Role of human papillomavirus testing in reducing the number of surgical treatments for precancerous cervical lesions

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Summary

Purpose of investigation: To determine whether the addition of the Hybrid Capture II (HC II) test *(Digene Corp., Gaithersburg, MD, USA)* to cytological, colposcopical and histological results could reduce the number of surgical treatment procedures for precancerous cervical lesions.

Methods: Surgical treatment of precancerous cervical lesions was performed in 181 women. Priorly, the women were tested for high-risk human papillomavirus (HPV). Sensitivity, specificity, positive and negative predictive value were calculated to assess the performance characteristics of HC II in the detection of cervical intraepithelial neoplasia grade 2 or worse (CIN 2+) and grade 3 or worse (CIN 3+).

Results: Eighty (44.2%) women had a histological result < CIN 2; 117 (64.6%) women had < CIN 3. Fifty-three (29.3%) women with < CIN 2 tested HPV negative; 69 (38.1%) women with < CIN 3 tested HPV negative ($p < 0.05$). The sensitivity of HC II for detecting CIN 2+ and CIN 3+ was 76.2% and 87.5%, respectively.

Conclusion: A high proportion of women were overtreated probably due to cytological and histological overestimations. HPV testing would reduce the number of unnecessary surgical treatments and should be used as an additional screening tool.

Key words: Human papillomavirus; Precancerous cervical lesion; Surgical treatment; HPV testing.

Introduction

The incidence of cervical cancer in Slovenia in the year 2000 was 19.6/100,000 women; it ranked seventh of all cancers in women with a relative frequency of 5%. There were 200 new cases of cervical cancer diagnosed in that year [1, 2].

Because the incidence of cervical cancer in Slovenia is high compared to developed countries (approximately 14/100,000 women) [3-5], the decision for introduction of surgical treatment is often made earlier than required [6, 7]. Recurrent abnormal cytological results may influence the gynaecologist to introduce surgical treatment although the histological result is less than cervical intraepithelial neoplasia grade 2 (< CIN 2), which is considered a low-risk histological result [8, 9].

It is known that persistent infection with certain human papillomavirus (HPV) genotypes is a necessary etiological factor for the development of cervical cancer [10, 11]. Therefore, additional testing of cervical smears to detect HPV provides useful information which help the clinician decide on the type of treatment or follow-up the woman needs [12-14]. The Hybrid Capture II (HC II) HPV DNA detection test (Digene Corp., Gaithersburg, MD, USA) is widely used for this purpose [6, 7, 15-20], and has proven to be a useful tool for detecting CIN 2 or worse histological results (CIN 2+), reaching a sensitivity of up to 98% [16, 21, 22].

Despite the national recommendations to manage women with abnormal cytological smears with an additional HC II-HPV DNA test, it is still not sufficiently and widely used [7, 23].

The aim of this study was to find out whether the use of the HC II HPV DNA test, complementary to cytology, colposcopy and histology, could reduce the number of surgical treatment procedures in Slovenian women.

Methods

Our study was carried out at the Department of Obstetrics and Gynaecology, University Medical Centre Ljubljana, between January and December 2003.

One hundred and ninety-seven women, who came to the Department for surgical treatment of precancerous cervical lesions detected by cytology, colposcopy and confirmed by histology, were included in the study. Each woman willing to participate in the study gave a written informed consent. The study design was approved by the national medical ethics committee.

Before surgical treatment, cervical smears for cytology and HC II were taken; for the latter we used the *Female Swab Specimen Collection Kit™ (Digene Corp., Gaithersburg, MD, USA).* Women were treated by laser vaporization (LV), loop electrosurgical excision procedure (LEEP) or cold knife conization. If LEEP or cold knife conization was performed, material for histological analysis was obtained during the procedure. If LV was performed, we considered the histological result previously obtained by colposcopically directed biopsy in the analysis. The

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pathologist was aware of the patient's history and her cytological diagnosis, but was blinded to her HPV status.

The HPV testing was done with the HC II test. The probes for detection of high-risk genotypes of HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 were used. All samples were analysed according to the manufacturer's instructions. Results of the test were given as a relation between the relative light unit (RLU) of a specimen and the RLU of the cut-off value. Specimens with the RLU/cut-off value ratio of ≥ 1.0 were considered positive for one or more high-risk HPV genotypes.

Sensitivity, specificity, positive predictive value and negative predictive value were evaluated to demonstrate performance characteristics of the HC II test for detection of CIN 2+ and CIN 3 or worse (CIN 3+). For the assessment of variability, a 95% confidence interval (CI) was calculated. The Student's t-test was used to compare normally distributed variables. Differences were considered statistically significant if p values were < 0.05. Statistical analysis was made with the statistical package SPSS for Windows, Release 11.0, Standard Version (SPSS Inc., Chicago, Illinois, USA).

Results

Of the 197 included women, 16 (8.1%) did not have histological results because biopsy was not done or the material was unsatisfactory for evaluation, therefore 181 women were included in further analysis.

The mean women's age was 36.0 years (SD 9.32).

LV was performed in 69 (38.1%) cases, LEEP in 98 (54.2%) cases and cold knife conization in 14 (7.7%) cases.

Cytological and histological results together with the percentage of HPV infected women are summarised in Table 1.

Table 1. — *Cytological, histological and HPV results; number of women (percentages of HPV infected women in brackets).*

Histological results	Cytological results - dyskaryosis			Total
	Mild	Moderate	Severe +	
< CIN 2	55 (21.8%)	22 (59.1%)	3 (66.7%)	80 (33.8%)
CIN 2	18 (27.8%)	17 (82.4%)	2 (100.0%)	37 (56.8%)
CIN 3	11 (72.7%)	42 (88.1%)	7 (100.0%)	60 (86.7%)
Microinvasive carcinoma	0	1 (100.0%)	3 (100.0%)	4 (100.0%)
Total	84 (29.8%)	82 (79.3%)	15 (93.3%)	181

+ including glandular dyskaryosis and glandular cell carcinoma (n = 4).

HPV testing showed 104/181 (57.5%) women to be HPV positive.

At first we considered CIN 2+ and then CIN 3 to be the threshold histological result for the analysis.

Overall 80 (44.2%) women had histological diagnosis of < CIN 2. In this group 53 (66.2% of women with < CIN 2, or 29.3% of the total) women tested HPV negative. In the group of 101 women with a histological diagnosis of CIN 2+, 24 (23.8% of women with CIN 2+ or 13.3% of the total) tested HPV negative. The difference in HPV-negative women between these two groups (< CIN 2 and CIN 2+) was statistically significant ($p < 0.05$).

Overall 117 (64.6%) women had a histological diagnosis of < CIN 3. In this group 69 (59.0% of women with < CIN 3 or 38.1% of the total) women tested HPV negative. In the group of 64 women with a histological diagnosis of CIN 3+, eight (12.5% of women with CIN 3+ or 4.4% of the total) tested HPV-negative. The difference in HPV negative women between the two groups (< CIN 3 and CIN 3+) was again statistically significant ($p < 0.05$).

Relations between HPV results and histological results are shown in Table 2.

Table 2. — *HPV infection in relation to histological results.*

Histological	HPV +	HPV –	Total
< CIN 2	27 (14.9%)	53 (29.3%)	80 (44.2%)
CIN 2	21 (11.6%)	16 (8.8%)	37 (20.4%)
CIN 3	52 (28.7%)	8 (4.4%)	60 (33.1%)
Microinvasive carcinoma	4 (2.2%)	0	4 (2.2%)
Total	104 (57.5%)	77 (42.5%)	181 (100.0%)

HPV infections in women with < CIN 2, CIN 2+, < CIN 3 and CIN 3+ are shown in Figures 1 and 2.

Significantly more women with CIN 3+ were HPV infected compared to the women with CIN 2+ (87.5% vs 76.2%; $p < 0.05$).

Sensitivity, specificity, positive predictive value and negative predictive value were evaluated to demonstrate performance characteristics of the HC II test. We considered a positive HPV result and CIN 2+ to be a true positive result; a positive HPV result and < CIN 2 to be a false-positive result; a negative HPV result and CIN 2+ to be a false-negative result and negative HPV result and < CIN 2 to be a true negative result.

As the calculated sensitivity, specificity, positive and negative predictive values were different from those in the literature [16, 21], we calculated these parameters for CIN 3+ as well. The results are shown in Table 3.

Table 3. — *Performance characteristics of the HPV test in detection of CIN 2+ and CIN 3+.*

HPV test	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)
CIN 2+	76.2 (69.6-82.1)	66.2 (57.8-73.6)	74.0 (67.5-79.7)	68.8 (60.1-76.5)
CIN 3+	87.5 (78.1-93.8)	59.0 (53.8-62.4)	53.8 (48.1-57.7)	89.6 (81.8-94.9)

Discussion

In this study, the 181 women, who fulfilled the criteria for the analysis, had undergone surgical treatment for precancerous cervical lesions diagnosed by cytology, colposcopy and confirmed by histology.

Our results show that 53 of the 181 (29.3 %) women that underwent surgical treatment had a histological diagnosis of < CIN 2 and tested HPV negative. This is the number of women that would be spared surgical treatment if HPV testing had been used in addition to cytology and colposcopically directed biopsy. If we considered CIN 3 as the threshold histological result, 69 (38.1%) would be spared surgical treatment.

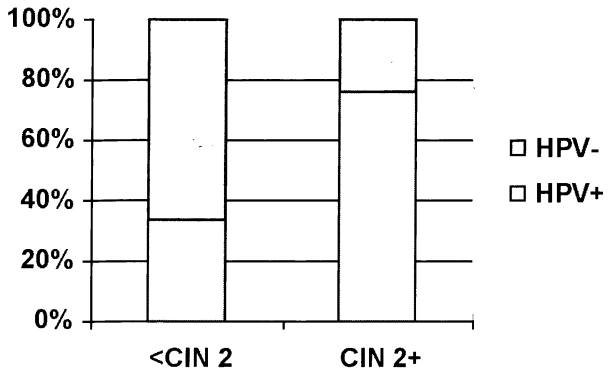

Figure 1. — HPV results in relation to histological results < CIN 2 and CIN 2+.

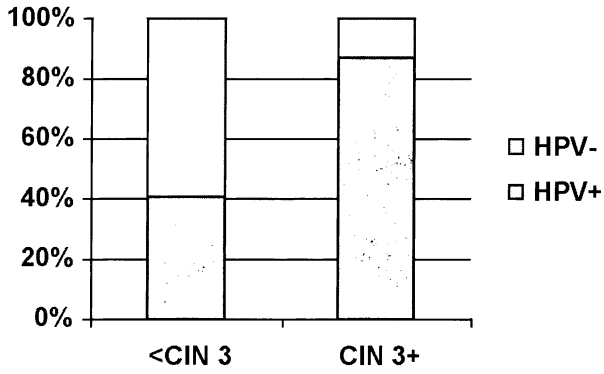

Figure 2. — HPV results in relation to histological results < CIN 3 and CIN 3+.

Moreover, 22 women (12.1% of the total) with a cytological diagnosis of moderate dyskaryosis had a histological diagnosis < CIN 2, and three women (1.7% of the total) with a cytological diagnosis of severe dyskaryosis or worse had a histological result < CIN 2. This indicates that some smears were assessed to have higher-grade dyskaryosis than they really had.

In our study the percentages of HPV-positive women with pathological cytological and histological results were lower than those found by Cuzick *et al.* [21], presumably because of cytological and histological overestimation: 21.8% of women in our study vs 79% of women in their study had mild dyskaryosis and < CIN 2, 27.8% vs 100% had mild dyskaryosis and CIN 2, 72.7% vs 100 % had mild dyskaryosis and CIN 3; 59.1% vs 100% had moderate dyskaryosis and < CIN 2, 82.4% vs 100% had moderate dyskaryosis and CIN 2, 87.2% vs 92% had moderate dyskaryosis and CIN 3 [21].

The percentage of HPV-positive women with severe dyskaryosis, regardless of the histological result, was similar in both studies. This indicates that we have problems mainly with low-grade cervical lesions. High-grade lesions are properly diagnosed and managed.

We calculated that the sensitivity of the HC II test for the detection of CIN 2+ was 76.2% and specificity 66.2%. The performance characteristics were poorer than in other studies [16, 22], where the sensitivity reached 98% and specificity 95.3%. Therefore we calculated these parameters for detection of CIN 3+, but the performance characteristics were still poor.

As shown in our study, there were almost one-third of women with a cytological diagnosis of mild dyskaryosis and more than 10% of those with a histological diagnosis of CIN 2+, who tested HPV negative. Since it is known that permanent HPV infection is one of the factors leading to the development of cervical cancer, women without HPV infection are not threatened by cervical cancer [10]. We have shown that cytological as well as histological results are often overestimated, therefore the additional usage of HPV testing would be of great value especially in the management of women with cervical cytological abnormalities diagnosed as mild or moderate dyskaryosis. In Slovenia, HPV testing would reduce the number of unnecessary surgical treatments and should therefore be used as an additional screening tool.

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