [12]. In that study high-risk HPV DNA positivity was observed in 94% of women having a cytological diagnosis of high-grade squamous intraepithelial lesion. Further HPV16 and/or 18 were detected in 56.2% (CI 44.1%-67.8%) of cytological high-grade squamous intraepithelial lesions (59.4% of HPV-positive ones).

The new vaccines against HPV are directed towards HPV 16 and 18, which together are responsible for about 70% of cervical cancers. Considering the hypothesized cross protection against other HPV types it could be interesting to understand which are the most common types found in histologically confirmed high grade cervical lesions in our population.

The results of this study should be also discussed in terms of clinical relevance.

In any sizeable population, even among women with evidence of cytologic abnormalities, there will be a few cases of cervical precancer that will test high-risk HPV negative for one or more reasons [13]. In our series HPV testing was negative in about 14% of high grade cervical lesions. This is the first study showing a high rate of HPV DNA negative results in women with high grade cervical lesions (CIN 2+).

Carcinogenic HPV E6/E7 mRNA is a potentially useful biomarker for detection of cervical precancer and cancer [14]. Repeated type-specific testing for HPV mRNA may identify young women with a persistent transforming infection being at increased risk for severe dysplasia [15]. Although integration of some HR-HPV types is not always necessary for progression of squamous intraepithelial lesions, mRNA testing is useful to analyze the physical state of HPV DNA sequences and predict the progression of these lesions [16]. Nevertheless, in our study more than one fourth of CIN2+ women who tested positive for HPV DNA 16/18/31/33/45 was negative for mRNA of the same five types, while more than 10% of lesions less than CIN2 were E6/E7 positive from HPV 16/18/31/33/45. In these situations, it seems possible to hypothesize that mRNA testing reflects the replication stage of HR HPV other than its physical state.

Although HPV DNA and mRNA negative results should be evaluated with caution, since they could represent false negatives or latent infection, high risk HPV DNA positivity should be assessed carefully with colposcopy before performing excisional treatments, particularly in adolescents but also in patients who want child, since they may reflect transient situations.

It would be of great interest to study the behaviour of those mRNA positive patients with a low grade cervical lesions. In this case a repeated mRNA test after 6-12 months could be able to predict how many of these women will develop a high grade lesion and how many will regress or persist as low grade disease [14, 17, 18]. On the other hand it could be interesting to follow-up with 6-month cytology and colposcopy up to 2 years those HPV DNA positive, mRNA negative adolescents with CIN 2+, to understand if they really are transient [19]. Actually, based on significant regression of CIN 2 among adolescent women, primary management in this population should consist of cytologic and colposcopic follow-up [20-22].

In this context, the concomitant analysis of surrogate markers of HPV infection like $p16^{INK4a}$ would be also helpful to better clarify the potential of progression of low grade lesions with mRNA positivity and that of regression of high grade lesions with mRNA negativity [23].

In our study more than 60% of low grade lesions tested positive for high risk types and more than 10% had a positive mRNA test for the five most common carcinogenetic HPV types. Identifying women with HPV and even early CIN lesions who will not develop cervical cancer may seem effective to clinician and patient yet have minimal impact on cervical cancer morbidity or mortality [24]. Primary HPV screening with cytology triage finds more CIN lesions compared to conventional screening but mild lesions are overrepresented. This is likely to result in overdiagnoses and treatment since most mild lesions are regressive [10].

Treatment for false-positive results carries significant potential for harm, risk of preterm delivery and other pregnancy complications subsequently [25, 26] and this is particularly true for adolescents and young women, since the age of first pregnancy is often postponed in developed countries.

Today in Italy, like in many other European countries, HPV testing is extensively used by clinicians even in those cases where it would not be useful in managing an abnormal cytology result. This will notably increase costs and anxiety, without improving the management of these cases. The detection of the virus in already established cervical lesions does not add crucial information in the work up of these women.

Newer technologies seem attractive but adoption in clinical practice is premature, until more data are obtained. Probably in the very next future, the widespread adoption of HPV vaccination may drive down HPV prevalence rates, improving accuracy of these new tests [24].

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