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RESEARCH

The Value of HPV-HR DNA Testing During the Follow-Up After Treatment of CIN3/AIS

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Introduction

The fact that up to 30 % of women treated for a preinvasive cervical disease are diagnosed with a residual/recurrent disease including an invasive carcinoma during follow-up, requires close surveillance of these patients [1]. The majority of cases (80–90 %) are detected during the first 2 years after conization [2]. This in part justifies the anxiety of patients and gynecologists in fearing insufficient treatment or recurrence of the disease and that can lead to over-surveillance and over-treatment. We must keep in mind that these patients are mostly of childbearing age and desire preservation of fertility. Repeated treatments on the cervix can cause negative sequelae in their reproductive health [3].

Cytology, colposcopy and HPV-HR DNA testing (HPV test) during the follow-up have some downfalls compared to primary screening. Removal of the transformation zone at conization and scarring after surgery often makes colposcopy unsatisfactory and unreliable, especially when the cold-knife method is used [4]. Cytology is burdened with reparative and proliferative healing process of the epithelium. Conization removes the dysplastic lesion but not necessarily all the normal epithelium infected by HPV. Being aware of this we must use these tools in the best cost-effective protocol, which will be most accurate in diagnosis and sparing in treatment. Studies have shown a higher sensitivity in detection of residual/recurrent disease with the use of combined testing (cytology and HPV) [5, 6]. Combined testing raises the cost of follow-up and in some countries like Croatia, with a tradition of high quality cytology this is unacceptable. Therefore we performed this study with intention to improve diagnostic accuracy of the follow-up protocol after conization keeping it safe and cost-effective and at the same time reducing the number of overtreated patients.

Materials and Methods

The study was conducted at the Department of Obstetrics and Gynecology, Clinical Hospital Center Zagreb from 1st January 2008 until 1st January 2014. One hundred and thirty five patients treated with cold-knife conization (CKC) or large loop excision of the transformation zone (LLETZ) for a preinvasive cervical disease, were enrolled in the study. The study inclusion criteria were: cervical intraepithelial neoplasia grade 3 (CIN3) and/or adenocarcinoma in situ (AIS) confirmed at conization; no previous history of cervical pathology; not pregnant; no concomitant cancer or immunosuppressive disease. All of the participants signed an informed consent before entering the study. Control visits were scheduled at 3–6 month, 9–12 month and 18–24 month intervals. If testing results were negative during the first 2 years, follow up visits were re-scheduled yearly. The follow-up consisted of cytology, colposcopy with biopsy if needed and HPV testing. Only patients with at least two cytology smears and one HPV test during the

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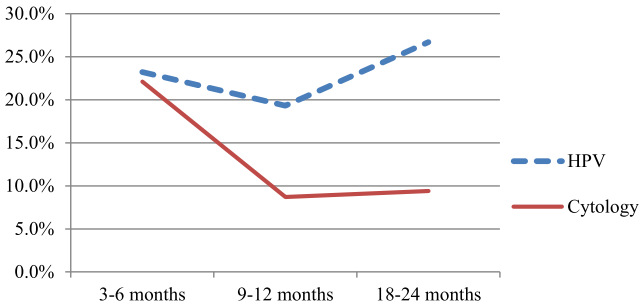

Fig. 1 The percentage of positive cytology smears and HPV tests during the follow-up

first two post-treatment years remained in the study. The end-point of the study was a secondary treatment (reconization or hysterectomy) or disease free period of at least 24 months. Indication for a second treatment was based on a positive cytology smear (HSIL/AGC/AIS) and unsatisfactory or positive colopscopy finding. The cytology smear was taken using liquid based cytology (LBC) ThinPrep© (Hologic, Inc Corp. Mariborough, MA) or the conventional Papanicolaou. Cervical smears were classified according to the Bethesda System 2006 - Zagreb modification and dichotomized into positive (≥H-SIL/AGC/AIS) or negative [7]. Since HPV testing was used for the evaluation of the current follow-up policy, test results were blinded until the analysis. The HPV test was performed by EIA PCR using a HPV-general-primer-mediated PCR. A β-globin PCR was performed to ascertain the quality of the target DNA. All 14 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) were tested for in one assay. Where possible, genotyping was performed before conization by Line immuno Probe Assay (Inno-LiPA) genotyping v2 system (Innogenetics, Gent, Belgium).

The statistic analysis was performed using the χ^2 test in analysis of frequency tables for independent categorical variables. Additionally, McNemar's test for trends was used to assess follow-up differences. Kappa coefficients (k=coefficient of agreement) were calculated to analyze consistencies and agreement between test results during the follow-up period. Performance indicators and diagnostic accuracy were calculated using the contingency tables for sensitivity, specificity, positive (PPV) and negative predictive value (NPV), with corresponding 95 % confidence intervals (CI). In all tests P values below 0.05 were regarded as statistically significant. Calculations were performed using the StatsDirect Statistical Software version 2.7.8 (www.statsdirect.com).

Results

Data was analyzed for 114 patients, since 21 were lost to follow-up. The median follow-up time was 41 months (5–72 months). Cone histology showed CIN3 in 101 (88.6 %) and AIS in 13 (11.45 %) patients. In six patients with AIS there were also CIN3 changes (46.2 %). Most of the patients were younger than 40 (64.9 %; median 35.0, interquartile range 30.0–43.3; range 18–66 years) and 31 (27.2 %) patients were nulliparous prior to conization. Histology diagnosis (CIN3/AIS) didn't differ significantly between age groups (P=0.402) however, the number of women younger than 30 was more frequent in the AIS group (38.5 % vs 21.8 %). All of the women that had HPV testing before conization (N=39) were HPV positive. Thirty-one (79.5 %) patients had a monoinfection, but a multiple infection was more common in patients younger than 40 (P=0.045). The most common

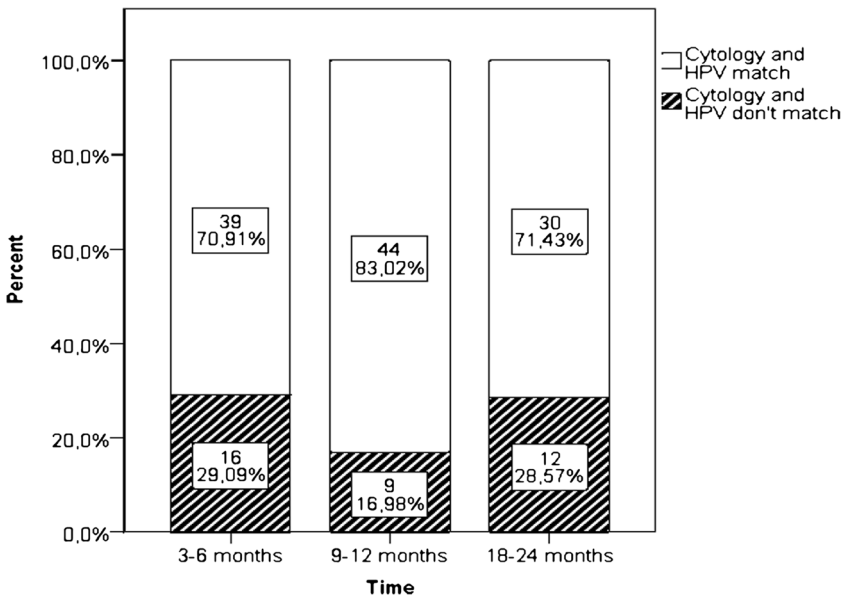

Fig. 2 Distribution of concordant and non-concordant test results during the follow-up

genotype isolated was HPV16 in both types of infection, being present in 25 (64.2 %) patients. Genotype distribution was related to histology since HPV18 had only been isolated in AIS specimens (P=0.036).

The primary treatment was by cold-knife conization in 98 (86 %) and LLETZ in 16 (14 %) patients. Positive margins were found in 20 (17.5 %) cone specimens and were more common in the LLETZ group (P=0.024). During the follow-up 11 (9.6 %) patients had a second surgery due to a suspicion of residual/recurrent disease (Table 1). In four patients reoperation was done (36.4 %) during the first 6 months after conization and in seven (63.6 %) during the following 18 months. Four (36.4 %) patients had hysterectomy and seven (63.6 %) had reconization done. Histology revealed no evidence of preinvasive or invasive diseases in five (45.5 %) of these patients. Four patients were confirmed with CIN 2,3 and two had an invasive carcinoma.

Margin involvement was not related to a decision to perform a second treatment (P=0.084). Still, in patients with positive cytology those with involved margins were more likely to have a residual/recurrent disease (P=0.022). There was no correlation between the type of HPV infection before conisation and the residual/recurrent disease (P=0.581).

Figure 1 shows distribution of positive cytology and HPV during the follow-up. The highest frequency of positive cytology smears was in the 3–6 month interval (P<0.001). There was no difference in HPV test results distribution between follow-up intervals. In all three intervals HPV test was more frequently positive than cytology smears and the biggest difference was in the 18–24 month interval (P=0.010). There was a high concordance of cytology and HPV test results during the whole follow-up period (Fig. 2). Cytology smears were most consistent between 3–6 month and 9–12 month intervals (k=0.458, P<0.001). HPV test results between follow-up intervals were inconsistent and had a low coefficient of agreement. Best agreement was found between HPV at 3–6 month interval and cytology at 9–12 month interval follow-up (k=0.559, P<0.001).

Table 2 Performance of cytology and HPV-HR DNA test in detection of residual/recurrent disease

	Cytology % (CI)	HPV % (CI)
Sensitivity	100 (54.1–100)	100 (54.1–100)
Specificity	88.9 (81–94.1)	79.6 (70.8–86.8)
PPV	33.3 (13.3–59)	21.4 (8.3–41)
NPV	100 (96.2–100)	100 (95.8–100)

Both cytology (P<0.001) and HPV test (P=0.015) results correlated with a decision to perform a second treatment. Cytology had a PPV of 61.1 % (CI 35.8–82,7) and a NPV of 100 % (CI 96.3–100), whereas HPV test had a PPV of 21.4 % (CI 8.3–41) and a NPV of 94.2 % (CI 87–98.1). The performance of cytology and HPV test in detecting a residual/recurrent disease is shown in Table 2. There was no residual/recurrent disease when posttreatment cytology or HPV test was negative. In the group of patients with positive cytology 33.3 % had a residual/recurrent disease, whereas among those HPV positive 21.4 % had a residual/recurrent disease. All of the patients who had a residual/recurrent disease were HPV positive, and 75 % of disease-free patients were HPV negative.

Discussion and Conclusion

Women treated for a preinvasive cervical disease are mostly of reproductive age and still desire pregnancy, as were the majority of women in our study, therefore the approach in treatment and follow-up has to be safe and sparing. A negative follow-up test result, whether it'd be only cytology, only HPV or a combined test, give a reassuring low risk

Table 1 Distribution of reoperated patients during the follow-up

Case no.	Age at conization	PHD 1	Cytology prior to 2nd treatment	HPV infection prior to 2nd treatment	Interval between treatments (months)	PHD 2
1	38	CIN 3	HSIL	Positive	11	Positive
2	31	CIN3	HSIL	Positive	10	Positive
3	44	CIN3	HSIL	Negative	11	Negative
4	43	CIN3	HSIL	Positive	7	Positive
5	46	CIN3	HSIL	Negative	5	Negative
6	54	CIN3	HSIL	Positive	24	Positive
7	37	AIS	AGC	Negative	5	Negative
8	31	AIS	HSIL, AGC	Positive	19	Positive
9	43	AIS	HSIL, AIS	Negative	5	Negative
10	31	AIS	AGC	Negative	5	Negative
11	38	CIN3	HSIL	Positive	8	Positive

of 5-year recurrence of CIN2+ lesion [8]. In our study both cytology and HPV showed a good performance in detecting a residual/recurrent disease (Table 2) with sensitivity of 100 % and NPV of 100 % for both tests. Women not reoperated remained disease free up to 72 months (4–7 years) after conization. However, in the reoperated group 45.5 % had no residual/recurrent disease in the histology report. We can argue that these patients were unnecessarily exposed to peri- and postoperative morbidity. Some other studies also found a relatively high number of reoperated patients who had no residual/recurrent disease confirmed [4, 9]. The decision for a second treatment in our study relied upon cytology and colposcopy protocol, mainly (81.2 %) during the first year of follow-up. Histology reports were mostly negative in those reoperated 6 months after conization, which confirms findings of some other authors that this protocol is more often false-positive in the early follow-up period [10].

During the whole follow-up period the concordance of cytology and HPV test results was high (Fig. 2) and almost 70 % of patients had both tests negative, which corresponds to results of other studies (14). Given the high sensitivity and concordance in our results we conclude that in our setting, with poorer resources and high quality cytology, it is not cost-effective to perform both tests to all women after conization. Gok et al. suggested that patients at the highest risk for residual/recurrent disease are those with HPV16 infection prior to conization and that they should have a combined test done 6 months after conization [11]. Considering that HPV infection is alluded in biopsy proven CIN/AIS lesions and the fact that most women in our study tested before conization had HPV16 and/or 18 positive (74.5 %), we don't think genotyping improves the quality of follow-up but only raises the cost. Given that HPV test results between follow-up intervals in our study had a low coefficient of agreement and that natural history of viral infection depends on individual's characteristics [12] it is questionable if we can rely on one-fold testing without the reassurance of cytology. The highest incidence of a positive HPV test was during the second year of follow-up (P=0.010), which can be explained by individual's sexual behavior. Protocols including the HPV test have shown that the optimal HPV clearance after conization is 6 months post-treatment [13].

Many studies have shown that combined testing has the highest sensitivity in detecting a residual/recurrent disease [14]. According to our results both tests show a very good performance in detecting a residual/recurrent disease so depending on the socio-economic aspect both can be used as a primary follow-up tool after conization. In our study specificity of cytology (88.9 %) was higher than of HPV (79.6 %) in detecting a residual/recurrent disease thus the choice of cytology as the first follow-up test after conization in our setting is obvious, whereas others can choose the HPV test instead. In each case if the chosen test is positive the other one has to be performed as well before deciding on a further procedure. In our study HPV test would have improved the specificity of cytology/colposcopy-based protocol in the group of reoperated patients since all of the patients with proven residual/recurrent disease were HPV positive whilst those with negative histology were HPV negative. Time interval from conization didn't influence test performance in this group (P<0.001). In all follow-up intervals in case of positive cytology and/or inconclusive colposcopy a negative HPV test can give reassurance and reduce anxiety without hurrying with a second treatment. Cytology can then be repeated in another 3–4 months, considering there is no suspicion of invasive disease. In these cases LBC has advantage compared to conventional PAP smear since there is no need to invite the patients for another visit.

The follow-up after conization must ensure safety and reassurance of both patients and gynecologists, but at the same time it must be cost-effective. Mentioned downfalls of surveillance tools can lead to overtreatment therefore, if cytology or HPV is positive we strongly recommend that the other test be performed as well before any other procedure. Results of our study suggest that in our setting the best approach is to have a first control cytology smear 6 months after conization. HPV test should be done in patients with a positive smear any time during follow-up as the point of decision for a second treatment. With this approach we could considerably decrease the number of reoperated patients and co-morbidities without decreasing the quality of follow-up.

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