ORIGINAL

Receipt: 2017 August 23
Accepted: 2018 June 18
Published: 2018 October 2

STUDY OF CONIZATIONS OF THE CERVIX AFTER FIVE YEARS OF CERVICAL CANCER SCREENING WITH CO-TESTING

Rosa Oncins Torres (1), Mª Ángeles Aragón Sanz (2), Eduardo Clemente Roldán (3), Mª Dolores Comes García (1), Gorka Muñiz Unamunzaga (1), Lorena Guardia Dodorico (2) y Víctor Vallés Gallego (3)

(1) Service of Pathology. Barbastro Hospital. Barbastro. Huesca. Spain.

(2) Service of Gynaecology and Obstetrics. Barbastro Hospital. Barbastro. Huesca. Spain.

(3) Primary Care of Barbastro Sector. Barbastro. Huesca. Spain.

ABSTRACT

Background: Uterine Cervical Cancer (UCC) screening has changed with the introduction of the High Risk Human Papilloma Virus test (HRH-PV) and its evaluation is necessary. The objective of this study is to analyze the effectiveness of UCC screening with activities aimed at early detection and treatment to modify the natural history of the process and improve its prognosis.

Methods: Cytology and HR-HPV (co-testing) were performed according to the SEGO protocol of 2010 between 2011 and 2015 with follow-up until 2017. The HR-HPV DNA test was HC2 Hybrid Capture (Digene®) at the beginning (16.1% of the cases) and Cobas 4800 (Roche®) afterwards. Target population: Barbastro´s health area. The initial treatment was conization with loop (LLETZ). Sensitivity and Positive Predictive Value of tests were studied, as well as the association between demographic and pathological variables.

Results: 238 high-grade dysplasias (HSIL) or more (CIN2+) were detected with a mean age of 37.9±10.3 years and 60.0% were genotype 16 and/or 18 positive. 220 patients (92.4%) underwent conization completed thereafter with reconization or hysterectomy in 25 cases (11.4%). HSIL was diagnosed in 220 cases (92.4%) and invasive carcinoma in 18 (7.6%), 7 microinvasive (2.9%). 14.4% of cones had no HSIL (negative cone) and 83.2% got free margins. 52.0% had involvement in a single quadrant and the mean horizontal extension was 3.5±3.1mm. Only in 14 (6.7%) patients the disease (HR-HPV positive) persisted after treatment. A statistically significant association was found in our cases between affected borders and age over 45 years (p=0.005).

Conclusions: The co-test has detected small preinvasive lesions, localized in a single quadrant and microinvasive cancers . Loop conization was effective, achieving the cure of 93.3% of the patients.

Key words: Conization, Screening, Cervical intraepithelial neoplasia, Human papillomavirus, Co-testing, Margin.

Estudio de piezas de conización tras cinco años de cribado de cáncer de cérvix con co-test

Fundamentos: El cribado del cáncer de cérvix uterino (CCU) ha cambiado con la introducción del test del virus del Papiloma Humano de alto riesgo (VPH-AR) y es necesaria su evaluación. El objetivo de este estudio fue analizar la eficacia del cribado del CCU con las actividades orientadas a la detección y tratamiento precoz para modificar la historia natural del proceso y mejorar su pronóstico.

Métodos: Se realizó un cribado con citología y VPH-AR (co-test) según el protocolo SEGO de 2010 entre los años 2011 y 2015 con seguimiento hasta 2017. El test de ADN VPH-AR fue Captura de Híbridos HC2 (Digene ®) al inicio (16,1% de los casos) y Cobas 4800 (Roche®) después. La población diana fue el Área de salud de Barbastro. El tratamiento inicial fue la conización con asa (LLETZ). Se estudió la sensibilidad y el valor predictivo positivo de los test, así como la asociación entre variables demográficas y patológicas.

Resultados: Se detectaron 238 displasias de alto grado (HSIL) o mayor con una media de edad de 37,9±10,3 años y el 60,0% fueron positivas a los genotipos 16 y/o 18. Se conizaron 220 pacientes (92,4%) y en 25 (11,4%) se precisó reconización o histerectomía. Se diagnosticó HSIL en 220 pacientes (92,4%) y carcinoma invasor en 18 (7,6%), 7 microinvasores (2,9%). En el 14,4% de los conos no se halló HSIL (conos blancos) y el 83,2% tuvo bordes libres. El 52,0% tenía afectación en un solo cuadrante y el tamaño tuvo de media 3,5±3,1mm. Sólo 14 pacientes (6,7%) continuaban enfermas (VPH-AR positivo) tras tratamiento. Se halló, en nuestros casos, asociación estadísticamente significativa entre bordes afectados y edad mayor de 45 años (p=0,005).

Conclusiones: El co-test ha detectado lesiones preinvasoras, pequeñas, localizadas en un solo cuadrante y carcinomas microinvasores. La conización con asa fue eficaz logrando la curación del 93,3% de las pacientes.

Palabras clave: Conización, Cribado, Neoplasia intraepitelial cervical, Virus del papiloma humano, Co-test, Margen.

Correspondence:
Rosa Oncins Torres
Service of Pathology
Barbastro Hospital
Nacional Road 240 s/n
22300 Barbastro (Huesca)
roncins@salud.aragon.es

Suggested citation: Oncins Torres R, Aragón Sanz MA, Clemente Roldán E, Comes García MD, Muñiz Unamunzaga G, Guardia Dodorico L, Vallés Gallego V. Study of conizations of the cervix after five years of cervical cancer screening with co-testing. Rev Esp de Salud Pública.2018;92: October 2 e201810045.

INTRODUCTION

The necessary presence of Human Papillomavirus (HPV) for the development of uterine cervical cancer (UCC)[1] has led scientific societies to include the screening of high-risk papillomavirus (hrHPV) test, combined or not with gynecological cytology. The name given to the joint performance of the cytology and hrHPV test[2] is co-testing (double test).

Due to its generalization, the screening of UCC in previous decades has led to a decrease in the incidence and mortality of this tumor, although it has been opportunistic. Among the hrHPV screening tests, those able to detect the DNA of the virus (hrHPV DNA) are the ones recommended by the ASCO (American Society of Clinical Oncology) guide[3]. The pre-invasive disease is called High-Grade Lesion (HSIL)[4] equivalent to Cervical Intraepithelial Neoplasia (CIN) grade 2 or 3. The treatment for HSIL is conization, usually with a loop of diathermy or LEEP ("Loop Electrosurgical Procedure") that manages to control the disease in most cases[5]. Therefore, screening will identify and treat early stages to decrease the risk of invasive cancer. Colposcopy, cytology and the hrHPV test[2,5] are useful for monitoring the cure. The goal of this study was to describe the efficacy of UCC cancer screening with co-testing, early diagnosis and conization treatment of all patients with pre-invasive or invasive lesions treated during five years, in the area attended by the Hospital of Barbastro to improve its prognosis.

SUBJECTS AND METHODS

A retrospective descriptive study of HSIL and invasive cancers; diagnosed from January 1st, 2011 to December 31st, 2015 and their follow-up until June 30th, 2017.

The Health sector population of Barbastro, located in the eastern area of the province of Huesca, which consists of 107,428 inhabitants (52,535 women), mostly rural, aging and dispersed, attended by 15 Primary Care teams. The Health area covered by the Hospital of Barbastro has an organized opportunistic screening. The target population for UCC screening was 27,401 women between 25 and 64 years of age. The immigrant population is 14%. The 2010 Protocol of the Spanish Society of Gynecology (SEGO) was followed[2]. Women under 30 years of age were screened with cytology every 3 years and the hrHPV DNA test and gynecological cytology were performed every 5 years for patients between 30 and 64 years of age. See figure 1a for the indication of cytology, HPV test or co-test. The primary care midwives carried out conventional cytology and HPV test and sent them to the Hospital's Pathology Service. This service has been accredited with the ISO 15185 standards for conventional cytology since 2011 and subsequently for the hrHPV DNA test and for liquid cytology. The result of the cytology was reported according to the Bethesda System[6,7]. The hrHPV DNA test was performed with HC2 (Quiagen®) that reported positive or negative results during the first 10 months of the study (16.1% of cases); and cobas 4800 (Roche®) which provided information about the results for genotype 16 and 18 and the group of other 12 genotypes (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) the rest of the time. The sampling and application of the protocol have already been described in other articles[8,9]. The patients in whom lesions were detected in their first screening test were identified as "diagnosis by the first test" and they differed from the group of patients who attended the program on a regular basis, identified as "diagnosis by screening" The group of "diagnosis by follow-up" corresponded to the screened patients who were followed by an abnormal cytology or/and positive HPV (figure 1b).

HSIL treatment was conization with LLEP that was completed with reconization and hysterectomy with or without double anexectomy, according to the diagnosis of conization and other benign diagnoses of the patient.

Conization specimens were submitted in a fresh state to Pathology, oriented with a silk

Figure 1a
Screening and monitoring algorithm (citology, HPV testing or co-testing).
Based on Protocol SEGO 2010[2]

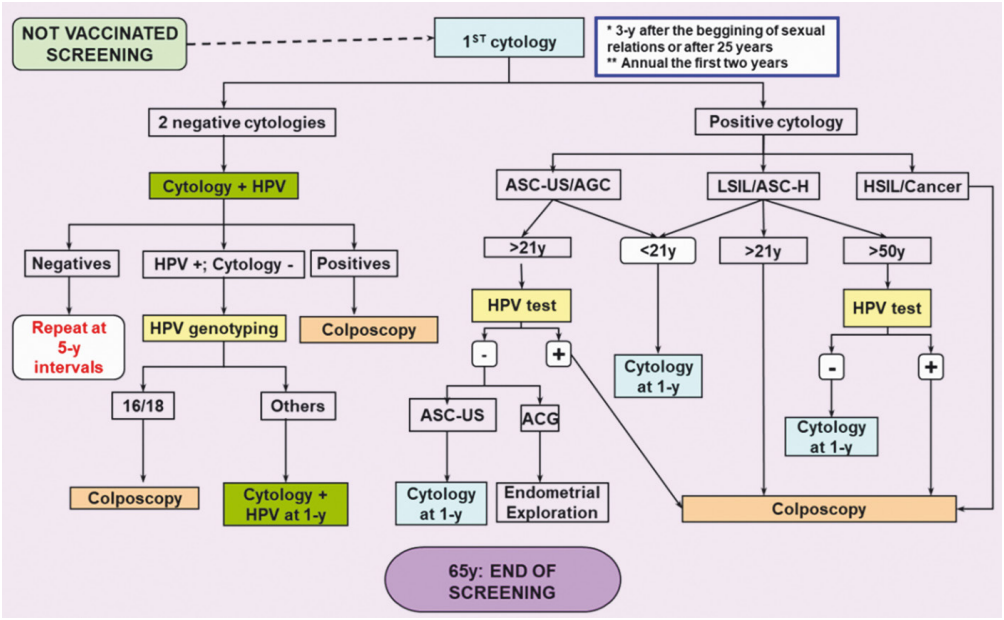
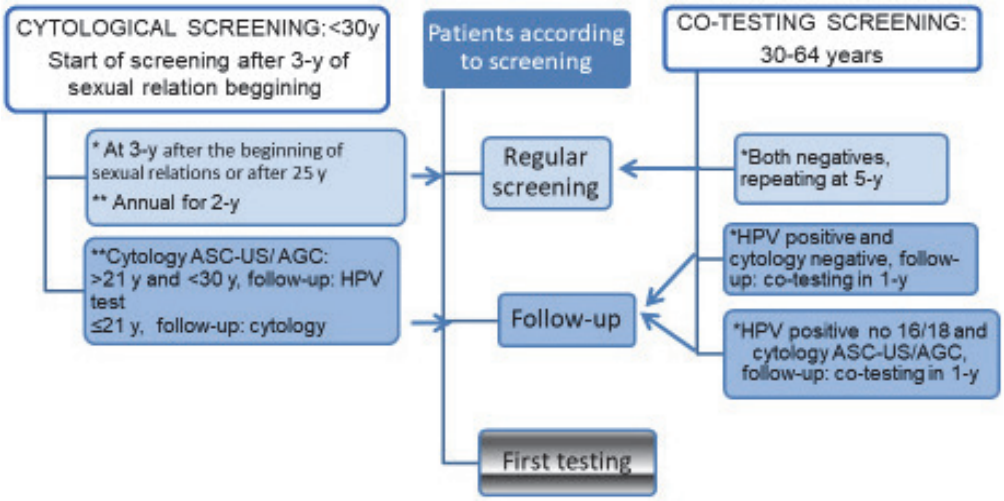


Figure 1b
Distribution of patients by regular screening, after follow-up or in their first testing



suture at the 12 o´clock position. The margin was painted with India ink and sections were taken according to clock hours. The endocervical curettage performed after the conization was also received. $p16^{INK4a}$ was used as a complementary technique for diagnosis or for margin evaluation in cases with artifact or doubtful diagnosis.

Invasive cases were referred for treatment to their reference hospital either for radical surgery or radiotherapy. Chemotherapy was administered in our own hospital.

The following variables were recorded: Age (grouped into the categories of ≤45 years and higher than 45 years); nationality (classified into Spanish and foreigners); result of cytology (grouped into low-grade intraepithelial lesion-LSIL- or less and high-grade intraepithelial lesion -HSIL- or higher, according to the Bethesda System); result of the HPV test for genotype 16 and/or 18 (alone or associated with multiinfection with other viruses) were grouped as risk virus; and the histological result of the biopsy that was diagnosed as intraepithelial neoplasia (CIN), was classified in three grades (CIN 1, 2 and 3) and reclassified according to the LATS[4] terminology in LSIL (CIN1) and HSIL (CIN2/3). CIN3 included adenocarcinoma in situ (AIS). Subsequently, HSIL or higher cases (invasive cancer) were grouped as CIN2+. The conization result being benign, LSIL, HSIL or invasive; In addition, the affected quadrants were recorded (grouped into one quadrant versus more than one quadrant); the size (maximum horizontal extension expressed in mm), grouped into less than 1mm versus 1mm or more; and the state of the margins. When the lesion did not reach the ink, negative margin was considered. If the endocervical curettage was valuable and not involved after a positive cone margin, the case was considered negative margin. The margin was indeterminate when dysplasia was followed by an ulceration that reached the margin without appreciating healthy mucosa between the lesion and the margin, these cases were considered positive in the follow-up. The margin was finally grouped into negative and positive. The date of diagnosis, conization, first post-treatment control and last control were recorded. In invasive tumors, the stage was also recorded, and also the date of last control or death.

The follow-up until 2015, according to 2006 SEGO Protocol[5] was controlled with co-test at 3 months if positive margin and at 6 if negative. If both tests were negative, the follow-up continued with annual cytology for 2 years. Since January 2015, follow-up was updated according to the new Oncoguía Protocol[10] with co-test at 6 months, second control at 24 months and third at three years if negative margin. When margin was positive, the first control was performed at four months, the second control at one year and the third at two years. In the study, the criteria were unified and the variable was recorded as first control, second and third; It was also specified if there had been an HPV study and the date of the last test.

Patients were considered cured when the last cytology and/or HPV test were negative and sick when the last cytology was ASC-US or higher and/or the HPV test positive. In the event that the biopsy was negative after cytology with lesion, they were considered cured if the HPV was also negative. When the second or third HPV control was positive after the first negative, it was considered reinfection.

The data were extracted from the Pathological Anatomy database and from the Minimum Data Set (MBDS) of the hospital. Subsequently, the patients were anonymized with numerical codes. The statistical study was performed with SPSS. The continuous variables were studied with means, standard deviation, minimum, maximum and 95% confidence interval (95% CI). The categorical variables with frequencies and percentages.

Both the sensitivity and positive predictive value of the cytology and the HPV test were studied, and were expressed in percentages

with confidence intervals. The sensitivity of the cytology was calculated: TP/TP+FN, where TP (True Positive) were the abnormal cytologies (LSIL, HSIL, carcinoma -ASC and AGC were excluded-) with CIN2+ biopsy and FN (False Negative) corresponded to CIN2+ biopsies with negative cytology in the last 3 years. The HPV sensitivity was calculated in the same way, the positive results of the test were true positive when correlated with CIN2+ and FN biopsies were the negative tests with CIN2+ biopsy. The Positive Predictive Value (PPV) was calculated using the formula PPV=TP/TP+FP, where TP (True Positive) were the abnormal cytologies (LSIL, HSIL, carcinoma -ASC and AGC were excluded-) with a CIN2+ biopsy. FP (False Positives) were abnormal cytologies with negative biopsy.

Positive cases with HC2 were included in the study of the sensitivity and PPV of the HPV test. They were excluded only when the genotype was required. Student's T test and Chi-square Test ($\chi 2$) were used to study the association between quantitative and qualitative variables, respectively. It was determined that there were statistically significant differences if p was less than 0.05. All the variables were analyzed using the Kolmogorov-Smirnov test to determine their normality

RESULTS

22,743 cytologies and 17,111 hrHPV tests are performed along the 5 year study. A total of 238 CIN2+ cases are diagnosed, 220 of which are intraepithelial and 18 invasive.

Table 1 shows the epidemiological, clinical and histological characteristics of all cases. Romania is the most frequent foreign country of origin with 31 patients (13.7%) followed by Colombia with 5 (2.21%). Among foreign patients 45 (19.91%) are European and 17 (7.52%) Latin American. Out of 37 (15.5%) cases followed by positive HPV with normal cytology, only one patient is younger than 30 (there are a total of 53 cases under 30, 22.3%, in the CIN2+ group). Sensitivity of cytology is 79.4 (95% CI: 71.9-82.9) and HPV test 98.7 (95% CI: 95.5-99.3). The PPV: 78.8 (95% CI: 73.9-83.1). 112 cases (50.9%) were studied with $p16^{INK4a}$.

Figure 2 shows progressive improving of screening coverage. Table 2 shows treatment and follow-up data from HSIL lesions and microinvasive carcinomas. Conization is the initial treatment in 213 out of 220 patients who were diagnosed with intraepithelial lesions (96.8%) and in all patients with microinvasive carcinoma. Untreated patients were due to their own personal choice because of their age and/or pregnancy wishes. No conization is performed in any case with minor HSIL biopsy diagnosis and there is no hysterectomy as first-choice treatment for HSIL. Residual HSIL is found in 4 patients submitted for repeated conizations and in 5 for hysterectomies. 14 (6.7%) patients treated for HSIL have persistent disease. Microinvasive carcinoma is diagnosed after repeated conization or hysterectomy because it is underrepresented in biopsy and in cone specimen. None of the patients with microinvasive carcinoma are sick during the follow-up, in part because the majority of them (6 out of 7 patients) undergo a hysterectomy.

Invasive cases from stage IB are described in table 3. Figure 3 shows HPV genotypes in HSIL (figure 3a) where 58% are positive for oncogenic viruses 16/18 which add up to 87% when being invasive (figure 3b) and 49% in follow-up. Thirty-three cases in figure 3a and two cases in figure 3b are not shown due to unknown genotype. There are also 4 cases of negative HPV: 2 pre-invasive, negative with cobas 4800® and HC2, but positive with $p16^{INK4a}$ in biopsy; and two invasive, one squamous carcinoma and one adenocarcinoma. The last one is a true negative being $p16^{INK4a}$ negative and also PCR (PGMY09 / PGMY11 and GP5+/GP6+). Figure 3c shows the distribution of genotypes in the follow-up. The fact that risk genotypes (16 and/or 18) are less frequent in follow-up than in HSIL and invasive cases (48.8%) can be seen.

Table 1
Epidemiological characteristics of all diagnosed cases in the cervical cancer screening programme, and separately intraepithelial (HSIL) and invasive

VARIABLES		ALL CIN2+ CASES		HSIL		INVASIVE	
Age (yr) (mean, standard deviation, minimum-maximun)		37,9±10,3 (20-85)		37,0±8,7 (20-66)		49,1±18,7 (23-85)	
Lesion size (mm) (mean, standard deviation, minimum-maximun)		3,5±3,1 (0,5 a 15,0)		3,4±3,0 (0,5 a 15,0)		7,0±2,6 (1,0 a 10,0)	
VARIABLES		**N**	**%**	**N**	**%**	**N**	**%**
Age (yr)	≤45	185	77,7	176	80,0	9	50,0
	>45	53	22,3	44	20,0	9	50,0
	Total	238	100	220	100	18	100
Nº of CIN2+ per year	1º	38	16,0	35	15,9	3	16,7
	2º	45	18,9	40	18,2	5	27,8
	3º	49	20,6	46	20,9	3	16,7
	4º	57	23,9	53	24,1	4	22,2
	5º	49	20,6	46	20,9	3	16,7
	Total	238	100	220	100	18	100
Country of origin	Spanish	162	71,4	151	71,9	11	64,7
	Foreign	65	28,6	59	28,1	6	35,3
	Total	227	100	210	100	17	100
Catchment	Screening diagnosis	102	45,0	54	46,3	7	43,7
	First testing diagnosis	43	18,9	35	16,6	8	50,0
	Follow-up for abnormal cytology	45	19,8	45	20,5		
	Follow-up for abnormal cytology and positive HPV	37	16,3	35	16,6	1	6,3
	Total	227	100	169	100	16	100
HPV genotypes	HPV 16 and/or 18	111	60,0	100	57,8	11	91,7
	HPV others no 16/18	74	40,0	73	42,2	1	8,3
	Total	185	100	173	100	12	100

HSIL: High-grade Intraepithelial Lesion. N: Number of cases. %: Percentage; HPV: Papilloma Virus; CIN: Cervical Intraepithelial Neoplasia (CIN 2/3/AIS)

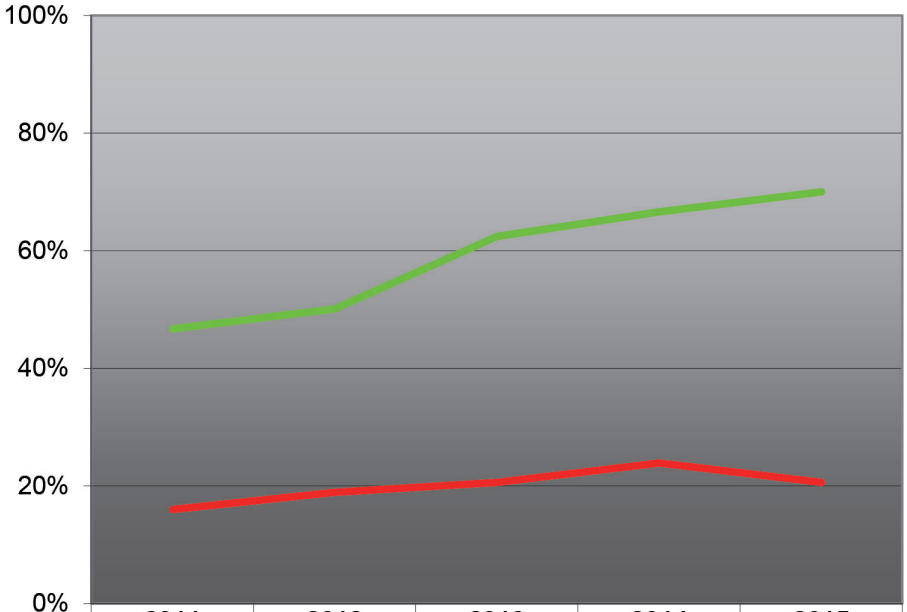
The bivariate analysis is shown in table 4. Patients over 45 years old, foreign or with a single involved quadrant are at greater risk of positive margins; all of them with statistical significance. No association is found among the horizontal extension of the lesion or genotype 16 and/or 18. However, genotype 16 and/or 18 do show a significant association when a single quadrant is involved in contrast to more than one. The average follow-up time since conization was 29.5 months (95% CI: 27.3-31.6).

Table 2
Conization and follow-up results, by HSIL and microinvasive

CONIZATION DATA (N= 220)		HSIL		MICROINVASIVE	
VARIABLES		N	%	N	%
Cone diagnosis	CIN1/cervicitis	29	13,6		
	CIN2	39	18,3	1[(a)]	14,3
	CIN3	143	67,2	2[(a)]	28,5
	AIS	2	0,9		
	Carcinoma microinvasor[(b)]			4	57,2
	Total	213	100	7	100
Margin cone status	positive	24	11,8	5	71,4
	undeterminate	9	4,4		
	negative	150	76,4	2[(c)]	28,6
	Positive but negative EC	15	7,4		
	Total	203	100	7	100
Quadrant involvement	One	91	52,0	3	42,9
	Two	51	29,1	1	14,3
	Three	8	4,6	1	14,3
	Four	25	14,3	2	28,5
	Total	175	100	7	100
Lesion size (mean, standard deviation, minimum-maximun confident interval 95%)		35±3,1 mm (<1-15) IC 95%: 2,8-3,8		6,8±1,2 mm (1-10) IC 95%: 3,6-10,0	
FOLLOW-UP DATA		HSIL		MICROINVASIVES	
Reinterventions		N	%	N	%
Reconization		7	28,0	1	14,3
HT/HTDA		18	72,0	6	85,7
Total		24	100	7	100
Cured patients, N= 192; negative HPV and cytology	Cured in first control (N=207)	166	80,2	3	60
	Cured in second control (N=171)	135	78,9	4	80
	Cured in third control (N=90)	80	88,9	4	100
Patients with residual disease in the end of follow-up (N=208)		14	6,7	0	0
Reinfections (N=208)		8	3,8	0	0

HSIL: High-grade IntraepithelialLlesion, CIN: Cervical Intraepithelial Neoplasia; AIS: Adenocarcinoma in situ; EC: Endocervical Curettage. HPV: Papilloma Virus; HT/HTDA: Hysterectomy or Hysterectomy with double adnexectomy; [(a)] Microinvasion was diagnosed at reintervention [(b)] Included 1Carcinoma adenosquamous microinvasive, 2 Microinvasive adenocarcinomas. The rest were squamous microinvasive carcinomas [(c)] Disease was detected at follow-up and reintervention was needed.

Figure 2
Evolution of program coverage and CIN2+ cases detected during the 5 year study (women aged 25 to 64)



	2011	2012	2013	2014	2015
Coverage	46,7%	50,1%	62,4%	66,6%	70,0%
%CIN2+	16,0%	18,9%	20,6%	23,9%	20,6%

Table 3
Invasive cases description, IB stage or more

Total patients		11
65 year old patients or more (outside the screening age)		4
VARIABLE		N (%)
Histologic type	Squamous	8 (72,7)
	Adenocarcinomas	3 (27,3)
Stage	IB	2 (18,2)
	II	4 (36,3)
	III	3 (27,3)
	IV	2 (18,2)
Treatment[a]	Surgery with radio and/or chemotherapy	3 (27,3)
	Radio and chemotherapy	6 (54,6)
Survival (months)	Mean and range of all	21,0 (1,1 a 41,4)
	Mean and range of deaths	9,8 (1,1 a 17,7)

[a]1 case wasn´t treated for age and another only underwent chemotherapy

Figure 3
Distribution of hrHPV genotypes in HSIL (3a), invasive (3b) and in the first follow-up control after conization (3c)

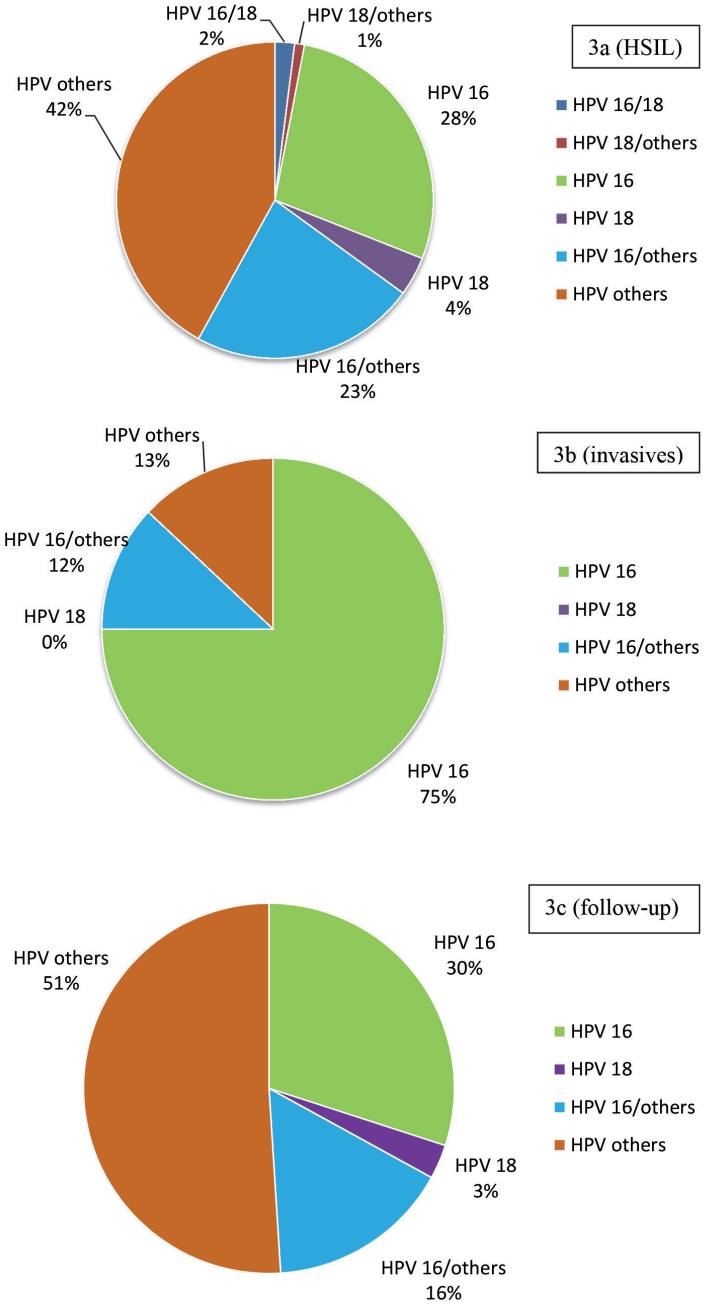


Tabla 4
Bivariate study margin status, HPV genotype and post-treatment status, according to demographic and pathological features, for all the cases

MARGIN STATUS						
VARIABLES		Positive		Negative		
		N	%	N	%	p
Grouped age (years)	≤45	25	61,0	140	81,4	**0,005**
	>45	16	39,0	32	18,6	
Country of origin	Spanish	22	55,0	115	72,8	**0,024**
	Foreign	18	45,0	43	27,2	
Histological diagnosis	CIN	33	80,5	170	98,8	**0,000**
	Invasor	8	19,5	2	1,2	
Quadrant	1	24	68,6	60	43,8	**0,009**
	>1	11	31,4	77	56,2	
Extension	<1mm	5	16,1	15	12,9	0,645
	≥1mm	26	83,9	101	87,1	
HPV Genotype	16 and/or 18	18	60,0	81	58,7	0,895
	No 16 and/or 18	12	40,0	57	41,1	
HPV GENOTYPE						
VARIABLES		Genotype 16/18		Genotype no 16/18		
		N	%	N	%	p
Quadrant	1	38	54,3	17	34,0	**0,028**
	>1	32	45,7	33	66,0	
CURE						
VARIABLES		Sicked		Cured		
		N	%	N	%	p
Grouped age (years)	≤45	19	76,0	135	81,3	0,537
	>45	6	24,0	31	18,7	
Country of origin	Spanish	15	62,5	115	71,9	0,494
	Foreign	9	37,5	45	28,1	
Histological diagnosis	CIN	24	96,0	159	95,8	0,960
	Invasive	1	4,0	7	4,2	
Quadrant	1	5	50,0	74	47,4	0,875
	>1	5	50,0	82	52,4	
Extension	<1mm	4	18,2	18	81,8	0,319
	≥1mm	10	10,5	85	89,5	
Margin status	positive	6	42,9	31	16,4	**0,013**
	negative	8	57,1	158	83,6	
HPV Genotype	16 and/or 18	12	66,7	75	58,6	0,513
	No 16 and/or 18	6	33,3	53	41,4	

CIN: Cervical Intraepithelial Neoplasia. HPV: Papilloma Virus.

DISCUSSION

Co-testing screening has detected small preinvasive lesions, in a single quadrant and microinvasive carcinomas. Conization is effective, achieving the cure of most of the patients.

Our series highlights the large number of HSIL with small lesions and microinvasive carcinomas. HSIL cases have increased considerably compared to the years prior to co-testing screening[13] and it is attributed especially to the greater sensitivity of the HPV testing compared to cytology[14]; also favored by the progressive coverage increase, given that increased diagnoses go side by side with coverage. About invasive carcinomas, a large percentage of them are detected in non-screened women, as found by other authors[15] and in a higher age range than in HSIL (4 patients were found outside the screening age range). The false negatives of cytology have been easily detected by the HPV testing, result similar to those obtained by Park[16] who detected 16.5% after a co-testing study (15.5% in our series). The false negatives of the HPV testing were detected by cytology and verified with the biopsy and the immunohistochemical technique of $p16^{INK4a}$ [17]. They are explained by rare HPV genotypes (low or undetermined risk)[18] or by the machines´ sensitivity threshold . This technique also helps in the detection of hidden or minimal CIN2+ and positive HPV testing[4], for CIN2 and CIN3 classification[19] and for margins evaluation particularly when erosion or artifact are found[20]. An accurrate diagnosis is very important because treatment can lead to later obstetric problems, so overtreatment should be avoided. CIN3 is for some the true high-grade intraepithelial neoplasia while a large part of the CIN2 regress[21]. However, CIN2 is the treatment threshold and our study has followed this classification[19], so that the results of CIN2 are shown separately to facilitate other comparisons.

In the study of the sensitivity of the tests, the purpose is to detect positive cases in healthy population and to find invasive HPV-negative carcinomas is less important. The test choice is important to evaluate the results and its application for future screening with a hrHPV DNA as primary screening testing[3] and 16 and 18 genotyping as an appropriate choice for positive cases triage[12]. The HPV testing change was due to an easier use of Cobas, genotyping 16/18 in one step and its approval by the FDA for co-testing in 2011. Although it was a limitation in the statistical study of genotyped cases, it allowed us to verify negative HPV cases with both tests. The sensitivity of the HPV testing was superior to that of cytology even with these negative HPV cases. Intraepithelial neoplasms are typically from young women and invasive cancer develops in the third decade and a later age[1]. Numerous studies have shown that HPV prevalence gradually decreases 2-8% in the population over 40 years of age; and persists in older than 50 showing a more indeterminate risk of cervical cancer in that group in most studies[1]. The average age of HSIL is about 12 years before than the invasive cases. The 9 cases of invasive carcinoma in patients under 45 years old found in our study refer mostly to microinvasive cases.

The finding that foreign patients have a higher risk of CIN2+, especially those of Romanian origin, is explained by the high incidence of cervical cancer in Romania (39.4 cases per 100,000 inhabitants per year)[22] and in other eastern countries of Europe[23]. The high incidence is attributed to the failure of the screening programs in these countries, both organized and opportunistic.

The conization with LLEP is the most widespread treatment and there are very few differences with the cases treated with cold knife[24]. The conization quality indicators would be the finding of CIN2+ in more than 85% of the cases treated and clear margins in more than 80% of the conizations[5,10,25]. It is not specified how the cones with ulcerated margins are classified, nor the affected

margin cones and free endocervical curettage. In our study, the results show data very close to the proposed indicator in Oncoguía from 2014 SEGO.

After a mean follow-up of 29.5 months the number of patients with residual disease after treatment is low, and it is within the range cited in the reference protocol that we apply[10] (average 15% and range between 5 and 25%). The association of age (over 45 years old) with positive margin that we find in our study is reflected in other publications. The association of age, tumor size and depth of the cone with positive margin has been published by Bae[26]. Tasci[27] associates it with the affectation of two or more quadrants (up to 80% margin involvement if more than two quadrants are affected). Güdücü[28] found association between positive margin and more than two thirds of the LEEP specimen involvement, and also with glandular endocervical involvement and multicentricity (in CIN3 specimens) but not with age.

We do not find an explanation for the "paradoxical" association between positive margin and a single quadrant involvement which may be due to the difficulty of locating small lesions. We have also found no explanation for the finding that the genotypes 16/18 are associated with less extension of the lesion (involvement of one quadrant versus more than one).

Whereas authors found an association between the genotype 16/18 and residual disease[29], our finding shows only a trend in our series without being significant, probably because of smaller sample size in follow-up. Kliemann[30] finds an association between the extension of the lesion and the positive margin. The sizes it refers to are 6.12 ± 3.25mm vs. 10.6 ± 4.45mm that clearly are greater than ours (3.4mm in HSIL and 6.8mm in the invasive). Pirtea[31] also finds an association between genotype 16 and age (greater than 36.5 years old) in the follow-up of patients conizised by HSIL.

The follow-up of patients with HPV testing, with or without cytology, is recommended for its sensitivity, specificity and accuracy of results[32]. It is also known that positive margin is associated with post-treatment virus persistence[10], as in our study. Residual disease despite clear margin can be attributed to lesion multifocality appearing in 23% of cases with clear margins.

The number of invasive cases has not decreased in recent years which is probably explained by the program´s increased coverage alongside the attendance of foreign patients from countries with ineffective screening programmes, who have contributed to the increase in invasive carcinomas (35.3 % of invasive cases are in woman from foreign countries).

The limitations of this study have neither included the findings of colposcopy, even being aware of its usefulness, nor data on vaccination. The use of two different techniques for the determination of HPV is another limitation of this study.

The study shows the applicability of the current recommendations and the effectiveness of the co-testing in diagnosis and follow-up. In addition, a round of co-testing, monitored and supported in Primary Care, serves as a reference for applying future protocols based on primary testing with hrHPV. The increase in sensitivity has resulted in an increase in conizations with small lesions and microinvasive carcinomas and has achieved the cure of most of the patients, which is the purpose of screening.

The implementation of the hrHPV DNA test in the Health Sector of Barbastro, has meant an increase in the number of HSIL detected and invasive of which a 50% are in incipient stage; attributable to the greater sensitivity of the HPV testing.

Genotype 16 is mainly responsible for invasive carcinomas and most pre-invasive carcinomas.

ACKNOWLEDGMENTS

The authors thank Diego Ablanedo for his valuable contribution in English translation.

REFERENCES

1. Bosch FX, Lorincz A, Muñoz N, Meijer CJ, Shah KV. The causal relation between human papillomavirus and cervical cancer. J Clin Pathol. 2002;55:244-265.

2. Cortés J, Martinón-Torres F, Ramón y Cajal JM, Gil A, Velasco J, Abizanda M, et al. Prevención primaria y secundaria de los cánceres de cuello de útero y vulva: recomendaciones para la práctica clínica. Prog Obstet Ginecol 2010;53 (supl 1):1-19.

3. Jeronimo J, Castle PE, Temin S, Shastri SS. Secondary Prevention of Cervical Cancer: American Society of Clinical Oncology Resource-Stratified Clinical Practice Guideline Summary. Published online ahead of print October 12, 2016. J Glob Oncol. doi:10.1200/JGO.2016.006577.

4. Darragh TM, Colgan TJ, Thomas Cox J, Heller DS, Henry MR, Luff RD, et al; Members of the LAST Project Work Groups. The Lower Anogenital Squamous Terminology Standardization project for HPV-associated lesions: background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. Int J Gynecol Pathol. 2013;32:76-115.

5. Puig-Tintoré LM, Cortés J, Castellsague X, Torné A, Ordi J, de Sanjosé S et al. Prevención del cáncer de cuello uterino ante la vacunación frente al virus del papiloma humano. Prog Obstet Ginecol. 2006;49 (Extraordinario 2).

6. Solomon D, Davey D, Kurman R, Moriarty A, O'Connor D, Prey M, et al. The 2001 Bethesda System: terminology for reporting results of cervical cytology. JAMA. 2002;287:2114-2119.

7. Nayar R, Wilbur DC. The Pap Test and Bethesda 2014. Acta Cytologica. 2015;59:121-132.

8. Oncíns Torres R, Aragón Sanz MA, Comes García MD, Vallés Gallego V, Cortés Ramas A. Evaluación de un nuevo protocolo de cribado de cáncer cervical con citología convencional y test del virus del papiloma humano. Prog Obstet Ginecol. 2014;57:14-19.

9. Comes MD, Oncins R, Clemente E, Aragón MA, Cortés A, Vallés V, et al. Prevalence of human papillomavirus and genotype distribution in women undergoing cervical cancer screening in the area of Barbastro, Spain. Rev Esp Patol. 2016;49:208-13.

10. Oncoguía SEGO: Prevención del cáncer de cuello de útero. Guías de práctica clínica en cáncer ginecológico y mamario. Publicaciones SEGO, Octubre 2014.

11. Queiro Verdes T, Puñal Riobóo J. Desarrollo de actividades de la Red Española de Agencias de Evaluación de Tecnologías y Prestaciones del SNS. Axencia de Avaliación de Tecnoloxías Sanitarias de Galicia; 2013. Informes de evaluación de tecnologías sanitarias: avalia-t Núm. 2013/01. Métodos automatizados de lectura de citología cervical uterina. http://www.sergas.es/Docs/Avalia-t/avalia-t201301Lecturaautomatizada.pdf.

12. Luttmer R, Berkhof J, Dijkstra MG, Kemenade FJ, Snijders PJF, Heideman DAM, et al. Comparing triage algorithms using HPV DNA genotyping, HPV E7mRNA detection and cytology in high-risk HPV DNA-positive women. J Clin Virol. 2015;67:59-66.

13. Aragón Sanz MÁ, Vallés Gállego V, Clemente Roldán E, Oncíns Torres R, Comes García MD, González Ballano I, et al. Estrategias para la implantación del cribado poblacional de cáncer de cuello uterino con test del virus del papiloma humano. Prog Obstet Ginecol. 2016;59(6):377-382.

14. Cuzick J, Clavel C, Petry K-U, Meijer C, Hoyer H, Ratnam S, et al. Overview of the European and North American studies on HPV testing in primary cervical cancer screening. Int J Cancer. 2006;119:1095–1101.

15. Ibáñez R, Alejo M, Combalia N, Tarroch X, Autonell J, Codina L, et al. Underscreened Women Remain Overrepresented in the Pool of Cervical Cancer Cases in Spain: A Need to Rethink the Screening Interventions. Biomed Res Int. 2015;2015:605375. doi: 10.1155/2015/605375. pmid:26180804.

16. Park IU, Wojtal N, Silverberg MJ, Bauer HM, Hurley LB, Manos MM (2015). Cytology and Human Papillomavirus Co-Test Results Preceding Incident High-Grade Cervical Intraepithelial Neoplasia. PLoS ONE. 10(3): e0118938. doi:10.1371/ journal.pone.0118938.

17. Ordi J, García S, del Pino M, Landolfi S, Alonso I, Quintó L, et al. p16 INK4a immunostaining identifies occult CIN lesions in HPV-positive women. Int J Gynecol Pathol. 2009;28:90-97.

18. Petry KU, Cox JT, Johnson K, Quint W, Ridder R, Sideri M, et al. Evaluating HPV negative CIN2+ in the ATHENA trial. International Journal of Cancer 2016;138:2932-2939.

19. Reuschenbach M, Wentzensen N, Dijkstra MG, von Knebel Doeberitz M, Arbyn M. p16INK4a immunohistochemistry in cervical biopsy specimens: a systematic review and meta-analysis of the interobserver agreement. Am J Clin Pathol. 2014;142:767-72.

20. Kim TH, Han JH, Shin E, Noh JH, Kim HS, Song YS. Clinical Implication of p16, Ki-67, and Proliferating Cell Nuclear Antigen Expression in Cervical Neoplasia: Improvement of Diagnostic Accuracy for High-grade Squamous Intraepithelial Lesion and Prediction of Resection Margin Involvement on Conization Specimen. J Cancer Prev. 2015;20:70-7.

21. Castle PE, Schiffman M, Wheeler CM, Solomon D. Evidence for frequent regression of cervical intraepithelial neoplasia–grade 2. Obstet Gynecol. 2009;113:18-25.

22. Bruni L, Barrionuevo-Rosas L, Albero G, Aldea M, Serrano B, Valencia S, et al. ICO Information Centre on HPV and Cancer (HPV Information Centre). Human Papillomavirus and Related Diseases in Romania. Summary Report 2016-02-26.

23. Arbyn M, Antoine J, Magi M, Smailyte G, Stengrevics A, Suteu O, et al. Trends in cervical cancer incidence and mortality in the Baltic countries, Bulgaria and Romania. Int J Cancer. 2011;128:1899–1907.

24. Santesso N, Mustafa RA, Wiercioch W, Kehar R, Gandhi S, Chen Y, et al. Systematic reviews and meta-analyses of benefits and harms of cryotherapy, LEEP, and cold knife conization to treat cervical intraepithelial neoplasia. Int J Gynecol Obstet. 2015;132:266-71.

25. Moss EL, Arbyn M, Dollery E, Leeson S, Petry KU, Nieminen P, et al. European Federation of Colposcopy quality standards Delphi consultation. Eur J Obstet Gynecol Reprod Biol. 2013;170:255-8.

26. Bae HS, Chung YW, Kim T, Lee KW, Song JY. The appropriate cone depth to avoid endocervical margin involvement is dependent on age and disease severity. Acta Obstet Gynecol Scand. 2013;92:185-92.

27. Tasci T, Turan T, Ureyen I, Karalok A, Kalyoncu R, Boran N, et al. Is there any predictor for residual disease after cervical conization with positive surgical margins for HSIL or microinvasive cervical cancer? J Low Genit Tract Dis. 2015;19:115-8.

28. Güdücü N, Sidar G, Başsüllü N, Türkmen I, Dünder I. Endocervical glandular involvement, multicentricity, and extent of the disease are features of high-grade cervical intraepithelial neoplasia. Ann Diagn Pathol. 2013;17(4):345-6.

29. Kang WD, Ju UC, Kim SM. A human papillomavirus (HPV)-16 or HPV-18 genotype is a reliable predictor of residual disease in a subsequent hysterectomy following a loop electrosurgical excision procedure for cervical intraepithelial neoplasia 3. J Gynecol Oncol. 2016;27:e2. doi: 10.3802/jgo.2016.27.e2.

30. Kliemann LM, Silva M, Reinheimer M, Rivoire WA, Capp E, Dos Reis R. Minimal cold knife conization height for high-grade cervical squamous intraepithelial lesion treatment. Eur J Obstet Gynecol Reprod Biol. 2012;165:342-6.

31. Pirtea L, Grigoraş D, Matusz P, Pirtea M, Moleriu L, Tudor A, et al. Age and HPV type as risk factors for HPV persistence after loop excision in patients with high grade cervical lesions: an observational study. BMC Surgery. 2016;16:70.

32. Mariani L, Sandri MT, Preti M, Origoni M, Costa S, Cristoforoni P, et al. HPV-Testing in Follow-up of Patients Treated for CIN2+ Lesions. J Cancer. 2016; 7(1):107-114. doi: 10.7150/jca.13503.