EJGO European Journal of Gynaecological Oncology

Original Articles

Human papillomavirus combined with cytology and margin status identifies patients at risk for recurrence after conization for high-grade cervical intraepithelial neoplasia

Y. Ruano, M. Torrents, F.J. Ferrer

Department of Obstetrics and Gynecology, Asturias University Hospital (HUCA), Oviedo city, Asturias (Spain)

Summary

Objective: To compare the ability of cytology, human papillomavirus (HPV) testing and co-testing to identify recurrence of patients treated by loop electrosurgical excision procedure (LEEP) for cervical intraepithelial neoplasia (CIN) 2-3. *Materials and Methods:* Retrospective analysis (R.A.): the medical records of 372 women treated for CIN 2-3 were reviewed. Resection margin, HPV typing, Pap smears, and biopsies post-LEEP were collected. Prospective analysis (P.A.): 97 women were followed post-LEEP by cytology, HPV test and colposcopy every six months. *Results:* Positive margins were found to be an independent risk factor for recurrent disease (OR 0.192; 95% CI 0.074-0.497 in R.A. and OR 0.096; 95% CI 0.023-0.392 in P.A.). HPV testing showed less sensitivity than cytology (69% vs 84%, respectively in R.A. and 80% vs 100% in P.A.). Co-testing predicted recurrent disease at a sensitivity of 90.6% in R.A. and 100% in P.A. *Conclusion:* Co-testing is the best option in follow-up protocols after treatment for CIN 2-3. If margins are free and co-testing is negative at six and 12 months, 18 months visit could be avoided.

Key words: Human papillomavirus; Conization; Cervical intraepithelial neoplasia; Surveillance; Recurrence.

Introduction

The relationship between high risk human papillomavirus (HR-HPV) and high-grade cervical intraepithelial neoplasia (CIN 2-3) or invasive cervical cancer has been clearly demonstrated [1].

The loop electrosurgical excision procedure (LEEP) is the standard procedure for conservative treatment of CIN 2-3. However, residual or recurrent disease occurs in 1.5% to 48% of patients [2-18].

The most important factors reported as being associated with recurrent CIN after conization include cone margin status and persistent HR-HPV infection [5, 18]. Other factors such as smoking, immunosuppression or age have been related to a higher risk in some series [15, 19] but not in others [2,3].

Cervical cytology has a relatively low sensitivity in the follow-up period (20-100% in different studies) [12, 18, 20, 21]. Due to this limitation of the Pap test, there is a growing interest in HPV-testing as a surveillance tool that, alone or in conjunction with cytology, may increase sensitivity and negative predictive value (NPV) for identifying women at high risk of recurrence.

Studies which investigate the potential role of HPV testing during the post-treatment period are profoundly heterogeneous, making it difficult to draw clear conclusions. Because of this, there is no uniform follow-up protocol.

The aim of this study was to compare the ability of cytology, HPV testing and co-testing to identify recurrence of patients treated by LEEP for CIN 2-3. The authors also investigated whether any factors can predict, prior to follow-up, the eventual development of recurrent disease.

Materials and Methods

The study was divided into two analyses: retrospective and prospective analysis.

RETROSPECTIVE ANALYSIS

Study population, variables and follow-up

The medical records of 939 women were reviewed. These subjects underwent LEEP at the Department of Gynecology of the Asturias University Hospital (HUCA), Spain, between 1995 and 2009. Inclusion criteria were histologically confirmed CIN 2-3 in the conization specimen or in the initial colposcopy-directed biopsy. Women were excluded if (a) CIN 2-3 was not diagnosed on histology, (b) HPV test results pre or post-conization and many epidemiological patient characteristics were not available, (c) no follow-up visits were available, and d) hysterectomy post-LEEP was immediately performed because large/multifocal CIN 2-3 or invasion in the cone was described. The remaining 372 women comprised the analytic population (Figure 1).

Revised manuscript accepted for publication March 6, 2014

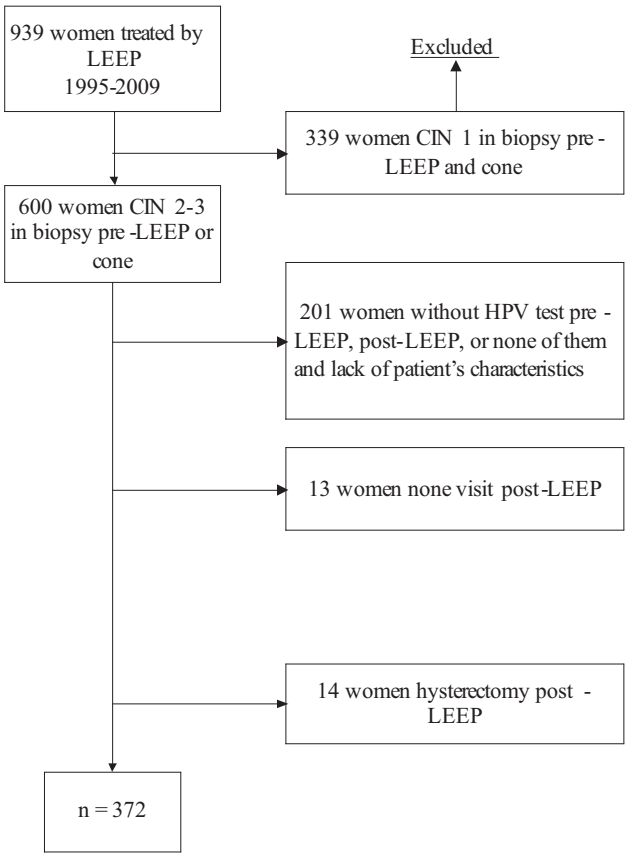

Figure 1. — Study population. Retrospective analysis.

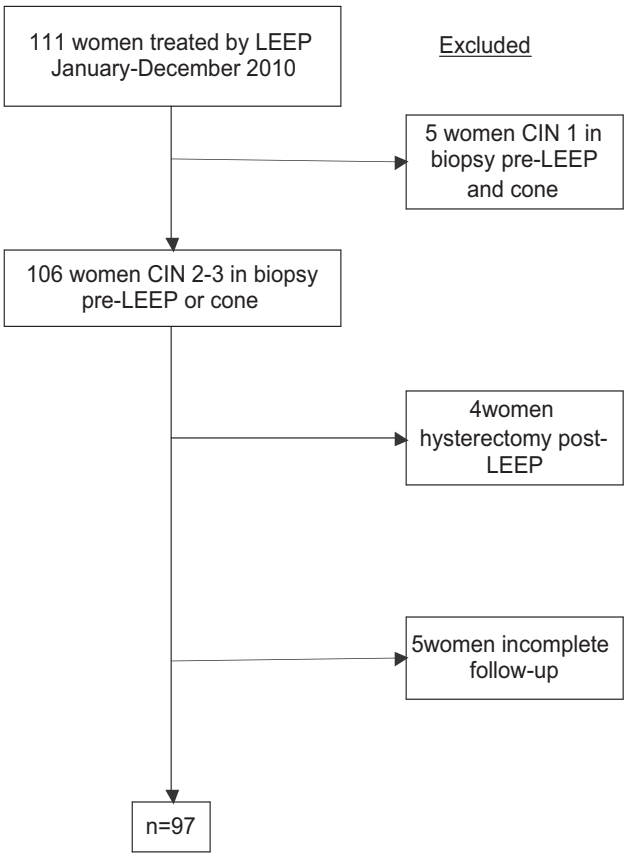

Figure 2. — Study population. Prospective analysis.

Patient characteristics were collected: age at conization, menopausal status, parity, smoking habits, immunosuppression or chronic disease, other sexually transmitted infections (STI), age at first intercourse, and mode of contraception.

Clinical data reviewed were: resection margin, HPV test results pre- and post-conization, HPV typing, follow-up Pap smears, and punch biopsies.

Mean follow-up time was 66 months (range 4-181, median 57). The average number of follow-up Pap smears per patient was 11.6 (range 1-22, median 5) but HPV testing was not so common in the first years of this study, hence the average number of follow-up HPV tests was 2.6 (range 0-19, median 2). Histologically confirmed presence of CIN 2+ was considered as recurrent disease.

Loop excision

All procedures were performed in an outpatient setting by three experienced surgeons. The electrosurgical procedure was performed with a LEEP system which was set to 50-52 W for cut and coagulation and 60-62 W for cauterization. Wire electrodes were used, cone biopsy excisor beginning at the 12 o'clock position or loop electrode for the "cowboy-hat" procedure: an ectocervical flat sweep followed by a deeper endocervical sweep using a smaller loop. The 12 o'clock position and margins were marked by ink. A ball electrode was used to cauterize the margins of the defect and achieve hemostasis after excision.

HPV testing

The real time polymerase chain reaction (PCR) method was used to detect viral DNA. In brief, after amplification, PCR products were analyzed by electrophoresis and hybridized with radiolabeled generic probes. HPV-DNA amplicons (L1 positive) were hybridized with type-specific oligonucleotide probes [6, 11, 16, 18, 31, 33, 45 and 58]. From 2002 HPV genotyping by direct DNA sequencing was used for those samples that could not be typed with hybridization.

PROSPECTIVE ANALYSIS

The authors collected 111 women with histologically confirmed CIN 2-3 who were treated by LEEP in the Department of Gynecology of the Asturias University Hospital between January and December 2010. Inclusion/exclusion criteria and variables were the same as in the retrospective analysis. The remaining 97 women comprised the analytic population (Figure 2).

All women were prospectively followed-up and scheduled for visits every six months after treatment, until July 2012. On each visit, a Pap smear, HPV test, and colposcopy were performed. Mean follow-up time was 24 months (range 19-30, median 24 months). The average number of follow-up pap smears and HPV tests per patient was 3.4 (range 1-5, median 3).

The study protocol was approved by the Regional Clinical Research Ethics Committee of Principado de Asturias and all patients signed the informed consent. LEEPs were performed by "cowboy-hat" procedure.

From May 2011, Cobas 4800 validated PCR was used for HPV testing. The Cobas 4800 system uses amplification of target DNA by PCR and subsequent nucleic acid hybridization for the detection of 14 HR-HPV types in a single analysis: 16, 18 or other HR-HPV [31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68].

Statistical analysis

Data were analyzed with the SPSS 17.0 software. The correlation between treatment failure with clinical factors (categorical variables) was determined by Fisher's exact test using two-by-two tables. Chi-square testing for non-categorical variables was used. A p value of < 0.05 was statistically significant. Odds ratio (OR) and 95% confidence intervals (95% CI) were calculated by logistic regression at multivariate analysis. The diagnostic accuracy of cytology, HPV testing, and co-testing as detection tools after conization was determined by the sensitivity, specificity, positive predictive value (PPV) and NPV of the test.

Results

RETROSPECTIVE ANALYSIS

Characteristics of the study group

The mean age of the studied population (n=372) was 35.6 years (range 18-80.5). HPV testing pre-treatment was available in 351 women: it was negative in 101 (29%) and positive in 250 (71%). HPV type 16 was the most prevalent type at baseline (64%), followed by types 33 (6.4%) and 18 (5.6%). Among women with initial negative HPV test (101), 84 remained negative during follow-up after treatment (83%), but 17 (17%) again acquired an HPV infection (we defined this situation as "new infection").

Among the 250 women with initial positive HPV test, 25 acquired "another infection" by an HPV type that was different from the type detected pre-LEEP, regardless of whether there was clearance of the initial infection or not.

Recurrent disease and HPV clearance

During follow-up, 32 patients of 372 (8.6%) developed recurrent disease. Most recurrences (68.7%) were identified within the first year after treatment (mean time 19 months, range 3-140 months, median 7).

Among the 238 women who were HPV positive before the procedure and for whom a post-treatment HPV test was available, 216 (91%) became negative during follow-up for the same type detected at baseline. HPV was cleared within the first year post-LEEP in 151 patients (151/216, 70%). In six women (3%), it was cleared beyond the first year of follow-up and in 59 (27%) clearance time could not be determined because of a lack of HPV tests available. There was no significant difference in clearance time depending on HPV type.

Among the 22 women (22/238, 9%) with persistent HPV infection post-LEEP, type 16 was identified most frequently (18/22, 82%), nevertheless no significant difference between the clearance rates of different HPV types was found.

In three of the 17 (17.5%) women with "new infection" the HPV was not cleared during follow-up. These three persistent "new infection" caused recurrent disease.

Among the 25 women with "another infection", four remained positive during follow-up post-LEEP.

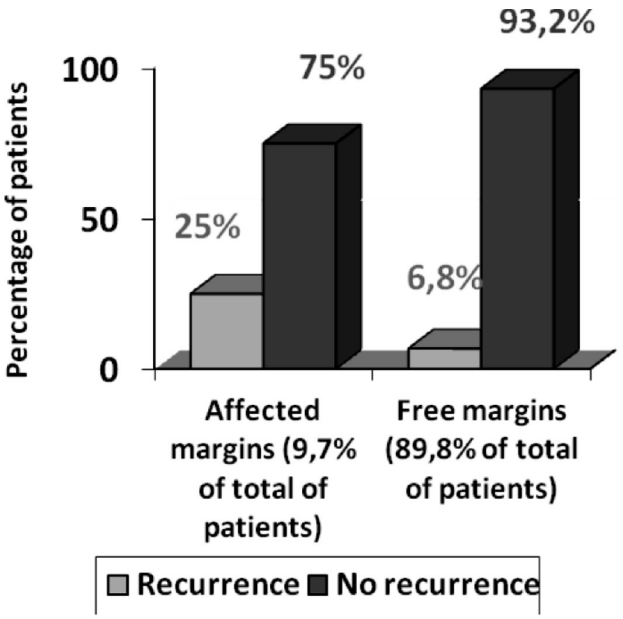

Figure 3. — Recurrence rate related to margin status. Retrospective analysis.

Margins and other predictors of recurrent disease

Surgical margins were positive in 36 cases (36/372, 9.7%). Of the overall group of women with negative margins, 23 developed recurrent disease (23/334, 6.8%). Nevertheless, among the 36 cases with incomplete resection of CIN, there were nine recurrences (25%), showing a significant difference (p = 0.00024, Figure 3).

Patient characteristics are listed in Table 1. Higher age at conization and menopausal status were associated with significantly higher risk of recurrent disease. Positive margins (OR 0.192; 95% CI 0.074 to 0.497) and higher age at conization (OR 1.061; 95% CI 1.019 to 1.104) resulted to be independent from other risk factors at multivariate analysis.

Identification of recurrent disease

Table 2 shows the sensitivity, specificity, PPV, and NPV for recurrent disease of HPV testing, cytology and co-testing.

- *HPV testing:* 32 women had persistent HPV infection post-LEEP (25 the same initial type, three "new infection" and four "another infection") and 316 cleared baseline/other HPV types or remained negative during follow-up. Twenty-two of the 32 recurrences of this study were diagnosed among the women with persistent HPV infection.
- Cytology: 27 of 32 recurrences had some positive cytology (≥ ASCUS) during follow-up prior to the confirmatory biopsy (CIN2+). The remaining five women with residual disease had completely negative cytological surveillance.

Table 1. — *Patient characteristics related to recurrence rate. Retrospective analysis.*

Patients' characteristics (Retrospective analysis)		No recurrences (%)	Statistical significance
Smoking	222 smokers	21 (9,4%)	$p = 0.547$
	144 non-smokers	11 (7,6%)	(NS)
	6 no data		
Menopausal status	23 menopausal	5 (21,7%)	$p = 0.020$
	349 non-menopausal	27 (7,7%)	
Age	103 women ≥40 years	16 (15,5%)	$p = 0.003$
	269 women <40 years	16 (6%)	
	25 women ≥50 years	5 (20%)	$p = 0.035$
	347 women <50 years	27 (7,8%)	
Parity	35 women ≥3 V. deliveries	5 (14,28%)	$p = 0.207$
	337 women <3 V. deliveries	27 (8%)	(NS)
Other STI	42 women yes	3 (7,14%)	$p = 0.716$
	329 women no	29 (8,81%)	(NS)
	1 woman no data		
Age at first intercourse	11 women <15 years	2 (18,18%)	$p = 0.273$
	314 women ≥15 years	27 (8,6%)	(NS)
	47 women no data		
	230 women <20 years	20 (8,7%)	$p = 0.82$
	95 women ≥20 years	9 (9,4%)	(NS)
Immunosuppression or chronic disease	76 women yes	10 (13,15%)	$p = 0.09$
	295 women no	21 (7,11%)	(NS)
	1 woman no data		
Mode of contraception	138 women hormonal	11 (8%)	$p = 0.807$
	107 women barrier	7 (6,5%)	(NS)
	114 women no H-no B	12 (10,5%)	
	13 women no data		

V. deliveries (vaginal deliveries), NS (not significant).
No H-no B (no Hormonal-no Barrier).

Table 2. — *Sensitivity, specificity, PPV and NPV of HPV test, cytology, and co-testing as predictors of recurrent disease. Retrospective analysis.*

	Sensitivity %	Specificity %	PPV %	NPV %
HPV test	68,75	96,83	68,75	96,83
Cytology	84,37	84,7	34,17	98,3
Co-testing	90,62	72,53	24,57	98,74

– Co-testing: 29 of 32 recurrences had positive cytology or HPV testing during follow-up (positive co-testing), but all pap smears and HPV tests post-LEEP were negative (negative co-testing) in the other three women with recurrent disease prior to the confirmatory biopsy.

PROSPECTIVE ANALYSIS

Characteristics of the study group

The mean age of the studied population (n=97) was 40 years (range 22-71). HPV tests pre-treatment were negative in 29 women (30%) and positive in 68 (70%). HPV type 16 was the most prevalent type at baseline (41%), followed by types 31 (13%) and 18 (7%). Among women with an initial negative HPV test, 21 remained negative during follow-up after treatment (72.4%), but 8 (27.6%) acquired a "new infection". Among the 68 women with an initial positive HPV test, 12 acquired "another infection".

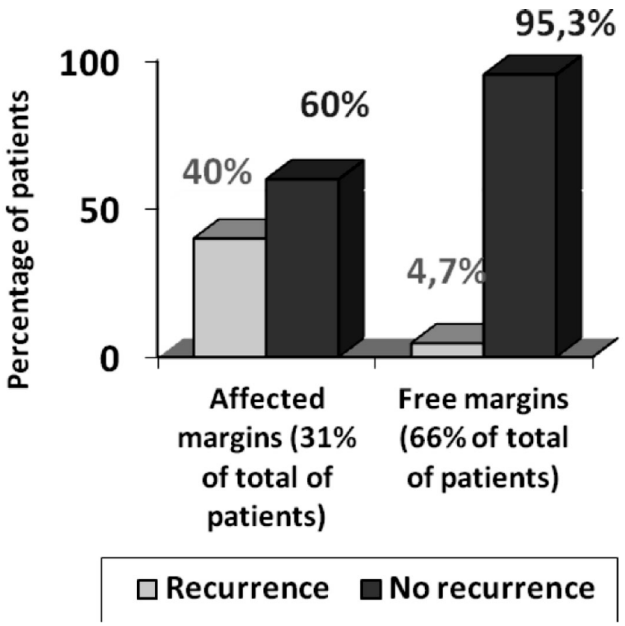

Figure 4. — Recurrence rate related to margin status. Prospective analysis.

Recurrent disease and HPV clearance

During follow-up, 15 patients of 97 (15.4%) developed recurrent disease. Most recurrences (60%) were identified within the first 6 months after treatment (mean time 10 months, range 4-24, median 6).

Among the 68 women who were HPV positive before the procedure, 56 (82%) became negative during follow-up for the same type detected at baseline. HPV was cleared within the first six months post-LEEP in 51 patients (51/56, 91%). In the remaining five women (9%) it was cleared between six and 12 months after treatment. There was no significant difference in clearance time depending on HPV type.

Among the 12 women with persistent HPV infection post-LEEP, type 16 was identified most frequent (67%), nevertheless no significant difference between the clearance rates of different HPV types was found.

In three of the eight (37.5%) women with "new infection" the HPV was not cleared during follow-up. These three persistent "new infection" caused recurrent disease. Among the 12 women with "another infection", three remained positive during follow-up post-LEEP.

Margins and other predictors of recurrent disease

Surgical margins were positive in 30 cases (30/97, 31%). Of the overall group of women with negative margins, three developed recurrent disease (3/64, 4.7%). Nevertheless,

Table 3. — *Patient characteristics related to recurrence rate. Prospective analysis.*

Patients' characteristics (Retrospective analysis)		No recurrences (%)	Statistical significance
Smoking	52 smokers	9 (17,3%)	$p = 0.59$
	45 non-smokers	6 (13,3%)	(NS)
Menopausal status	16 menopausal	3 (19%)	$p = 0.7$
	81 non-menopausal	12 (15%)	(NS)
Age	46 women ≥40 years	11 (24%)	$p = 0.03$
	51 women <40 years	4 (8%)	
	14 women ≥50 years	2 (14,3%)	$p = 0.9$
	83 women <50 years	13 (16%)	(NS)
Parity	10 women ≥3 V. deliveries	1 (10%)	$p = 0.61$
	87 women <3 V. deliveries	14 (16%)	(NS)
Other STI	16 women YES	6 (37,5%)	$p = 0.007$
	81 women NO	9 (11%)	
Age at first intercourse	3 women <15 years	1 (33,3%)	$p = 0.38$
	94 women ≥15 years	14 (15%)	(NS)
	73 women <20 years	14 (19%)	$p = 0.077$
	24 women ≥20 years	1 (4%)	(NS)
Immunosuppression or chronic disease	30 women yes	6 (20%)	$p = 0.4$
	67 women no	9 (13,4%)	(NS)
Mode of contraception	35 women hormonal	5 (14,3%)	$p = 0.826$
	26 women barrier	5 (19,2%)	(NS)
	36 women no H-no B	5 (14%)	

V. deliveries (vaginal deliveries), NS (not significant).
No H-no B (no Hormonal-no Barrier).

Table 4. — *Sensitivity, specificity, PPV and NPV of HPV test, cytology, and co-testing as predictors of recurrent disease. Prospective analysis.*

	Sensitivity %	Specificity %	PPV %	NPV %
HPV test	80	92,7	66,6	96,2
Cytology	100	81,7	50	100
Co-testing	100	66	35	100

among the 30 cases with incomplete resection of CIN, there were 12 recurrences (40%), showing a significant difference ($p = 0.000013$, Figure 4).

Patient characteristics are listed in Table 3. Higher age at conization and other STI were associated with significantly higher risk of recurrent disease.

Positive margins (OR 0.096; 95% CI 0.023 to 0.392) and other STI (OR 0.252; 95% CI 0.064 to 0.998) resulted to be independent from other risk factors at multivariate analysis.

Identification of recurrent disease

Table 4 shows the sensitivity, specificity, PPV, and NPV for recurrent disease of HPV testing, cytology, and co-testing.

– *HPV testing: 18* women had persistent HPV infection post-LEEP (12 the same initial type, three "new infection" and three "another infection*"*) and 79 cleared baseline/other HPV types or remained negative during follow-up. Twelve of the 15 recurrences of this study were diagnosed among the women with persistent HPV infection.
– Cytology: The 15 women with recurrent disease had some positive cytology yielding a sensitivity of 100%
– Co-testing: When both tests were combined, all women with recurrent disease were detected (sensitivity and NPV 100%).

Follow-up post-treatment

Among the 36 women with free margins that were followed for at least two years, 24 had negative co-testing in every visit: if co-test was negative at six and 12 months, then it was negative at 18-24 or 30 months visits. The remaining 12 patients, including the three women with free margins and recurrence, had positive cytology or HPV test at six to 12 months.

Nevertheless, one patient with positive margins and normal co-testing at six to 12 months developed high grade squamous intraepithelial lesion at 18 months post-LEEP.

Discussion

The present data showed 70-71% of women HPV-positive pre-LEEP, a slightly lower rate than described before, although the present authors agree with other authors in describing type 16 as the most prevalent [6, 10, 12, 13, 18].

The recurrence rate in the prospective analysis was much higher than in the retrospective one (15.4% *vs.* 8.6%). This is probably due to conization during the year 2010 being more conservative, above all in women with childbearing desires and to a higher mean age of patients in that period. Nonetheless, both rates are included in the range described by others (3-17%) considering only studies that define recurrence as CIN 2-3 histologically confirmed [8, 9, 12, 14-16]. Most reviewed studies found a peak incidence of recurrence within the first two years post-treatment, but some of them [9,14] have a precisely two year follow-up. In the few publications with long term follow-up [10, 15, 22, 23] recurrent cases are described beyond the first two years. In particular Chua *et al.* [23] found 9/26 recurrences two to eight years post-treatment. Eight of 32 recurrences in our retrospective analysis occurred between two and 11 years after LEEP.

HPV clearance rate (82-91%) and clearance time after treatment (most in first 6-12 months) in the present study are similar to previous investigations [3,13].

Age and positive surgical margins were found to be risk factors for recurrent disease, although age was not independent in prospective analysis. Women over 35-40-50 years had an increased recurrence rate in many studies, perhaps because of an abnormal immunity or higher HPV persistence [8, 18, 22, 24]. Incomplete excision of CIN is a risk factor but an inexact predictor for recurrence: most women with affected margins will never present recurrent disease and, on the other hand, a small but significant number of patients with free margins will develop recurrence [2, 5, 7, 16]. It is important to emphasize that the increased recurrence rate in the prospec-

tive analysis did not represent an increased invasive cervical cancer rate in the Department of Gynecology (HUCA), although follow-up time in this analysis was not very long.

Cytology showed very high sensitivity and NPV for recurrent disease in this study. In particular, both were calculated as 100% in the prospective analysis, although this could be due to a small *n*. Published cytology sensitivity ranges from 20 to 100% [2, 12, 18, 20, 21]. In contrast with most previous reports [6-8,12, 16, 17, 23], HPV showed less sensitivity than pap smear in our hospital (69% *vs* 84% and 80% *vs* 100%), but NPV was similar, very high in both tests (97% *vs* 98% and 96% *vs* 100%). In spite of our HPV sensitivity being lower than described by others (83-100%) [2, 4, 5, 8, 9, 11, 12, 17, 18, 21], in the large case-control study of Acladious *et al.* [25] HPV only showed 47% sensitivity six months post-conization. Nonetheless the present authors hope that the Cobas 4800 PCR will improve HPV predictive power in the near future in Asturias University Hospital.

In respect to co-testing, it is clear in the present study and others [6, 7, 9, 21, 25] that combining cytology and HPV increases sensitivity and NPV and, although specificity and PPV decrease, the former are more important in this context of a potentially lethal disease. In this way, it is possible to select a group of patients with very low risk of recurrent disease that could be returned early to routine screening.

However, Strander *et al.* [26] published that 76% of recurrences more than two years post-LEEP occurred in women that had cleared HPV after treatment, so most probably these patients were infected with HR-HPV at a later stage. The host conditions that once led to the initial CIN usually persist and these women have an increased risk for re-infection and persistence with HR-HPV. They concluded that this is the probable cause for the limited value of HPV for the design of long term follow-up and criticize other small prospective studies that publish a very high NPV for HPV but with a fairly short follow-up period and a minimal number of recurrences [12, 27].

Proposed surveillance protocols

Several reports [12, 15, 21, 27] conclude that risk of recurrent disease is so low in women with negative co-testing at six months that retesting at 12 months can be omitted and scheduled again at 24 months. The need for fewer tests would be a cost benefit and would be more convenient for the patient. Only when co-testing is negative during at least 24 months should women be referred to a routine screening program.

Kitchener *et al.* [28] recently published a large prospective study (n=917). They found that the risk of residual CIN 2-3 is negligible in women who were HPV/Pap negative at six months so these patients could be safely returned to three- or five-yearly recall.

Some authors advise caution and emphasize that more relaxed follow-up in HPV test-negative women still awaits further evaluation in larger studies with longer follow-up times [14, 29].

On the basis of the present prospective analysis, the authors recommend that patients with free margins and negative co-testing at six and 12 months could omit the 18 months visit and retest at 24 months. Nevertheless, if margins are positive, visits should be at 6-12-18-24 months with cytology and HPV testing. In the present retrospective analysis, tests were not done at fixed time intervals therefore the authors cannot conclude the same, but it should be noted that the three women with false negative co-testing that developed recurrent disease were diagnosed thanks to routine colposcopy in the Department of Gynecology (HUCA). Furthermore, the retrospective analysis had a large study population (n=372) and a long-term follow-up (up to 15 years), therefore the authors could confirm that eight of 32 recurrences (25%) developed more than two years post-LEEP. Among these eight long-term recurrences, three (37.5%) were caused by persistent "new/another infections", results in concordance with Strander *et al.* [26]. Another three of these recurrences occurred, surprisingly, in women who tested negative for HPV pre-LEEP and every visit post-LEEP, and the remaining three long-term recurrences were caused by persistent same HPV-type infection present before treatment.

Thus, the present authors agree with several authors [22, 24, 30] that long-term risk of recurrent dysplasia or invasive cancer remains higher among women treated for CIN 2-3, hence it is important not to stop routine screening at age 65 as in the general population. More intensive follow-up is proposed, preferably for at least 20-25 years after conization, particularly for those women older than 35-40 years at treatment. Moreover, the authors consider that the available evidence is not strong enough to abbreviate surveillance at the Colposcopy Department to less than two years.

The Spanish Society for Colposcopy and Cervical Pathology maintains the follow-up protocol published in 2006 [31]: co-testing and colposcopy at three to six months followed by two annual Pap smears. If these are negative, women are returned to cervical screening.

The American Society for Colposcopy and Cervical Pathology has recently updated its guidelines [32]. In its 2006 recommendations, if a single HPV test or cytology at six and 12 months were negative, women were returned early to screening. Current guidelines suggest co-testing at 12 and 24 months and if both are negative, it is repeated three years later. Then, women are returned to screening for at least 20 years.

Conclusion

HR-HPV testing, in combination with cytology, is the best option in follow-up protocols after treatment for CIN 2-3. The authors also recommend routine colposcopy during the first two years post-conization. If margins are free and co-testing is negative at six and 12 months, 18 months visit could be avoided.

References

[1] Bosch F.X., Lorincz A., Munoz N., Meijer C.J., Shah K.V.: "The causal relation between human papillomavirus and cervical cancer". *J. Clin. Pathol.*, 2002, *55*, 244.

[2] Jeong N.H., Lee N.W., Kim H.J., Kim T., Lee K.W.: "High-risk human papillomavirus testing for monitoring patients treated for high-grade cervical intraepithelial neoplasia". *J. Obstet. Gynaecol. Res.*, 2009, *35*, 706.

[3] Brismar S., Johansson B., Borjesson M., Arbyn M., Andersson S.: "Follow-up after treatment of cervical intraepithelial neoplasia by human papillomavirus genotyping". *Am. J. Obstet. Gynecol.*, 2009, *201*, 17.

[4] Park J.Y., Bae J., Lim M.C., Lim S.Y., Lee D.O., Kang S., *et al.*: "Role of high risk-human papilloma virus test in the follow-up of patients who underwent conization of the cervix for cervical intraepithelial neoplasia". *J. Gynecol. Oncol.*, 2009, *20*, 86.

[5] Prato B., Ghelardi A., Gadducci A., Marchetti I., Di Cristofano C., Di Coscio G., *et al.*: "Correlation of recurrence rates and times with posttreatment human papillomavirus status in patients treated with loop electrosurgical excision procedure conization for cervical squamous intraepithelial lesions". *Int. J. Gynecol. Cancer*, 2008, *18*, 90.

[6] Bae J.H., Kim C.J., Park T.C., Namkoong S.E., Park J.S.: "Persistence of human papillomavirus as a predictor for treatment failure after loop electrosurgical excision procedure". *Int. J. Gynecol. Cancer*, 2007, *17*, 1271.

[7] 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.

[8] Verguts J., Bronselaer B., Donders G., Arbyn M., Van Eldere J., Drijkoningen M., *et al.*: "Prediction of recurrence after treatment for high-grade cervical intraepithelial neoplasia: the role of human papillomavirus testing and age at conisation". *BJOG*, 2006, *113*, 1303.

[9] Kreimer A.R., Guido R.S., Solomon D., Schiffman M., Wacholder S., Jeronimo J., *et al.*: "Human papillomavirus testing following loop electrosurgical excision procedure identifies women at risk for posttreatment cervical intraepithelial neoplasia grade 2 or 3 disease". *Cancer Epidemiol. Biomarkers Prev.*, 2006, *15*, 908.

[10] Nagai N., Mukai K., Oshita T., Shiroyama Y., Ohama K.: "Human papillomavirus DNA status after loop excision for cervical intraepithelial neoplasia grade III - A prospective study". *Int. J. Mol. Med.*, 2004, *13*, 589.

[11] Houfflin Debarge V., Collinet P., Vinatier D., Ego A., Dewilde A., Boman F., *et al.*: "Value of human papillomavirus testing after conization by loop electrosurgical excision for high-grade squamous intraepithelial lesions". *Gynecol. Oncol.*, 2003, *90*, 587.

[12] Nobbenhuis M.A., Meijer C.J., van den Brule A.J., Rozendaal L., Voorhorst F.J., Risse E.K., *et al.*: "Addition of high-risk HPV testing improves the current guidelines on follow-up after treatment for cervical intraepithelial neoplasia". *Br. J. Cancer,* 2001, *84*, 796.

[13] Nagai Y., Maehama T., Asato T., Kanazawa K.: "Persistence of human papillomavirus infection after therapeutic conization for CIN 3: is it an alarm for disease recurrence?" *Gynecol. Oncol.*, 2000, *79*, 294.

[14] Sarian L.O., Derchain S.F., Andrade L.A., Tambascia J., Morais S.S., Syrjanen K.J.: "HPV DNA test and Pap smear in detection of residual and recurrent disease following loop electrosurgical excision procedure of high-grade cervical intraepithelial neoplasia". *Gynecol. Oncol.*, 2004, *94*, 181.

[15] Kocken M., Helmerhorst T.J., Berkhof J., Louwers J.A., Nobbenhuis M.A., Bais A.G., *et al.*: "Risk of recurrent high-grade cervical intraepithelial neoplasia after successful treatment: a long-term multi-cohort study". *Lancet Oncol.*, 2011, *12*, 441.

[16] Kreimer A.R., Schiffman M., Herrero R., Hildesheim A., Gonzalez P., Burk R.D., *et al.*: "Long-term risk of recurrent cervical human papillomavirus infection and precancer and cancer following excisional treatment". *Int. J. Cancer*, 2012, *131*, 211.

[17] Heymans J., Benoy I.H., Poppe W., Depuydt C.E.: "Type-specific HPV geno-typing improves detection of recurrent high-grade cervical neoplasia after conisation". *Int. J. Cancer*, 2011, *129*, 903.

[18] Baloglu A., Uysal D., Bezircioglu I., Bicer M., Inci A.: "Residual and recurrent disease rates following LEEP treatment in high-grade cervical intraepithelial lesions". *Arch. Gynecol. Obstet.*, 2010, *282*, 69.

[19] Brockmeyer A.D., Wright J.D., Gao F., Powell M.A.: "Persistent and recurrent cervical dysplasia after loop electrosurgical excision procedure". *Am. J. Obstet. Gynecol.*, 2005, *192*, 1379.

[20] Paraskevaidis E., Arbyn M., Sotiriadis A., Diakomanolis E., Martin-Hirsch P., Koliopoulos G., *et al.*: "The role of HPV DNA testing in the follow-up period after treatment for CIN: a systematic review of the literature". *Cancer Treat. Rev.*, 2004, *30*, 205.

[21] 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.

[22] Melnikow J., McGahan C., Sawaya G.F., Ehlen T., Coldman A.: "Cervical intraepithelial neoplasia outcomes after treatment: long-term follow-up from the British Columbia Cohort Study". *J. Natl. Cancer Inst.*, 2009, *101*, 721.

[23] Chua K.L., Hjerpe A.: "Human papillomavirus analysis as a prognostic marker following conization of the cervix uteri". *Gynecol. Oncol.*, 1997, *66*, 108.

[24] Strander B., Andersson-Ellstrom A., Milsom I., Sparen P.: "Long term risk of invasive cancer after treatment for cervical intraepithelial neoplasia grade 3: population based cohort study". *BMJ*, 2007, *335*, 1077.

[25] Acladious N.N., Sutton C., Mandal D., Hopkins R., Zaklama M., Kitchener H.: "Persistent human papillomavirus infection and smoking increase risk of failure of treatment of cervical intraepithelial neoplasia (CIN)". *Int. J. Cancer,* 2002, *98*, 435.

[26] Strander B., Ryd W., Wallin K.L., Warleby B., Zheng B., Milsom I., *et al.*: "Does HPV-status 6-12 months after treatment of high grade dysplasia in the uterine cervix predict long term recurrence?" *Eur. J. Cancer*, 2007, *43*, 1849.

[27] Zielinski G.D., Rozendaal L., Voorhorst F.J., Berkhof J., Snijders P.J., Risse E.J., *et al.*: "HPV testing can reduce the number of follow-up visits in women treated for cervical intraepithelial neoplasia grade 3". *Gynecol. Oncol.*, 2003, *91*, 67.

[28] Kitchener H.C., Walker P.G., Nelson L., Hadwin R., Patnick J., Anthony G.B., *et al.*: "HPV testing as an adjunct to cytology in the follow up of women treated for cervical intraepithelial neoplasia". *BJOG*, 2008, *115*, 1001.

[29] Aschkenazi-Steinberg S.O., Spitzer B.J., Spitzer M., Lesser M.: "The clinical usefulness of human papillomavirus testing in the follow-up of women after treatment for cervical intraepithelial neoplasia". *J. Low. Genit. Tract Dis.*, 2004, *8*, 304.

[30] Soutter W.P., de Barros L.A., Fletcher A., Monaghan J.M., Duncan I.D., Paraskevaidis E., *et al.*: "Invasive cervical cancer after conservative therapy for cervical intraepithelial neoplasia". *Lancet*, 1997, *349*, 978.

[31] Comino R., Cararach M., Puig-Tintoré L.M., Cortés J., Torné A., Dexeus S., *et al.*: "Documentos de consenso SEGO 2006. Prevención del cáncer de cérvix uterino" *In: Documentos de consenso SEGO 2006*. Meditex, Madrid, SEGO, 2007, 123.

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

Address reprint requests to:
Y. RUANO RODRÍGUEZ, M.D.
Fray Ceferino Nº 55, 5º A. 33011.
Oviedo, Asturias (Spain)
e-mail: yolandaruano@yahoo.es