EJGO European Journal of Gynaecological Oncology

Impact of the human papillomavirus vaccination on patients who underwent conization for high-grade cervical intraepithelial neoplasia

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Summary

Objectives: To test whether the human papillomavirus (HPV) vaccination in patients undergoing loop diathermy conization (LEEP) for high-grade intraepithelial neoplasia (CIN 2-3) is effective in preventing recurrence of CIN 2-3 in our area. *Materials and Methods:* A retrospective review was conducted on 242 patients undergoing LEEP for CIN 2-3 and 42.6% received the HPV vaccine (bivalent or tetravalent) immediately before or after conization. Follow up was conducted at 3, 6, 12, 18, and 24 months to detect CIN 2-3 recurrence. *Results:* Regardless of the HPV type, 27 (11.1%) patients developed CIN 2-3 recurrence during post-LEEP follow up. Of the 70 vaccinated with bivalent vaccine, two (2.8%) showed recurrence, of the 33 vaccinated with tetravalent vaccine, three (9%), and of the 139 unvaccinated 61 (43.9%) developed recurrence. Of the patients infected with HPV genotypes 16/18, in the non-vaccinated group, 15 (21.7%) patients had recurrence, whereas in the vaccinated group, three (5.9%) were diagnosed with recurrence ($p < 0/05$). The multivariate logistic regression analysis showed that the vaccination acted as a protective factor for CIN 2-3 recurrence (OR: 0.360 (95% CI: 0.125-1.000; $p < 0.05$). However, neither the type of vaccine nor the time of vaccination showed a significant association with the onset of recurrence. *Conclusions:* The HPV vaccine appears to be a recommendable preventative strategy in reducing the risk of recurrent disease for patients treated for CIN 2-3.

Key words: Human papillomavirus; Vaccine; Cervical intraepithelial neoplasia; Recurrent disease; Conization; LEEP.

Introduction

Cervical cancer is the third leading cause of cancer in women globally [1] and it is caused by a persistent human papillomavirus (HPV) infection, which is linked to the carcinogenesis of the cervical epithelial cells [2]. HPV genotypes 16 and 18 cause approximately 70% of cervical cancers and 50% of precancerous cervical lesions [3]. The estimated annual incidence of cervical intraepithelial neoplasia (CIN) among women undergoing screening for cervical cancer is 0.4% for CIN 1 and 0.5% for CIN 2-3 [4]. To prevent the progression of these lesions to invasive cancer, women with CIN 2-3 are generally treated with loop diathermy conization (loop electrosurgical excision procedure: LEEP) [5]. However, the risk of invasive cancer up to 10-20 years later is four to five times more likely among these women that have been treated than in those in the general population [6], and the level of CIN recurrence is 5-17% for any of the ablation or excision treatments [7]. Therefore, these patients continue to be part of an at-risk group that requires close follow up. To date, there are two widely studied, prophylactic vaccines, based on non-infectious, recombinant virus-like particles (VLP), made with viral capsid L1 protein of the viral genotypes 16 and 18 (bivalent vaccine) [8, 9] and 6, 11, 16, and 18 (tetravalent vaccine) [10, 11], which are designed to prevent disease associated with HPV. Through a great number of trials, both vaccines have demonstrated to be highly effective in the prevention of CIN 2-3, adenocarcinoma in situ, and vaginal and vulvar intraepithelial neoplasia (VAIN and VIN 1-3) [12], especially in 16-26-year-old women uninfected by vaccine types. Furthermore the tetravalent vaccine has shown effectiveness in reducing the incidence of genital

Revised manuscript accepted for publication February 8, 2018

warts. Recently, in 2015, a vaccine with a broader antigenic spectrum has been placed on the market. This nine-valent vaccine [13] aims to protect against five more higher risk HPV genotypes, not included in the first generation of vaccines (31, 33, 45, 52, and 58) and of similar effectiveness to the tetravalent. However, this is currently not yet available for special population trials.

The HPV vaccine appears to be safe and effective in preventing infection in women older than 25 years of age, however in general, the benefits are less than in younger women [14]. A meta-analysis on women who had received the tetravalent vaccine, who had also been surgically treated for HPV-related disease, had a reduction in the occurrence of recurrent disease [15]. A study where the tetravalent vaccine was administered to patients after CIN 2-3 conization, demonstrated the effectiveness of the HPV vaccine in preventing recurrent CIN 2-3 disease [16].

The present study aims to assess if the HPV vaccination, with either of the two vaccines (bivalent or tetravalent), immediately before or after conization with LEEP in CIN 2-3 can be effective in reducing the risk of recurrence of high-grade post-LEEP cervical lesions.

Materials and Methods

This is a retrospective review of the digitized medical records of 264 patients, who were between 18 and 65 years of age, diagnosed with CIN 2-3 and who had undergone loop diathermy conization (LEEP) in the Obstetrics and Gynecology Department at the Santa Lucia of Cartagena University Hospital (HUSL) between January 2011 and May 2015.

The 242 patients who were included in the study fit the following criteria: 1) CIN 2-3 histologically confirmed after conization (LEEP), 2) patients diagnosed with HPV pre-conization, 3) patients who had not received the bivalent or tetravalent vaccine before developing CIN, and 4) patients who underwent follow-up for a minimum of two years. Twelve patients were excluded for hysterectomies post conization and ten for residual CIN 2-3 disease.

Conization was performed with loop diathermy and local anesthetic, obtaining a piece that was referenced at 12 hours accompanied by endocervical curettage. Both samples were fixed in 4% formaldehyde solution for pathological study. All of the patients who were given conization and not previously vaccinated were randomly assigned to one of the two commercially available HPV vaccines. Patients were classified in two groups: unvaccinated and vaccinated, the latter were subdivided into two subgroups based on the type of vaccine received, bivalent or tetravalent. The first dose of anyone of both vaccines had been administered 0-1 month before or 0-1 month after conization. Patients underwent post-operative examination at 3, 6, 12, 18, and 24 months during the first two years and then annually at the Cervical Pathology Unit at HUSL to detect any recurrence of disease caused by HPV. During each visit, a liquid cytology, a HPV test, and a colposcopy with 5% acetic acid were performed in all patients. In the case of CIN 2-3 being diagnosed on the edges of the conization tissue or the curettage endocervical sample, an endocervical biopsy was taken at three and six months. At any time if the colposcopy was not adequate or the HPV test came back positive during the medical revision, an endocervical biopsy was carried out. If the result of the liquid cytology produced atypical cells of uncertain significance (ASCUS) or higher grade, a colposcopy-guided biopsy was taken. The criteria to define residual disease or recurrent disease was determined by the histological CIN 2-3 diagnosis in the colposcopy-guided biopsy or endo- cervical biopsy at three months of follow-up (residual disease) or at six months or more (recurrence). For the statistical analysis, the results of the cervical biopsy during follow up were grouped in negatives (normal, cervicitis or CIN I) or positives (CIN 2-3 or greater degree of lesion). The positive results in the cervical biopsy were considered as recurring disease.

An initial PCR technique was used for HPV analysis using an extraction package. The sample was obtained with an endocervical brush which was subsequently submerged and washed in the vial for PCR cell collection media. After the DNA extraction process, a PCR was performed in real time in a thermal cycler, employing fluorescent probes: HPV kit. This kit differentiated between the 16/18 HPV, non-16/18 HR-HPV (without specifying genotype) and negative genotypes. Low risk genotypes were not detected. The extracted DNAs were placed on another plate to perform a second technique for the determination of HPV genotypes by high resolution melting curve analysis using the HPV 28 detection kit, in a real-time thermocycler. With this method, 19 high-risk genotypes (16, 18, 26, 31, 33, 35, 39, 45, 52, 53, 56, 58, 66, 68, 69, 73, 82) and nine of low oncogenic risk (6, 11, 40, 42, 43, 44, 54, 61, 70) could be detected.

The primary event was the appearance of CIN 2-3 recurrence after conization. The normality of continuous variables was tested by Kolgomorov-Smirnov of Shapiro-Wilk tests, as appropriate continuous variables are presented as the median (interquartile range [IQR]) for non-normally distributed data or mean (standard deviation [SD]) for normally distributed data. Comparisons of group differences for continuous variables were made by the Mann-Whitney *U*-test or the Student's *t*-test, as appropriate. Categorical variables are presented as a number and percentage in each category. The significance of differences in percentages was tested by the Chi-squared test. Univariate and multivariate binary logistic analyses were performed with the previously defined variables for the prediction of recurrence disease and the odds ratios (OR) were displayed. The statistical analysis were performed using SPSS v. 20.0. All p-values ≤ 0.05 were considered statistically significant.

Results

A total of 242 patients met the requirements of the study and had follow up care of at least two years. The median age of all 242 patients was 36 years. Of these, 28 (11.6%) patients were between 18-25-years-old, 92 (38%) patients between 26-35-years-old, 62 (25.6%) patients between 35-45-years-old and 60 patients were older than 45 years; 88.8% of the patients were of Spanish nationality. The high grade cervical neoplasia (CIN 2-3) was histologically confirmed, 106 (43.8%) had a CIN 2 diagnosis, and 136 (56.2%) CIN 3 diagnosis. Of the 242 patients, 27 (11.1%) had recurrence. Of the patients with recurrence, the histology showed eight CIN 2 cases and 19 CIN 3 cases. The average time between recurrence and conization was 14.2 (6-24) months. Of the 242 patients included in the analysis, 103 (42.6%) patients had been vaccinated, of them 70 (68%) patients received the bivalent vaccine and 33 (32%)

Table 1. — *Description of the patient characteristics in the study by their vaccination status (unvaccinated, general vaccination, and vaccinated with bivalent or tetravalent vaccine).*

Characteristics n=242	Unvaccinated n=139	Vaccinated n=103	*p*	Bivalent n=70	Tetravalent n= 33	*p*
Age (years)						
Median (MD)	39	33	<0.001	33	33	0.932
Interquartile intervals (IQR)	(31-50)	(28-38)		(28-38.3)	(28.5-37.5)	
Geographic region of origin						
Spain	119 (85.6%)	96 (93.2%)	0.215	65 (92.9%)	31 (93.9%)	0.729
Latin America	10 (7.2%)	5 (4.9%)		4 (5.7%)	1 (3.0%)	
Northern Africa	2 (1.4%)	0		0	0	
Eastern Europe	8 (5.8%)	2(1.9%)		1 (1.4%)	1 (3.0%)	
Time of vaccine						
(0-1 month) before				17 (24.3%)	29 (87.9%)	< 0.001
(0-1 month) after				53 (75.7%)	4 (12.1%)	
Reason for conization						
CIN 2	55 (39.6%)	51 (49.5%)	0.12	32 (45.7%)	19 (57.6%)	0.261
CIN 3	84 (60.4%)	52 (50.5%)		38 (54.3%)	14 (42.4%)	
Cone margin						
Negative	103 (74.1%)	77 (74.8%)	0.908	54 (77.1%)	23 (69.7%)	0.659
Positive	36 (25.9%)	26 (25.2%)		16 (22.9%)	10 (30.3%)	
HPV baseline						
Negative	5 (3.6%)	3 (2.9%)	0.955	3 (4.3%)	0 (0.0%)	0.355
Vaccine (16/18)	69 (49.6%)	51 (49.5%)		36 (51.4%)	15 (45.5%)	
No vaccine	65 (46.8%)	49 (47.6%)		31 (44.3%)	18 (54.5%)	

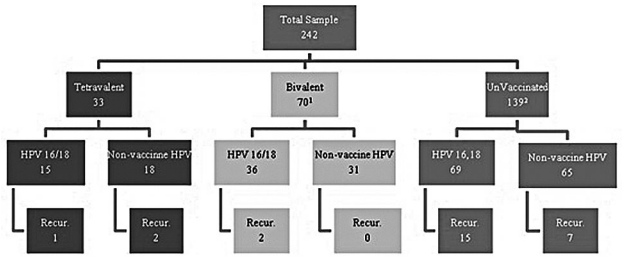

Figure 1. — Results from the population of the study. 1 includes three patients with negative HPV. 2 includes five patients with negative HPV.

patients the tetravalent vaccine. The moment of vaccination was 0-1 month before conization in 46 (44.6%) patients and 0-1 month after conization in 57 (55.4%) patients.

The baseline characteristics of the patients included in the different study groups were fairly homogeneous in terms of country of origin, reasons for conization, state of cone margins, and positivity for 16/18 genotypes HPV testing; 96.7% (234/242) of the total of patients were positive for high risk-HPV (HR-HPV), 51.4% (120/234) were positive for HPV 16/18, and 48.7% (114/234) were positive for other HR-HPV types. The median age was six years higher in the unvaccinated group, being identical in the two vaccinated groups (Table 1).

The design of the study to determine the effect of either of the two vaccines on CIN 2-3 recurrence after conization is shown in Figure 1. Recurrence was detected during follow-up in five (4.8%) of the vaccinated patients, versus 22 (15.8%) of the 139 unvaccinated patients. Among the vaccinated patients that showed recurrence, three (60%) had received the tetravalent vaccine and two (40%) the bivalent vaccine. Considering the type of HPV related to the primitive lesion, in the group of vaccinated patients with 16/18 genotypes HPV-related CIN, recurrence was detected in 6.7% (1/15) of the patients vaccinated with tetravalent vaccine and 5.5 % (2/36) of the bivalent-vaccinated patients. With regards to the vaccinated patients with CIN unrelated to vaccine genotypes (HPV different to 16/18), 11.1% (2/18) of those vaccinated with tetravalent vaccine and 0/31 of those vaccinated with the bivalent vaccine showed recurrence. In the unvaccinated population, recurrence was detected in 21.7% (15/69) of the positive to HPV 16/18 group and 10.8% (7/65) of those positive for non-vaccine HPV. For the patients positive for genotypes included in either vaccine (HPV 16/18), the group of non-vaccinated patients had a significantly higher level of recurrence than the vaccinated group, with either of the vaccines ($p < 0.05$). However, with respect to patients positive to other genotypes of HR-HPV, different to those included in the vaccines, there were no significant differences observed between the vaccinated and unvaccinated groups. All of the 27 patients that developed recurrence showed the same HPV genotype as before LEEP.

The patients that showed recurrence were similar in terms of the reason for conization, cone margin status, pre-LEEP HPV genotype, and type of vaccine received or moment of vaccination. The older patients at the time of conization and

Table 2. — *Description of the patient characteristics in the study in relation to recurrence/ non-recurrence post-conization*

Characteristics	No recurrence	Recurrence	*p*
Age (years)			
Median (MD)	34	42	0.005*
Interquartile range (IQR)	(29-44)	(32-51)	
CIN at LEEP			
CIN 2	98 (45.6%)	8 (29.6%)	0.115
CIN 3	117 (54.4%)	19 (70.4%)	
Cone margin			
Negative	163 (75.8%)	17 (63%)	0.149
Positive	52 (24.2%)	10 (37%)	
Vaccination status			
Unvaccinated	117 (54.4 %)	22 (81.5%)	0.007*
Vaccinated	98 (45.6 %)	5 (18.5%)	
Type of vaccination			
Bivalent	68 (69.4%)	2 (40%)	0.170
Tetravalent	30(30.6%)	3 (60%)	
Time of vaccination			
Before conization	43 (43.9 %)	3 (60 %)	0.479
After conization	55 (56.1%)	2 (40 %)	
HPV baseline			
Negative	8 (3.7%)	0 (3.7%)	0.134
Vaccine (16,18)	102 (47.4%)	18 (66.7%)	
Non vaccine	105 (48.8%)	9 (33.3%)	

those who had not been vaccinated had a significantly greater risk of recurrence ($p < 0.05$, Table 2).

The univariate analysis demonstrated a significant differences between the age variation at the time of conization and the vaccination variable ($p < 0.05$, Table 3). The multivariate lineal regression analysis showed that the only two variables that act as independent indicators of CIN 2-3 recurrence are age at the time of conization ($p < 0.05$, Table 3) and not being vaccinated for HPV ($p < 0.05$, Table 3).

Discussion

Despite timely treatment of CIN cases, cases of recurrent disease occur in 1.9-66.8% of patients, with an average rate of 15% [17-22]. The average time of recurrence is approximately 9-10 months, with a range of 3-23 months [23, 24]. The prevention of recurrence has an impact on the outcome of a future pregnancy, as most are young women and a second conization is associated with a two-fold higher likelihood of premature birth [25].

There are several factors that have been associated with an increased rate of recurrence, such as effects of cone margins [26], immunosuppression [27], and advanced age of the patient [28]. In the present sample, the average age of the unvaccinated group was six years older than the vaccinated group, due to the evident recommendation of the vaccine for women under 45 [14]. The present authors found that age has a significant influence on recurrence (1.037 per additional year of the patient at the time of conization). However, the effect of cone margins was not a predictor of risk for recurrence in the current study and could be explained by the LEEP procedure used, that inevitably leads to clotting on the ecto- and endocervix area in contact with cone margins. However, it was observed that when margins were negative, the vaccine clearly increased its efficacy in reducing the risk of recurrence ($p < 0.05$) compared to unvaccinated patients. Nevertheless, there were no significant differences in recurrence when margins were affected and the vaccine was administered.

After conization in most patients, HPV clearance occurs [29]. In recent publications, there is strong evidence that the presence of high risk-HPV post-treatment with LEEP can predict the likelihood of persistent or recurrent disease [30, 31]. The HPV test at six months of conization may be a marker of recurrent disease with greater sensitivity and specificity than cytology [32].

In this study, five of the 103 vaccinated patients (4.8%) developed recurrent disease and 22 of the 130 unvaccinated patients (15.8%) developed recurrence. The 27 patients that had recurrence were positive for the same HPV genotype in the PCR test as before conization, as was the same in other studies [16, 33]. The CIN 2-3 recurrence in patients with a vaccine-induced HPV lesion [16/18] was lower in the vaccinated group than in the unvaccinated group (4.8% and 21.7% respectively, $p < 0.05$), coinciding with the results of another study [16].

The literature demonstrates that HPV vaccines marketed to date do not appear to have a therapeutic effect by not influencing the course of cervical neoplastic disease in HPV-infected women at the time of vaccination [34]. There are some studies that have demonstrated the effectiveness of the vaccine in reducing CIN 2-3 recurrence after treatment [16]. The current study additionally analyzed the impact of the two vaccines (bivalent and tetravalent), separately, in women after conization, considering other variables such as the time of vaccination. Administration of either vaccine (bivalent or tetravalent) at the time of conization provided a significant reduction ($p < 0.05$) for CIN 2-3 recurrence. Vaccination also provided protection for recurrent disease related to vaccine genotypes (HPV 16/18) ($p < 0.05$). Neither the type of vaccine nor the time of vaccination showed a significant association with the onset of recurrence.

The present authors believe that the reduction of recurrent disease would be due to the prevention of HPV reinfection by the same HPV genotype as before the LEEP or by other HPV type. However, this should be analyzed in another study with long-term follow-up.

Although we do not yet have consensus guidelines, there are vaccination programs for women after CIN 2-3 conization. In the present area of health, the authors have been leaders in conducting this type of program in Spain. The data from the first two years of follow-up (April 2014 to April 2016) of the financed bivalent vaccine administration in the first post-LEEP month have already been evaluated

Table 3. — *Uni- and multivariate analyses.*

	Univariate analysis		Multivariate analysis	
Variable	OR (IC 95%)	*p*	OR (IC 95%)	*p*
Age (years)	1.050 (1.014-1.088)	0.006*	1.037 (1.0-1.076)	0.04*
Positive margins	1.844 (0.795-4.276)	0.154		
Vaccination	0.271 (0.099-0.743)	0.011*	0.360 (0.125-1.032)	0.03*
Type of vaccine (tetravalent *vs.* bivalent)	3.4 (0.54-21.41)	0.192		
Time of vaccination (after *vs.* before)	0.521 (0.083-3.529)	0.486		
Type of HPV baseline (16, 18 *vs.* other RA)	2.059 (0.884-4.794)	0.094		

and have achieved excellent results regarding the coverage and safety of this vaccine [35]. This could be the determining factor for the present program to have a greater proportion of bivalent vaccinated patients.

According to the results obtained, the HPV vaccination can be considered as a preventative strategy for patients being treated for CIN 2-3 in reducing the cases of recurrence and the overall risk of any related HPV disease that may appear long term in this group of patients.

References

[1] Ferlay J., Shin H.R., Bray F., Forman D., Mathers C., Parkin D.M.: "Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008". *Int. J. Cancer*, 2010, *127*, 2893.

[2] Cox J.T.: "Epidemiology of cervical intraepithelial neoplasia: the role of human papillomavirus". *Baillieres Clin. Obstet. Gynecol.*, 1995, *9*, 1.

[3] Konno R., Shin H.R., Kim Y.T., Song Y.S., Sasagawa T., Inoue M., *et al.*: "Human papillomavirus infection and cervical cancer prevention in Japan and Korea". *Vaccine*, 2008, *26*, 30.

[4] Insinga R.P., Glass A.G., Rush B.B.: "Diagnoses and outcomes in cervical cancer screening: a population based study". *Am. J. Obstet. Gynecol.*, 2004, *191*, 105.

[5] Wright T.C. Jr., Massad L.S., Dunton C.J., Spitzer M., Wilkinson E.J., Solomon D., *et al.*: "2006 consensus guidelines for the management of women with cervical intraepithelial neoplasia or adenocarcinoma in situ". *Am. J. Obstet. Gynecol.*, 2007, *197*, 340.

[6] Soutter W.P., Sasieni P., Panoskaltsis T.: "Long-term risk of invasive cervical cancer after treatment of squamous cervical intraepithelial neoplasia". *Int. J. Cancer*, 2006, *118*, 2048.

[7] Martin-Hirsch P.P., Paraskevaidis E., Bryant A., Dickinson H.O.: "Surgery for cervical intraepithelial neoplasia". *Cochrane Database Syst. Rev.*, 2013, *12*, CD001318.

[8] Paavonen J., Jenkins D., Bosch F.X., Naud P., Salmeron J., Wheeler C.M., *et al.*: "Efficacy of a prophylactic adjuvanted bivalent L1 virus-like-particle vaccine against infection with Human papillomavirus types 16 and 18 in young women: an interim analysis of a phase III double-blind, randomised controlled trial". *Lancet*, 2007, *369*, 2161.

[9] Paavonen J., Naud P., Salmeron J., Wheeler C.M., Chow S.N., Apter D., *et al.*: "Efficacy of Human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final analysis of a double-blind, randomised study in young women". *Lancet*, 2009, *374*, 301.

[10] FUTURE II Study Group: "Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions". *N. Engl. J. Med.*, 2007, *356*, 1915.

[11] Ault K.: "Effect of prophylactic human papillomavirus L1 virus-like-particle vaccine on risk of cervical intraepithelial neoplasia grade 2, grade 3 and adenocarcinoma in situ: a combined analysis of four randomised clinical trials". *Lancet*, 2007, *369*, 1861.

[12] Garland S., on behalf of the HPV PATRICIA Study Group: "Efficacy of the HPV-16/18 AS04-adjuvanted vaccine against vulvar/vaginal intraepithelial neoplasia". In: 27th International Papilloma Conference and Workshop. Berlin: 2011. [Cited 12 November 2017]. Available at: http://ipvsoc.org/event/27th-annual-international-papillomavirus-conference-clinical-workshops/

[13] Joura E.A., Giuliano A.R., Iversen O.E., Bouchard C., Mao C., Mehlsen J., *et al.*: "A 9-valent HPV vaccine against infection and intraepithelial neoplasia in women". *N. Engl. J. Med.*, 2015, *372*, 711.

[14] Skinner S.R., Szarewski A., Romanowski B., Garland S.M., Lazcano-Ponce E.5., Salmerón J., *et al.*: "Efficacy, safety, and immunogenicity of the human papillomavirus 16/18 AS04-adjuvanted vaccine in women older than 25 years: 4-year interim follow-up of the phase 3, double-blind, randomised controlled VIVIANE study". *Lancet*, 2014, *384*, 2213.

[15] Joura E.A., Garland S.M., Paavonen J., Ferris D.G., Perez G., Ault K.A.: "Effect of the human papillomavirus (HPV) quadrivalent vaccine in a subgroup of women with cervical and vulvar disease: retrospective pooled analysis of trial data". *BMJ*, 2012, *344*, 1401.

[16] Kang W.D., Choi H.S., Kim S.M.: "Is vaccination with quadrivalent HPV vaccine after loop electrosurgical excision procedure effective in preventing recurrence in patients with high-grade cervical intraepithelial neoplasia (CIN2–3)?" *Obstet. Gynecol.*, 2013, *130*, 264.

[17] Prendiville W., Cullimore J., Norman S.: "Large loop excision of the transforming zone (LLETZ): a new method of management for women with cervical intraepithelial neoplasia". *Br. J. Obstet. Gynecol.*, 1989, *96*, 1054.

[18] Lindeque B.G.: "Management of cervical premalignant lesions". *Best Pract. Res. Clin. Obstet. Gynecol.*, 2005, *19*, 545.

[19] Fogle R.H., Spann C.O., Easley K.A., Basil J.B.: "Predictors of cervical dysplasia after the loop electrosurgical excision procedure in an inner-city population". *J. Reprod. Med.*, 2004, *49*, 481.

[20] Gardeil F., Barry-Walsh C., Prendiville W., Clinch J., Turner M.J.: "Persistent intraepithelial neoplasia after excision for cervical intraepithelial neoplasia grade III". *Obstet. Gynecol.*, 1997, *89*, 419.

[21] Dietrich C.S., Yancey M., Miyazawa K., Williams D., Farley J.: "Risk factors for early cytological abnormalities after loop electrosurgical excision procedure". *Obstet. Gynecol.*, 2002, *99*, 188.

[22] Livasy C.A., Moore D.T., Van Le L.: "The clinical significance of a negative loop electrosurgical cone biopsy for high-grade dysplasia". *Obstet. Gynecol.*, 2004, *104*, 250.

[23] Carter J., Sim J., Land R., Dalrymple C., Abdel-Hadi M., Pather S.: "Recurrence after treatment for high-grade dysplasia: should we modify our post-treatment surveillance protocols?" *Aust. N. Z. J. Obstet. Gynecol.*, 2006, *46*, 360.

[24] Alonso I., Torne A., Puig-Tintore L.M., Esteve R., Quinto L., Campo E., *et al.*: "Pre-and post-conization high-risk HPV testing predicts residual/recurrent disease in patients treated for CIN 2-3". *Gynecol. Oncol.*, 2006, *103*, 631.

[25] Jakobsson M., Gissler M., Paavonen J., Tapper A.M.: "Loop electrosurgical excision procedure and the risk for preterm birth". *Obstet. Gynecol.*, 2009, *114*, 504.

[26] Robinson W.R., Lund E.D., Adams J.: "The predictive value of LEEP specimen margin status for residual/recurrent cervical intra- epithelial neoplasia". *Int. J. Gynecol. Cancer*, 1998, *8*, 109.

[27] Skinner E.N., Gehrig P.A., Van Le L.: "High-grade squamous intraepithelial lesions: abbreviating posttreatment surveillance". *Obstet.*

Gynecol., 2004, *103*, 488.
[28] Verguts J., Bronselaer B., Donders G., Arbyn M., Van Eldere J., Drijkoningen M., Poppe W.: "Prediction of recurrence after treatment for high-grade cervical intraepithelial neoplasia: the role of human papillomavirus testing and age at conization". *BJOG*, 2006, *113*, 1303.
[29] Elfgren K., Jacobs M., Walboomers J.M., Meijer C.J., Dillner J.: "Rate of human papillomavirus clearance after treatment of cervical intraepithelial neoplasia". *Obstet. Gynecol.*, 2002, *100*, 965.
[30] Paraskevaidis E., Koliopoulos G., Alamanos Y., Malamou-Mitsi V., Lolis E.D., Kitchener H.C.: "Human papillomavirus testing and the outcome of treatment for cervical intraepithelial neoplasia". *Obstet. Gynecol.*, 2001, *98*, 833.
[31] Chua K.L., Hjerpe A.: "Human papillomavirus analysis as a prognostic marker following conization of the cervix uteri". *Gynecol. Oncol.*, 1997, *66*, 101.
[32] Kocken M., Uijterwaal M.H., de Vries A.L., Berkhof J., Ket J.C., Helmerhorst T.J., *et al.*: "High- risk human papillomavirus testing versus cytology in predicting post-treatment disease in women treated for high-grade cervical disease: a systematic review and meta-analysis". *Gynecol. Oncol.*, 2012, *125*, 500.
[33] KangW.D., Oh M.J., Kim S.M., Nam J.H., Park C.S., Choi H.S.: "Significance of human papillo- mavirus genotyping with high-grade cervical intraepithelial neoplasia treated by a loop electrosurgical excision procedure". *Am. J. Obstet. Gynecol.*, 2010, *203*, 72.
[34] Haupt R.M., Wheeler C.M., Brown D.R., Garland S.M., Ferris D.G., Paavonen J.A., *et al.*: "Impact of an HPV6/11/16/18 L1 virus-like particle vaccine on progression to cervical intraepithelial neoplasia in seropositive women with HPV16/18 infection". *Int. J. Cancer*, 2011, *129*, 2632.
[35] Pérez- Martin J., Navarro-Alonso J.A., Cayuela-Fuentes J., Bernal-González P.: "Vaccination Program against HPV in Women with Excisional Treatment Due to Preneoplasic Cervical Lesions in the Region of Murcia: Results of the First Year". *Clin. Res. Infect. Dis.*, 2016, *3*, 1031.

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