



# The role of human papillomavirus (HPV) testing in the follow-up of patients after treatment for cervical intraepithelial neoplasia (CIN)

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## ABSTRACT

**Introduction:** The aim of this study was to examine the role of human papillomavirus testing in the follow-up after treatment for CIN, as a prognostic sign for residual/recurrent cervical precancerous lesions.

**Methods:** A hospital-based analysis was performed on 460 patients previously treated for CIN with cold knife conization, at the University Clinic for Gynecology and Obstetrics and General Hospital Remedika, in Skopje, Republic of Macedonia, in a period of 3 years. The patients were followed-up with HPV testing in addition to cytology, colposcopy and/or biopsy. The first after treatment HPV testing was performed 8 months after cold knife conization, proceeded by follow-up within 24 months after treatment, at 4 months intervals.

**Results:** Among 460 treated patients, at the first HPV and cytologic testing, 8 months after treatment, 69 (15%) were HPV+, and 391 (85%) HPV negative. From the 69 HPV+ patients, 41 (59.4%) were with cytologic abnormalities and 28 (40.6%) without abnormalities. 12 months after treatment, the number of HPV+ patients developing cytologic abnormalities raised to 45/70 (64.29%). Within the 24 months after treatment, the number of patients who had recurrent/ residual CIN from the HPV+ patients reached 50/71 (70.42%); which was 10.87% from all 460 treated patients.

**Conclusion:** Persistence or clearance of HPV especially 8 months after treatment even in patients with normal cytology, is an early valid prognostic marker of treatment failure, and is more accurate than cytology at the same follow-up intervals.

**Keywords:** human papillomavirus, uterine cervix carcinoma, HPV, cervical intraepithelial neoplasia

## INTRODUCTION

Cervical cancer with an estimated 530 000 new cases in 2008 is the third most common cancer in women, and the seventh overall. More than 85% of these cases occur in developing countries, where it accounts for 13% of all female cancers. The highest-

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Submitted 14 March 2013 / Accepted 15 August 2013



risk regions are Eastern and Western Africa [age-standardized rate (ASR) greater than 30 per 100 000], and the lowest are Western Asia, Northern America and Australia/New Zealand, where the rates are less than 6 per 100 000. Above all, in 2008, cervical cancer proved to be the reason for 275.000 deaths with the mortality incidence ratio of 52%, and an estimation of 88% of deaths in developing countries from which 53.000 in Africa, 31.700 in Latin America and the Caribbean, and 159.800 in Asia (1, 2).

Today, it is well-established that Human papillomavirus (HPV) infection is the most important cause of cervical cancer, with a special attention to HPV types 16 and 18, which proved to be the reason in 70% of the world cervical cancer cases. The World data show that around 11.4% of women are evaluated to capture cervical HPV infection at a given time. The same data presented that the prevalence of HPV 16 and/or 18 ranging from normal cytology is 3.8%; through 24.3% in low-grade cervical lesions; up to 51.1% in high-grade cervical lesion. The same types are blamed for about 70.9% of the most invasive cases (3). The DNA-HPV detection results of cervical infection are measured in all cervical morphological lesions ranging from normal findings up to invasive cervical cancer, showing that the prevalence of HPV increases with the malice of the lesion. HPV remains the cause of almost 100% of all cases of cervical cancer. The vaccine-preventable HPV-16 and -18, are still the reason for more than 70% of all cervical cancer cases in the world, especially in high-grade cervical lesions, 41-67% (4). After HPV-16/18, the six most common HPV types in all world regions, which account for an additional 20% of cervical cancers worldwide are the types: 31, 33, 35, 45, 52 and 58. The discovery of Human Papilloma Virus (HPV) infection to be the prime cause for this disease, gives a tremendous chance to prevent and early detect cervical neoplasia (5, 6). Recent studies demonstrated that HPV test combined with cytology may improve the early detection of both primary cervical neoplasia as well as recurrence of neoplasia after therapy, decreasing the need for more radical treatment (7, 8).

Cervical conization is defined as excision of a cone-shaped or cylindrical wedge from the uterine cervix that includes the transformation zone and all or a portion of the endocervical canal. It is used as a de-

finite diagnostic and treatment tool for squamous or glandular intraepithelial lesions and for excluding micro-invasive carcinomas.

There are several conization techniques: cold-knife (scalpel) conization, laser conization, or electro-surgical loop conization, each with certain benefits and disadvantages. The cleanest specimen margins for patho-histologic analysis is provided only by cold-knife conization. As an attempt to excise gross cervical tumors per vaginam, in the early 19<sup>th</sup> century, similar procedures to conization were used. In the late 20<sup>th</sup> century, first conization was used as a diagnostic tool for cervical lesions and later as treatment as well. Today the use of cold-knife conization as a diagnostic tool is reduced since wide spreading the colposcopically directed cervical biopsies combined with endocervical curettage as less invasive procedure with high diagnostic value. However, in selected situations, it is still very important diagnostic tool and accepted modality for management and treatment of CIN (9-12).

Conization site usually heals in 6 weeks. To determine treatment success and avoid possibility of residual or recurrent CIN, Papanicolaou tests should be performed every 4 months during the first and second postoperative years and every 6 months thereafter. A single follow-up Papanicolaou test shows positive results in fewer than 25% of women with residual disease, therefore we designed our study to determine the role of human HPV testing in the follow-up after treatment for CIN, as a valuable prognostic sign for residual/recurrent cervical precancerous lesions.

## METHODS

### Patients

A hospital-based analysis was performed on 460 patients previously treated for CIN with cold knife conization, at the University Clinic for Gynecology and Obstetrics and General Hospital Remedika, in Skopje, Republic of Macedonia, in a period of 3 years. The patients were followed-up with HPV testing in addition to cytology, colposcopy and/or biopsy. The first after treatment HPV testing was performed 8 months after cold knife conization, proceeded by follow-up within 24 months after treatment, at 4 months intervals.

**TABLE 1.** The role of HPV testing in the follow-up of patients 8 months after treatment for CIN

Values entered:	Condition: Recurrent/Residual CIN 8 months after treatment		Totals
	Absent	Present	
HPV Test Positive	28	41	69
HPV Test Negative	386	5	391
Totals	414	46	460
		95% Confidence Interval	
	Estimated Value	Lower Limit	Upper Limit
Prevalence	0.1	0.074864	0.132022
Sensitivity	0.891304	0.756386	0.959285
Specificity	0.932367	0.902598	0.953796
Positive predictive value	0.15	0.119293	0.186696
Negative predictive value	0.85	0.813304	0.880707
True Positives	0.594203	0.469205	0.708661
False Positives	0.405797	0.291339	0.530795
True Negatives	0.987212	0.968665	0.995283
False Negatives	0.012788	0.004717	0.031335
likelihood Ratios	13.178571	9.088179	19.109962

### Procedures

During each of these follow-up visits, patients received colposcopy, conventional PAP or liquid based PAP (CYTOFAST by HOSPITEX DIAGNOSTICS, Sesto Fiorentino, Italy) and HPV test specimens. Biopsy and/or endocervical curettage was performed to prove recurrent/residual lesion only if the previous test suggested low-grade or high-grade cervical lesions. Residual/recurrent disease was defined only if the CIN2+ lesion was histologically confirmed at least 8 months after treatment. After re-treatment, women received further follow-up tests, but were dropped out from the study. If women had histologically confirmed CIN1, follow-up continued without treatment ('wait and see').

Human papillomavirus DNA was detected by Polymerase Chain Reaction (PCR) method in the Laboratory for Molecular Biology, Institute of Biology, Faculty of Natural Sciences and Mathematics, Skopje, Macedonia. The material for analysis (exfoliated cells in medium) was analyzed 24-48 hours after sample collection. The cervical cells were collected and digested with an appropriate buffer containing Proteinase K and 0.5% SDS. The total DNA was isolated with NaCl/chloroform extraction and ethanol precipitation. The PCR amplification was performed with 3 pairs of consensus primers (MY09/11, GP5+/6+, HPVpU 1M/2R) specific for L1 and E6/E7 regions of the HPV genome (thermo-

cycler Perkin Elmer Geneamp PCR System 2400). Positive and negative controls were included in each of the tested series. The positive primers were genotyped and digested with 7 restriction endonucleases (AfaI, HaeIII, PstI, AccI, AvaII, BglII, AvaI) specific for "low-risk" HPV types (6, 11, 40, 42, 43, 44, 54, 55, 61, 70, 72, 81, MM8, CP6108) and "high-risk" HPV types (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82, MM4, MM7, MM9). The results were analyzed with agarose gel electrophoresis and visualised on UV transilluminator. The viral genotype was determined through the length of restriction fragments of the electrophoresis gel (12).

The results from the conventional PAP or liquid-based smears that were used for cytological analysis were interpreted according to the Bethesda classification III System, 2001.

### Statistical analysis

All data gathered during the follow-up period, were analyzed using the statistical package SPSS 20. We computed the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and likelihood ratio with 95% confidence intervals (95% CI), for cytology abnormalities development and HPV presence/persistence and the combination of these tests by making use of cross tabs.

**TABLE 2.** The role of HPV testing in the follow-up of patients 12 months after treatment for CIN

Values entered:	Condition: Recurrent/Residual CIN 12 months after treatment		Totals
	Absent	Present	
HPV Test Positive	25	45	70
HPV Test Negative	386	4	390
Totals	411	49	460
		95% Confidence Interval	
	Estimated Value	Lower Limit	Upper Limit
Prevalence	0.106522	0.080563	0.139246
Sensitivity	0.918367	0.795162	0.973524
Specificity	0.939173	0.910335	0.959457
Positive predictive value	0.152174	0.121257	0.189041
Negative predictive value	0.847826	0.810959	0.878743
True Positives	0.642857	0.518666	0.751266
False Positives	0.357143	0.248734	0.481334
True Negatives	0.989744	0.972124	0.996709
False Negatives	0.010256	0.003291	0.027876
likelihood Ratios	15.097959	10.232947	22.275926

## RESULTS

Among 460 treated patients, at the first HPV and cytologic testing, 8 months after treatment, 69 (15%) were HPV+, and 391 (85%) HPV negative. From the 69 HPV+ patients 41 (59.4%) were with cytologic abnormalities; and 28 (40.6%) without abnormalities.

Twelve months after treatment, the number of HPV+ patients developing cytologic abnormalities raised to 45/70 (64.29%). Within the 24 months after treatment, the number of patients who had recurrent/ residual CIN from the HPV+ patients reached 50/71 (70.42%); which was 10.87% from all 460 treated patients.

Eight months after treatment, HPV Prevalence, Sensitivity and Specificity are 15%; 89% and 93% respectively; followed by 15% Positive Predictive Value; 85% Negative Predictive Value; 59% True Positive Rate; 41% False Positive Rate; 99% True Negative Rate; 1% False Negative Rate. The Positive Likelihood Ratio is 13.18 (9.09-19.11) with 95% Confidence Interval (Table 1).

Twelve months after treatment, the HPV Prevalence, Sensitivity and Specificity are 11%; 92% and 94% respectively; followed by 15% Positive Predictive Value; 85% Negative Predictive Value; 64% True Positive Rate; 36% False Positive Rate; 99% True

Negative Rate; and 1% False Negative Rate. The Positive Likelihood Ratio is 15.10 (10.23-22.28) with 95% Confidence Interval (Table 2).

Twenty-four months after treatment, HPV Prevalence, Sensitivity and Specificity are 12%; 94% and 95% respectively; followed by 15% Positive Predictive Value; 85% Negative Predictive Value; 70% True Positive Rate; 30% False Positive Rate; 99% True Negative Rate and 1% False Negative Rate. The Positive Likelihood Ratio is 18.28 (11.99-27.87) with 95% Confidence Interval (Table 3).

## DISCUSSION

It is evidence-based that HPV, especially the high risk types, are the most important cause of cervical cancer (13, 14). It is also clear that there is a relation between persistent infection of HPV and CIN2+ lesions.

The aim of this study was to examine the role of HPV testing in the follow-up after treatment for CIN, as a prognostic sign for residual/recurrent cervical precancerous lesions. Based on many different large studies it was published that at least 95% of patients who have CIN can be cured by different conization techniques. However, cure rates as low as 60% have also been reported. Also, in patients with positive margins for precancerous lesion in the

**TABLE 3.** The role of HPV testing in the follow-up of patients 24 months after treatment for CIN

Values entered:	Condition: Recurrent/Residual CIN 24 months after treatment		Totals
	Absent	Present	
HPV Test Positive	21	50	71
HPV Test Negative	386	3	389
Totals	407	53	460
		95% Confidence Interval	
	Estimated Value	Lower Limit	Upper Limit
Prevalence	0.115217	0.088214	0.14883
Sensitivity	0.943396	0.833723	0.985267
Specificity	0.948403	0.920963	0.966989
Positive predictive value	0.154348	0.123224	0.191384
Negative predictive value	0.845652	0.808616	0.876776
True Positives	0.704225	0.582474	0.803664
False Positives	0.295775	0.196336	0.417526
True Negatives	0.992288	0.975702	0.998006
False Negatives	0.007712	0.001994	0.024298
likelihood Ratios	18.283917	11.992908	27.874943

conization specimen, according to Felix et al. the Recurrence or Persistence (16.5%) is significantly more often than Cure Rate (1.9%) (15). Since a single follow-up Papanicolaou test shows only in 25% positive results of residual disease, different studies showed that HPV tests combined with cytology offer clear advantage in the postoperative follow-up period, especially the redevelopment of CIN can be caused by the same HPV subtype that induced the initial disease (16, 17).

In our study the patients were followed-up with HPV testing in addition to cytology, colposcopy and/or biopsy. The first after treatment HPV testing was performed 8 months after cold knife conization, proceeded by follow-up within 24 months after treatment, at 4 months intervals.

Our results regarding the first detection of HPV in the post-conization period, declared that during the first 8 months after the end of therapy, HPV persisted in 69 of 460 treated patients (15%). Furthermore it pointed out that 59.4% of the HPV positive patients had cytologic abnormalities 8 months after treatment, and that number raised to 66.4% after 12 months and at the end reached 72.4%, 24 months after treatment. These results show that HPV seems to be of crucial importance in developing recurrent/residual CIN in the post-operative period.

Multiple studies recognised HPV-DNA test as

100% accurate in identifying development of CIN, in the follow-up period after treatment (18, 19). Other studies reported slightly lower sensitivity of HPV DNA test. One of those is the large Nobbenhuis and Paraskevaidis study reporting 93% sensitivity of HPV DNA test in detecting recurrence of CIN (20- 22).

Our study, in 8, 12 and 24 months after treatment showed similar sensitivity of 89, 92 and 94% respectively; but higher specificity at the same points of follow-up period (93, 94, and 95% respectively).

Specificity given by other authors of HPV tests for CIN detection are as follows: 88%-Nagai, 86%-Nobbenhuis, and 84%-Paraskevaidis (18, 20-22).

In our study, an increase of specificity and sensitivity was noticed in the further follow-up period points. Based on these observations, there is a need, the follow-up period to be increased in longer than 12 months. Diagnostic value of HPV testing in the follow-up of patients after treatment for CIN, as a method of detection of recurrent/residual neoplasia, increases with time and seems to be of great significance the hole period of 24 months after treatment. An attention should be drawn to significance of initial viral test (8 months after treatment) for prediction of recurrent/residual CIN, since 59.4% of the patients with CIN recurrence were HPV positive at the first follow-up visit after conization.

## CONCLUSION

Persistence or clearance of HPV especially 8 months after treatment even in patients with normal cytology, is an early valid prognostic marker of treatment failure, and is more accurate than cytology at the same follow-up intervals.

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