Int J Clin Exp Pathol 2016;9(8):8611-8616
www.ijcep.com /ISSN:1936-2625/IJCEP0031788

Original Article

Management of women aged 25-34 with diagnosis of ASCUS in the screening center of Latina

Caterina Chiappetta[1], Chiara Puggioni[1], Eugenio Lendaro[1], Jessica Cacciotti[1], Mirko Cirenza[1], Roberto Zaralli[1], Giovanna Migliore[1], Paola Bellardini[2], Vincenzo Petrozza[1], Carlo Della Rocca[1], Claudio Di Cristofano[1]

[1]UOC of Pathology, Department of Medical-Surgical Sciences and Bio-Technologies, Sapienza University of Rome, Polo Pontino, I.C.O.T, Latina, Italy; [2]Screening Unit, Local Health Unit of Latina, Italy

Received May 6, 2016; accepted June 22, 2016; Epub August 1, 2016; Published August 15, 2016

Abstract: In cervical cancer screening program of Latina (Italy) the hr-HPV as primary test is performed only on women aged 35-64 while women aged 25-34 are invited to perform PapTest. The aim of this study was to evaluate the impact of the application of the PapTest in women aged 25-34 and to evaluate the management of ASCUS. Women aged 25-34 were invited to perform PapTest according to the Italian guidelines; women with diagnosis of LSIL+ were referred to colposcopy while women with diagnosis of ASCUS were referred to hr-HPV test and only women resulted positive were referred to colposcopy. The 4.0% of women resulted positive to PapTest and the referral rate to colposcopy was 3.5%. The PPV value for CIN2+ at colposcopy was 7.2% and the Detection Rate (DR) for CIN2+ was 2.40‰. The ASCUS category was diagnosed in 41.8% of women resulted positive to PapTest and between them the 70.6% resulted positive to the hr-HPV test. The referral rate to colposcopy of women resulted positive to hr-HPV test was 1.1%. The PPV for CIN2+ at colposcopy and the DR of CIN2+ was 8.4% and 0.96‰ respectively. Between women with diagnosis of ASCUS, only 6 women showed a CIN2+ lesion (4 CIN2 and 2 CIN3). The present study showed that this algorithm, applied to women aged 25-34, obtained a good performance in term of test specificity (98%) and confirm that the application of hr-HPV test in the management of ASCUS leads to a decreased of inappropriate colposcopy due to transitory infection in young women.

Keywords: Cervical cancer, screening, PapTest, ASCUS

Introduction

The necessary condition so that cervical cancer can be developed is the persistent infection with an oncogenic human papillomavirus (HPV) type described as high-risk-HPV (hr-HPV) [1]. The hr-HPV test is a screening test to define the presence of an hr-HPV type in a sample of exfoliated cervical cells [2, 3]. This is used in the Italian screening programme as a preventive medicine intervention aimed at early diagnosis and improvement of prognosis of cervical cancer [4]. The rationale of hr-HPV test as a primary test with cytological triage is to perform before the more sensitive test and then the more specific test, in order to anticipate the diagnosis and to not increase the over-diagnosis of intraepithelial lesions [5-7]. It's well established that there is a high prevalence of HPV infection in young women, when they became sexually active, that tends to decrease with increasing of age [8] and in most cases, the infection caused by oncogenic HPV subtype, regresses spontaneously [9]; moreover, excisional treatment of cervical lesions is associated with increased risk of pregnancy-related morbidity and mortality [10].

Effectively, the NTCC (New Technologies for Cervical Cancer) study showed that the use of hr-HPV test applied to young women (aged 25-34) resulted in over-diagnosis and in increase of unnecessary treatment of regressive CIN2+ lesions (cervical intraepithelial neoplasia) [5, 6]. Therefore, the GISCi (Italian Association of Cervical Screening Programs) guidelines recommended to not carry out hr-HPV test before of 35 years of age and so to perform PapTest to women aged 25-34 [4]. Successively, the women (aged 25-34) with diagnosis of Low-grade Squamous Intraepithelial Lesion or worse (LSIL+) are referred to col-

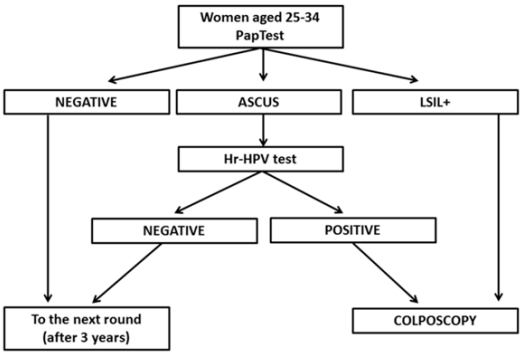

Figure 1. Algorithm of the cervical cancer screening program of women aged 25-34 in Latina district.

poscopy while women with diagnosis of ASCUS (Atypical Squamous Cells of Undetermined Significance) are referred to hr-HPV test and only women resulted positive to the molecular test were referred to colposcopy, otherwise women are recalled after three years as well as women with a negative PapTest (**Figure 1**). The triage of ASCUS with the hr-HPV test arises with the aim to discriminate between women with cytological abnormalities that are precursor of a lesion and benign alterations [11].

We aim to evaluate the outcome and impact of the application of the PapTest as a primary test and in particularly to evaluate the management of ASCUS in women aged 25-34 using data of 3 years (2012-2014) of the screening centre of Latina (Italy).

Materials and methods

Study population

Study populations have been enrolled by the Pathology Unit of ICOT Hospital, Department of Medical-Surgical Sciences and Bio-echnologies, Sapienza University of Rome and Screening Unit of Local Health Unit of Latina. From 2012 in the Latina district it was organized a new cervical screening; this program considers that women aged 25-34 are invited by mail to perform a PapTest (**Figure 1**) while women aged 35-64 are invited by mail to perform hr-HPV test according to GISCi algorithm [4, 12].

In this study we evaluate women aged 25-34 that were invited to perform a PapTest from April 2012 to December 2014. Cytological and histological diagnosis were reported according to 2001 Bethesda System [13] evaluated by one cytologist and two pathologists. Women with diagnosis of ASCUS were referred to hr-HPV test. The women resulted negative to hr-HPV test were recalled after three years as well as women resulted negative to PapTest. Indeed, women with diagnosis of LSIL+ or that resulted positive to hr-HPV test were referred to colposcopy.

The colposcopy was performed by two gynaecologists of the screening unit while colposcopy biopsies were read by two pathologist and women with diagnosis of CIN2 or more severe (CIN2+) were referred to excisional treatment.

Cytology: PapTest

The cervical cell samples were obtained by using a cytobrush and were put in PreservCyt solution; liquid-based cytology was performed by using the Sure Path system (BD SurePathTM, Franklin Lakes, NJ, USA). One slide per woman was prepared according to the supplier's instructions.

Hybrid capture 2 high-risk HPV DNA test (HC2)

Exfoliated cervical cells were collected using a cytobrush and eluted in the Sample Transport Medium (STM, Qiagen, Hilden, DE). Cervical specimens were denatured to disrupt the virus and release the target DNA. The RNA probes were diluted in a probe diluent and once loaded all the samples, calibrators, controls and reagents, the hybridization phase began according to supplier's instructions. The chemiluminescent reaction was measured by luminometer (DML instrument, Qiagen, Hilden, DE) and the emitted light was measured as RLU.

For each reaction were used three negative controls, three positive controls, one quality control for low-risk HPV (lr-HPV) and one quality control for hr-HPV. Samples that showed a RLU ≥ 1 pg/ml were considered positive.

Results

In the period of reference (April 2012 - December 2014) were enrolled 15,0517 women aged 25-64 and 44,415 (29.5%) were screened. Between them, 41,092 (27.3%) aged 25-34 and 6,249 (15.2%) were screened.

Between the women aged 25-34 the 4.0% (251/6249) resulted positive to PapTest (ASCUS or worse: ASCUS+) and the most fre-

Table 1. Results of the screening test performance of the HPV screening program of women aged 25-34

	2012	%	‰	2013	%	‰	2014	%	‰	Total	%	‰
Women Invitedaged 25-34	11484			14013			15595			41092		
Women Examinated	299/11484	2.6		2831/14013	20.2		3119/15595	20		6249/41092	15.2	
Proportion of positive PapTest	22/299	7.4		121/2831	4.3		108/3119	3.5		251/6249	4.0	
ASCUS	12/22	54.5		49/121	40.5		44/108	40.7		105/251	41.8	
LSIL	8/22	36.4		65/49	53.7		57/44	52.8		130/251	51.8	
ASCH	0/22	0		2/49	1.7		2/44	1.9		4/251	1.6	
HSIL	2/22	9.1		4/49	3.3		5/44	4.6		11/251	4.4	
Referral rate to colposcopy	19/299	6.4		112/2831	4.0		87/3119	2.8		218/6449	3.5	
Compliance with referral to colposcopy	18/19	94.7		106/112	94.6		85/87	97.7		209/218	95.9	
PPV for CIN2+	1/18	5.6		8/106	7.6		6/85	7.0		15/209	7.2	
Detection rate for CIN2+	1/299		3.34	8/2831		2.83	6/3119		1.92	15/6249		2.40

Table 3. Results of the screening test performance of the HPV screening program of women aged 25-34 with diagnosis of ASCUS

	2012	%	‰	2013	%	‰	2014	%	‰	Total	%	‰
Women Examinatedaged 25-34	299/11484	2.6		2831/14013	20.2		3119/15595	20		6249/41092	15.2	
Proportion of ASCUS	12/22	54.5		49/121	40.5		44/108	40.7		105/251	41.8	
Proportion of positive hr-HPV test	9/11	81.8		40/49	81.6		23/42	54.8		72/102	70.6	
Referral rate to colposcopy	9/299	3.0		40/2831	1.4		23/3119	0.7		72/6249	1.1	
Compliance with referral to colposcopy	9/9	100		39/40	97.5		23/23	100		71/72	98.6	
PPV for CIN2+	1/9	11.1		2/39	5.1		3/23	13.0		6/71	8.4	
Detection rate for CIN2+	1/299		3.34	2/2831		0.71	3/3119		0.96	6/6249		0.96

Table 2. Results of histological diagnosis

Histological diagnosis								
	2012	%	2013	%	2014	%	Total	%
CIN1	12/14	85.7	56/69	81.2	42/51	82.3	110/134	82.1
CIN2	1/14	7.1	4/69	5.8	2/51	3.9	7/13	5.2
CIN3	0/14	0	4/69	5.8	4/51	7.4	8/134	6.0

quent diagnostic category was LSIL (51.8%, 130/251) followed by ASCUS (41.8%, 105/251), HSIL (High grade Squamous Intraepithelial lesion, 4.4%, 11/251) and ASCH (Atypical Squamous Cell, High-grade not excluded, 1.6%, 4/251). The PapTest positive rate decreased from 2012 (7.4%) to 2014 (3.5%) and the frequency of diagnostic category was similar in 2013 and 2014; indeed, the most frequent diagnostic category was LSIL in 2013 (53.7%, 65/121) and 2014 (52.8%, 57/108) while was ASCUS in 2012 (54.5%, 12/22). The referral rate to colposcopy was 3.5% with a decrease during the three years (6.4%, 4% and 2.8%). During these three years the adhesion to colposcopy was 95.9% (**Table 1**). The positive predictive value (PPV) for CIN2+ at colposcopy was similar during 2013 and 2014 (7.6% and 7.0%) while during 2012 this value was lower (5.6%); however in average, the PPV value for CIN2+ at colposcopy was 7.2%. The detection rate (DR) for CIN2+ was 2.40‰ with a decrease from 2012 to 2014 (3.34‰, 2.83‰ and 1.92‰ respectively, **Table 1**). The most frequent histlogical category was CIN1 (82.1%) followed by CIN3 (6.0%) and CIN2 (5.2%) and even taking into account year by year the most frequent category was CIN1 (**Table 2**). The referral rate to next round (after three years) was 96.5% with a decrease during the first three years. The specificity value of this algorithm in women aged 25-64 was 98.2% with the same value during the first three years.

The ASCUS category was diagnosed in 41.8% (105/251) of women resulted positive to PapTest; this value was similar in 2013 and 2014 (40.5% and 40.7%) while was higher in 2012 (54.5%). The hr-HPV positive rate among women with diagnosis of ASCUS was 70.6% (72/102) and this rate was similar in the first two years (81.8% and 81.6%) while was lower in 2014 (54.8%). The referral rate to colposcopy of women resulted positive to hr-HPV test was 1.1% with a decrease during the three years (3.0%, 1.4% and 0.7%). There was a maximum adhesion to colposcopy in 2012 and 2014 while in 2013 the adhesion to colposcopy was 97.5% with an average of 98.6%.

The ASCUS PPV for CIN2+ at colposcopy was 8.4% and we observed different value among these period: 11.1%, 5.1% and 13% in 2012, 2013 and 2014 respectively. The ASCUS DR of CIN2+ relative to the total was 0.96‰; this value was similar in 2013 and 2014 (0.71‰ and 0.96‰) while was higher in 2012 (3.34‰, **Table 3**). Only a small number of CIN2+ lesions were diagnosed during this period: 4 CIN2 and 2 CIN3 lesions between women with diagnosis of ASCUS.

Discussion

Most HPV infections regress spontaneously and only a minority become persistent and progress in cancer lesion [9]. It has been showed that the molecular hr-HPV test is more sensitive than PapTest in identifying CIN2+ lesions but less specific; so, the application of this test to women aged 25-34 resulted in over-diagnosis and in increase of unnecessary treatment of regressive CIN2+ lesions [5-7]. In this study we applied the Italian guideline in the screening of cervical cancer [4] and we performed only PapTest to women aged 25-34 and performed the hr-HPV test for the triage of ASCUS category. The acceptance rate to perform PapTest was lower than the acceptance rate to perform hr-HPV test between women aged 35-64 while the adhesion to colposcopy value was equal to women aged 35-64 [12].

The PapTest positive rate (ASCUS+, 4.0%) was equal to the value observed in NTCC study performed on women of the same age (4.1%) [5, 6]. In our study we observed that the most frequently diagnosed category was LSIL (2.1%) unlike NTCC study [5, 6] where the most frequently represented category was ASCUS with a positive rate of 2.0%, value that is anyway below the limits set by the GISCi guidelines (<5%) [4]. Indeed, the ASCUS category includes women that are a low risk of developing a CIN2+ lesion and it doesn't describe a real diagnostic entity but rather includes morphological changes that are suggestive of squa-

mous intraephitelial lesion of low/indeterminate grade and that be confused with alterations of reactive, inflammatory and metaplastic nature [14] and for these reasons its interpretations is highly variable across the pathologists [15].

In our study the triage of ASCUS category, through the hr-HPV test, showed that the 71% of women with diagnosis of ASCUS presented were infected with the hr-HPV and this value was higher than NTCC study that include ASCUS+ category (60.6%) [16].

Indeed, considering only the ASCUS category, the referral rate to colposcopy (1.1%) was lower than the referral rate to colposcopy referred to the total population of young women (3.5%) and this value is consistent with NTCC study (4%) [5, 6].

The predictive positive value (PPV) for CIN2+ of women aged 25-34 was 7.2% and this value is lower than NTCC study (9%) [5, 6] while the PPV referred only to the ASCUS category was 8.4%. The DR of CIN2+ referred to the total population (2.4‰) was equal to the NTCC study [5, 6] while the ASCUS DR of CIN2+ was lower (0.96%).

Finally, the present study showed that this algorithm, applied to women aged 25-34, obtained a good performance in term of test specificity (98%). Moreover, this study confirm that the employment of hr-HPV test in the management of ASCUS can help the operators to confirm a cytological abnormalities caused by an HPV infection and to avoid unnecessary colposcopy in women aged 25-34.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Carlo Della Rocca, UOC of Pathology, Department of Medical-Surgical Sciences and Bio-Technologies, Sapienza University of Rome, Corso della Repubblica 79, Polo Pontino, I.C.O.T, Latina, Italy. E-mail: carlo.dellarocca@uniroma1.it

References

[1] Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer CJ and Muñoz N. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999; 189: 12-19.

[2] Meijer CJ, Berkhof J, Castle PE, Hesselink AT, Franco EL, Ronco G, Arbyn M, Bosch FX, Cuzick J, Dillner J, Heideman DA and Snijders PJ. Guidelines for Human papillomavirus DNA test requirements for primary cervical cancer screening in women 30 years and older. Int J Cancer 2009; 124: 516-520.

[3] Castle PE, Solomon D, Wheeler CM, Gravitt PE, Wacholder S and Shiffman M. Human papillomavirus genotype specificity of hyibrid capture 2. J Clin Microbiol 2008; 46: 2595-2604.

[4] Gruppo Italiano Screening Citologico (GISCi). Raccomandazioni sul test hr-HPV come test di screening primario e rivisitazione del ruolo del pap-test. Belluno: Evidenzia; 2010. pp. 19 (Italian). [www.gisci.it/documenti/documenti_gisci/documento_hpv.pdf].

[5] Ronco G, Giorgi-Rossi P, Carozzi F, Confortini M, Dalla Palma P, Del Mistro A, Gillio-Tos A, Minucci D, Naldoni C, Rizzolo R, Schincaglia P, Volante R, Zappa M, Zorzi M, Cuzick J and Segnan N; New Technologies for Cervical Cancer Screening Working Group. Results at recruitment from a randomized controlled trial comparing human papillomavirus testing alone with conventional cytology as the primary cervical cancer screening test. J Nat Cancer Inst 2008; 100: 492-501.

[6] Gillio-Tos A, De Marco L, Carozzi FM, Del Mistro A, Girlando S, Burroni E, Frayle-Salamanca H, Giorgi Rossi P, Pierotti P and Ronco G; New Technologies for Cervical Cancer Screening Working Group. Clinical impact of the analytical specificity of the hybrid capture 2 test: data from the New Technologies for Cervical Cancer (NTCC) study. J Clin Microbiol 2013; 51: 2901-2907.

[7] Giorgi-Rossi P, Franceschi S and Ronco G. HPV prevalence and accuracy of HPV testing to detect high-grade cervical intraepithelial neoplasia. Int J Cancer 2012; 130: 1387-1394.

[8] Baussano I, Franceschi S, Gillio-Tos A, Carozzi F, Confortini M, Dalla Palma P, De Lillo M, Del Mistro A, De Marco L, Naldoni C, Pierotti P, Schincaglia P, Segnan N, Zorzi M, Giorgi-Rossi P and Ronco G. Difference in overall and age-specific prevalence of high-risk human papillomavirus infection in Italy: evidence from NTCC trial. BMC Infect Dis 2013; 13: 238.

[9] Ho GY, Bierman R, Beardsley L, Chang CJ and Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. N Engl J Med 1998; 338: 423-428.

[10] Berretta R, Gizzo S, Dall'Asta A, Mazzone E, Monica M, Franchi L, Peri F, Patrelli TS and Bacchi Modena A. Risk of Preterm Delivery Associated with Prior Treatment of Cervical

Precancerous Lesion according to the Depth of the Cone. Dis Markers 2013; 35: 721-726.

[11] Massad LS, Einstein MH, Huh WK, Katki HA, Kinney WK, Schiffman M, Solomon D, Wentzensen N and Lawson HW; 2012 ASCCP Consensus Guidelines Conference. 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. Obstet Gynecol 2013; 121: 829-846.

[12] Chiappetta C, Lendaro E, Cacciotti J, Zaralli R, Puggioni C, Migliore G, Bellardini P, Porta N, Petrozza V, Della Rocca C and Di Cristofano C. Primary HPV test screening in cervical cancer: a two-year experience of a single screening center in Latina (Italy). Eur J Gynaecol Oncol 2015; 36: 569-573.

[13] Solomon D, Davey D, Kurman R, Moriarty A, O'Connor D, Prey M, Raab S, Sherman M, Wilbur D, Wright T Jr and Young N; Forum Group Members; Bethesda 2001 Workshop. The 2001 Bethesda System: terminology for reporting results of cervical cytology. JAMA 2002; 287: 2114.

[14] Caprara L, Monari F, Sassoli De Bianchi P, Amadori A and Bondi A. ASCUS in screening. Pathologica 2001; 93: 645-650.

[15] Ko V, Nanji S, Tambouret RH and Wilbur DC. Testing for HPV as an objective measure for quality assurance in gynecologic cytology: positive rates in equivocal and abnormal specimens and comparison with the ASCUS to SIL ratio. Cancer 2007; 111: 67-73.

[16] Giorgi Rossi P, Carozzi F, Collina G, Confortini M, Dalla Palma P, De Lillo M, Del Mistro A, Ghiringhello B, Gillio-Tos A, Maioli P, Pellegrini A, Schiboni ML, Segnan N, Zaffina L, Zorzi M, Ronco G; The NTCC Working Group. HPV Testing Is an Efficient Management Choice for Women With Inadequate Liquid-Based Cytology in Cervical Cancer Screening. Am J Clin Pathol 2012; 138: 65-71.