The significance of HPV in the follow-up period after treatment for CIN

J. Gallwas, *M.D.*; **N. Ditsch**, *M.D.*; **P. Hillemanns**, *M.D.*; **K. Friese**, *M.D.*; **C. Thaler**, *M.D.*; **C. Dannecker**, *M.D.*

Department of Obstetrics and Gynecology, University of Munich, Munich (Germany)

Summary

Purpose of investigation: High-risk anogenital human papillomavirus (HPV) infections are causally related to cervical cancer. Successful treatment of cervical intraepithelial neoplasia (CIN) results in complete eradication of HPV in most cases. There is an increasing interest regarding the role of HPV testing in the follow-up period after treatment for CIN. *Patients and Methods*: This retrospective study includes 107 women who underwent conization for histologically verified CIN. All of them had HPV testing pre- and postoperatively. HPV testing was carried out using a hybrid capture assay (HC2). The mean follow-up period was 21.4 months (range 2-76 months). The data were analyzed with respect to success of conization, HPV persistence/recurrence and CIN recurrence. Sensitivity, specificity and negative predictive value (NPV) of HPV testing were assessed and compared to the cytological results. *Results*: Preoperatively, 97 of 107 women were HPV positive. Ninety-seven conizations showed negative resection margins with 86 women becoming HPV negative. In the following months, nine of these HPV negative women became HPV positive again. Out of ten conizations with positive resection margins, six women became HPV negative. Recurrent CIN 2/3 lesions were observed in 11 women, nine of whom had persistent positive HPV testing throughout the entire study period. Regarding CIN recurrence HPV testing showed a sensitivity of 93%, a specificity of 85% and a NPV of 99%. *Conclusions:* The sensitivity of HPV testing concerning persistent or recurrent CIN as well as the NPV are high. The present data suggest that HPV testing should be integrated in a follow-up algorithm after treatment for CIN by conization.

Key words: CIN; Conization; LEEP; Follow-up.

Introduction

Approximately 500.000 women worldwide are annually diagnosed with invasive cervical carcinoma (ICC) and about 230,000 women die from the disease [1]. Although the incidence of ICC has declined over the last decade, the incidence of cervical intraepithelial neoplasia (CIN) has increased, especially in younger women. If untreated, 15-20% of these women will develop severe dysplasia and 5-10% invasive carcinoma [1-3]. About 15 of more than 40 genital mucosal types of HPV are known to be oncogenic, causing almost all ICC and cervical precancerous lesions, including CIN 3 [4-6]. Therefore, it appears reasonable that HPV-DNA detection in cervical samples would improve the performance of existing screening methods. In fact, it has been shown that HPV testing in combination with Pap tests are 96% to 100% sensitive for the detection of CIN [4, 6, 7]. Furthermore, it has been shown that HPV is eliminated after successful treatment of CIN whereas it persists in recurrent disease [6-8]. This implies a potential role of HPV testing in the follow-up period after treatment of CIN. Several studies suggested that HPV testing is useful in predicting the presence of residual CIN while others indicated that the presence of HPV after treatment resembles only a risk factor for residual CIN and that additional diagnostic procedures are indispensable [9-13].

We studied the value of HPV-DNA testing in the follow-up period after treatment of CIN. In particular we evaluated:

- If conization eradicates HPV.
- The sensitivity of HPV testing in the detection of persistent or recurrent CIN.
- If HPV testing should be combined with Pap tests in the follow-up.

Patients and Methods

Over a period of six years 385 women were admitted to the Department of Gynecology and Obstetrics, University of Munich for conization of histologically verified CIN 2/3 or because of a cervical smear showing Pap III to Pap IV dysplasia. One hundred and seven of these patients who underwent HPV testing pre- and postoperatively were included in this retrospective study.

The gynecological examinations were carried out at the Colposcopy Clinic of the Department of Gynecology and Obstetrics, University of Munich, and followed a specific sequence: two Pap smears were obtained, one from the ectocervix (cotton tip swab) and one from the endocervix (cytobrush). A HPV DNA sample was obtained from the cervix with a cytobrush. Standard colposcopy was performed with acetic acid (3%). Directed biopsies were taken from acetic acid positive areas. Cervical smears were classified according to the revised Munich classification which is the most widely used in Germany (Münchner Nomenklatur II): °I, normal cytology; °II, mild to moderate inflammatory, metaplastic or degenerative changes; °III, squamous or glandular cells of defined significance; °IIID, mild to moderate dysplasia; °IVa, severe dysplasia or carcinoma *in situ*; °IVb, carcinoma *in situ*, invasion cannot be ruled out; °V, invasive carcinoma. Histology was classified as follows: CIN 1, mild dysplasia; CIN 2, moderate dysplasia; CIN 3, severe dysplasia/carcinoma *in situ*.

HPV testing was carried out using the Hybrid Capture System 2 (HC2) (Digene, Gaithersburg, MA, USA). This

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test detects 13 different high-risk HPV types (16,18,31,33,35,39,45,51,52,56,58,59,68), and is approved by the FDA. It was run in accordance to the manufacturer's protocol. HPV-DNA analysis was quantitative and women with samples producing readings higher than the positive controls (1 pg/ml HPV DNA) were regarded as being HPV test positive.

Statistical analyses were performed using SPSS version 8.0 (SPSS Inc. Chicago IL, USA). Significant differences in proportions were assessed using the chi-square test.

Results

The 107 women who were included in this study had a mean age of 34.5 years (range 22-68 years). Electrosurgical loop conization (LEEP) was carried in 87 women (81%) while 20 women had cold-knife conization. The mean follow-up period was 21.4 months (range 2-76 months) with the first postoperative control after a median of four months (range 1 to 54 months). The women had between one and nine follow-up investigations (mean 2.7 investigations). The first follow-up HPV testing was carried out at 4.8 months (range 1 to 10.7 months) after conization.

Preoperative HPV testing showed that 97 women (91%) were high-risk HPV positive and ten women (9%) HPV negative. Preoperative cervical biopsies were available for 104 women revealing CIN 1 in 17 cases CIN 2 in 30 cases, CIN 3 in 55 cases as well as two negative findings. In these women as well as in those with CIN 1 conization was carried out because of persistent Pap IIID dysplasia in cervical cytology. Among the ten women with negative HPV testing preoperatively we observed two CIN 2 lesions, six CIN 1 lesions and two negative findings . However, all these women showed severe dysplasia in cervical cytology. Eighty-seven women (81%) underwent electrosurgical loop conization. An in sano resection was achieved in 78 women (89%). Seventy-seven women (88%) became HPV negative. Cold-knife conization was performed in 20 women (19%). Here, free resection margins were achieved in 19 patients (95%) and a negative HPV status in 17 patients (85%). There were no statistically significant differences between either group.

Operative histology revealed one negative finding, CIN 1 in 16 cases, CIN 2 in ten cases, and CIN 3 in 61 cases. A comparison between preoperative and postoperative histological findings is shown in Table 1.

Among the 97 women in whom free resection margins were achieved, 86 (87%) had a negative postoperative HPV test. In contrast, among the ten women that were considered as treatment failures, only six (60%) had a negative postoperative HPV test whereas four (40%) were positive for HPV (Table 2).

Regarding the follow-up, a permanent HPV eradication or a persistent negative HPV test were seen in 83 women. One of these women developed recurrent CIN 2/3. In 24 women a permanent eradication of CIN/HPV was not achieved. Fifteen women remained HPV positive throughout the course, whereas nine women became HPV positive again after having been negative in the initial follow-up period. Ten (42%) of these 24 women developed recurrent CIN 2/3.

Table 1. — *Comparison of preoperative cervical biopsies and final operative histology.*

Operative Histology	Preoperative Negative	CIN1	CIN2	CIN3	Histology Total
Negative	1	4	6	3	14
CIN1	0	9	5	2	16
CIN2	0	1	7	2	10
CIN3	1	3	11	46	61
Microinvasive carcinoma	0	0	1	2	3
Total	2	17	30	55	104

The difference between both groups was highly significant ($p < 0.001$). The sensitivity of HPV testing in detecting treatment failures was 93% with a specifity of 85%. The negative predictive value (NPV) of persistent negative HPV to predict recurrent/residual disease was 99% and the positive predictive value (PPV) 42%.

Postoperative cervical cytology showed inconspicious results in 61 women whereas 46 women developed a positive cytology (Pap IIID or higher). Recurrent CIN 2/3 was observed in ten patients with positive cytology and in one woman with negative cytology. Accordingly, in the present series cervical cytology reached a sensitivity of 91% and a specifity of 63%. The NPV to predict recurrent/residual disease was 98% and the PPV 22%.

Discussion

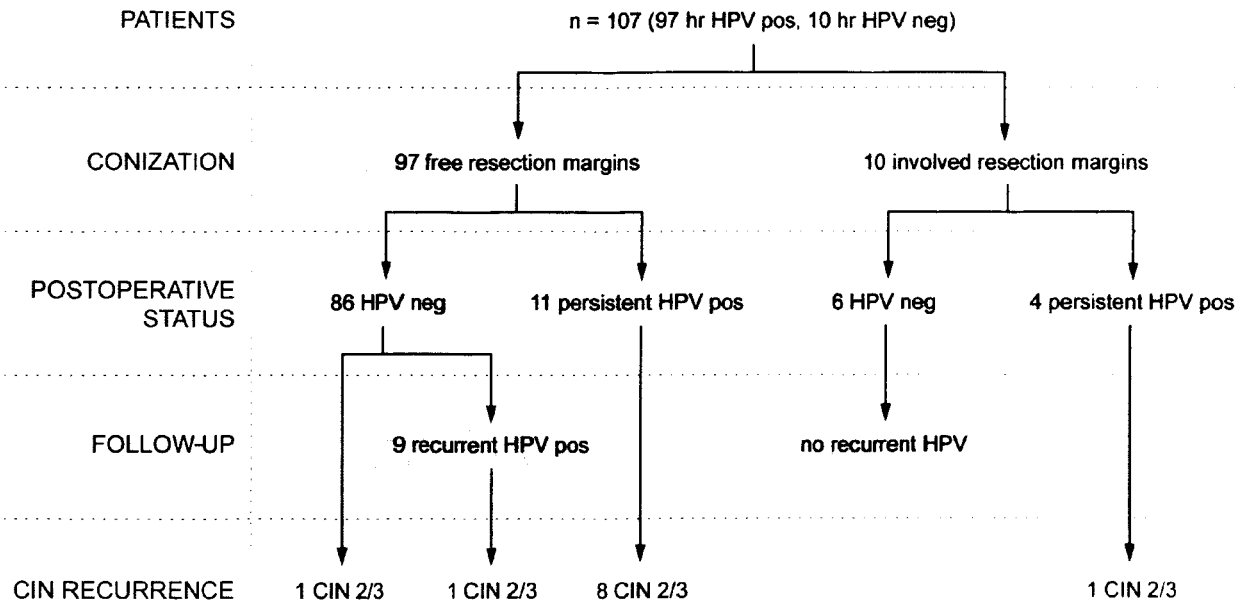
In recent years, several national societies have established specific guidelines for the follow-up after treatment of CIN. Most of these protocols include cytology, colposcopy and HPV testing at various intervals. However, they are difficult to compare as the approaches are different and have not been evaluated in randomized clinical trials.

The efficacy of cytology screening in detecting recurrent disease is controversial as various studies show inconsistent results. Its specifity lies above 95% with most recurrences being associated with pathological findings, though its main disadvantage is the dissatisfying sensitivity between 20 and 85% [10, 11, 14-16]. This degree of false negative follow-up cytology accounts for the interest in finding additional diagnostic tools such as HPV testing that either alone or in combination would increase the predictive value in detecting recurrent disease.

Systematic reviews by Paraskevaidis *et al.* [10] and Zielinski *et al.* [11] have found that the pooled sensitivity of HPV testing for detecting recurrent or persistent disease reaches 90% six months after treatment and remains at this level for at least 24 months. Some studies showed that the combination of HPV testing and cytology resulted in increased sensitivity [10].

In the present study ten women (9%), six of them with a CIN 1 lesion, had a negative HPV status initially. It is known that the prevalence of HPV rises with increasing

Table 2. — *HPV status and clinical course after conization for CIN in 107 women (hr: high risk).*



severity of the CIN lesion. By applying the PCR technique HPV was shown to be present in 78% of women with CIN 1, 86% of women with CIN 2 and 88% of women with CIN3 [6, 17]. Concerning the different tests available today, the clinical accuracy of hybridization tests such as the HC2 used in this study is at least equal to PCR-based assays [18].

The issue of whether HPV DNA becomes negative after conization is of relevance for the question of the usefulness of HPV testing in the follow-up period. In their review of the literature, subsuming 11 studies, Paraskevaidis *et al.* report, that among 672 women in whom CIN was treated successfully 566 (84%) showed negative postoperative HPV testing, whereas 106 (16%) remained HPV positive. Among the 204 women that were considered as treatment failures only 35 (17%) showed negative HPV testing, whereas 169 women (83%) were positive [10]. In accordance with these studies the present data indicate that conization to a high extent eradicates HPV. However, there exists a significant difference between negative and positive resection margins.

CIN positive excisional margins (non in sano resection) are accepted as a risk factor for recurrent disease, and it is more likely that these women redevelop abnormal cervical cytology. However, CIN-positive margins are not a reliable predictor of treatment failure as residual or recurrent disease can develop with both involved and clear margins [9-11]. This has also been shown in the present study, where most women with involved margins remained disease-free on follow-up although HPV eradication was significantly lower than after in sano excision ($p < 0.05$).

In subsuming 11 studies, Zielinski *et al.* found in their meta-analysis of combined testing for cytology and free resection margins a low HPV (92%, range 85-96%) when compared to that of combined testing for HPV and cytology (99%, range 98-100%) or HPV and resection margins (99%, range 95-100%) [11]. Although the sensitivities of combined testing for HPV and resection margins or cytology were comparable, the specifity of combined HPV testing and cytology (81%, range 77-84%) was much higher than that of HPV testing and resection margins (54%, range 47-61%). The authors therefore concluded that HPV testing in combination with cervical cytology represents the best combination to monitor women in the follow up period [11].

The ongoing European multicenter study has set the goal to reach a conclusion regarding the optimal follow-up algorithm in order to define a strategy that would ultimately diminish the incidence of post-treatment cervical carcinoma. Based on our experience and the available studies, the implementation of HPV testing in post-treatment screening programmes might lead to a decrease in the rate of false-negative results and to an extension of the screening intervals. Open questions remain in setting the length of optimal screening intervals and the combination or sequence of cytology and HPV testing.

In conclusion, involvement of the surgical margins and the presence of HPV are associated with a higher risk of recurrence. HPV testing does not seem to be obviously superior to cervical cytology screening but the combined tests increase the sensitivity of detecting persistent or recurrent CIN and seem to be more effective than either test alone or the resection margin status. Furthermore, the combination of both tests increases the NPV identifying those women with minimal risk for persistent or recurrent disease.

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Address reprint requests to:
C. DANNECKER, M.D.
Klinik und Poliklinik für Frauenheilkunde
und Geburtshilfe
Klinikum Großhadern
Marchioninistrasse 15
81377 München (Germany)
e-mail: Christian.Dannecker@med.uni-muenchen.de