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Primary HPV test screening in cervical cancer: a two-year experience of a single screening center in Latina (Italy)

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Summary

Objective: The aim of this study was to evaluate the effect and performance of the new algorithm in cervical cancer screening program in two years' experience of Latina (Italy). *Materials and Methods:* The female population was divided into two groups, the first group was referred to PAP test and the second one to hr-HPV test according to national guidelines. *Results:* In two years the participation mean rate increased among women aged 35-64 compared to women aged 25-34. The primary PAP test positive rate and hr-HPV test positive rate were 4.0% and 5.2%, respectively. The PAP test positive rate among hr-HPV+ women decreased from 2012 to 2013. Women with hr-HPV+/PAP+ were referred immediately to colposcopy and this rate was 1.2%. The predictive positive value for CIN2+ to colposcopy was 10.9% in 2012 and 9.1% in 2013, while the detection rate for CIN2+ was 1.6% in 2012 and 1.4% in 2013. *Conclusion:* The stratification of the female population leads to a decreased inappropriate therapeutic path while the combination of hr-HPV test with PAP test in woman aged 35-64 lets obtain high levels specificity and sensitivity results.

Key words: Cervical cancer screening; HPV; HC2.

Introduction

Cervical cancer is the second most common cancer in the female population and in Italy it is estimated to affect about 3,500 women/year [1]. The program cervical screening is a health intervention made on an asymptomatic population; it has the purpose of early diagnosis of cervical intraepithelial neoplasia (CIN) [2] before the appearance of symptoms by identifying the disease in the earlier stage of its natural history [1, 3]. The early diagnosis also allows to take appropriate therapeutic treatment promoting healing and reducing cervical cancer mortality [4-7]. Human papillomavirus (HPV) is a key factor, although not sufficient, for the cervical cancer development [8].

The International Agency for Research on Cancer has confirmed the oncogenic potential of 12 HPV types (16, 18, 31, 33, 34, 39, 45, 51, 52, 56, 59, and 59) in the development of cervical cancer, defining these as high-risk HPV (hr-HPV) [9].

The cytology cervical smear (PAP test) has always been considered the first level of investigation in cervical cancer screening. However, it was shown that the molecular hr-HPV test is more sensitive than the PAP test in identifying ≥ CIN2 lesions [10-11]. Hence, the use of molecular methods for the detection of hr-HPV cervical infections, such as primary test in cervical cancer screening programs, has recently been suggested t [3, 12, 13]. In these programs hr-HPV test is followed by PAP test only in women with hr-HPV positive test before referring them to colposcopy; this procedure increases the specificity of molecular testing [14-19]. Hence, addressing immediately to colposcopy, all positive hr-HPV women may be the cause of an indiscriminate increase of this procedures and, for this reason, women with positive hr-HPV test are referred to the PAP test [20]. The New Technologies for Cervical Cancer (NTCC) Italian study, carried out to evaluate the performance of the hr-HPV test and showed similar results to those of other cited studies [17, 18]. Moreover, the NTTC study has shown that hr-HPV test does not increase the over-diagnosis of CIN2 lesions compared with cytology in women over 35 years of age, while the over-diagnosis of CIN2 lesions increases in younger woman (< 35 years); therefore this study recommends to carry out the hr-HPV screening not before 35 years of age [10]. Based on these evidences, the National Center for the prevention and Control of diseases and the Health Ministry has taken into account the possibility to change the guidelines for the HPV screening. The aim of this descriptive study is to evaluate the effect and the quality of the application of the new guidelines on hr-HPV test screening using a two-year experience (2012-13) of a single screening center in Latina (Italy).

Materials and Methods

Study population

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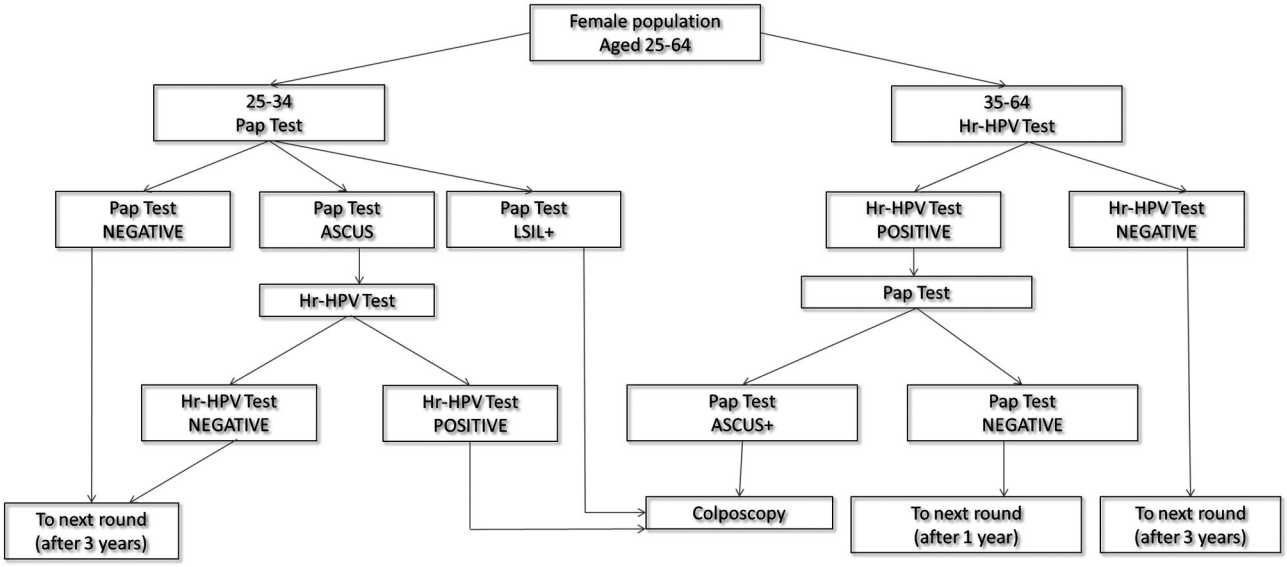

Figure 1. — Algorithm of the cervical cancer screening program in Latina district.

Rome and Screening Unit of Local Health Unit of Latina, have been running a new organized cervical-screening in the Latina district since 2012. The screening program plans to invite each year, about 30% of entire female population, aged 25-64, resident in the Latina district, with the aim of covering the whole population in three years (three years around time). Women aged 25-34 are invited by mail to perform a PAP test while women aged 35-64 are invited by mail to perform a hr-HPV test according to the screening algorithm of the Italian Association of Cervical Screening Programs (GISCi) guidelines [3] during 2012-2013. A double-sampling for PAP test and hr-HPV test was performed on all participating women except for women aged 25-34 who underwent only to PAP test. The screening algorithm is described in Figure 1. The women with a negative PAP test or hr-HPV test were advised to repeat the test after three years. The women positive to hr-HPV test were referred to PAP test; diagnosis were reported according to 2001 Bethesda System [2] evaluated by one cytologist and two pathologists. The colposcopy was performed by two gynecologists of the screening unit. Colposcopic biopsies were read by two pathologists and women with diagnosis of CIN2 or more severe were referred to excisional treatment. The authors present the preliminary data of program screening in the first two years (2012-2013).

Cytology

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. One slide per woman was prepared according to the supplier's instructions.

Hybrid capture test 2 (HC2)

Exfoliated cervical cells were collected using a cytobrush and eluted in the Sample Transport Medium. First of all, 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 and the emitted light was measured as RLU. For each reaction were used three negative controls, three positive controls, one quality control for lr-HPV and one quality control for hr-HPV. Samples that showed a RLU ≥ one pg/ml were considered positive.

Results

In 2012 the hr-HPV test was introduced as primary test for cervical cancer screening program. In 2012 there were enrolled 22,862 women (11,484 aged 25-34 and 11,378 aged 35-64), while in 2013 there were enrolled 62,923 women (14,013 aged 25-34 and 48,910 aged 35-64); the screening program involved in 2012 only Latina City and in 2013 the entire district too, hence this accounts for the difference in the number of enrolled women. From 2012 to 2013, 85,785 women were invited and 25,210 were screened (29.4%); women aged 25-34 that underwent a PAP test alone were 4,242 (16.8%) while women aged 35-64 that underwent an hr-HPV test were 20,968 (83.0%). The PAP test positive rate among women aged 25-34 was 4.0% (170/4242) and the hr-HPV positive rate among women aged 35-64 was 5.2% (1092/20968) (Table 1). Among the positive PAP test in 25-34 age group, the most frequent diagnostic category in 2012 was atypical squamous cells of undetermined significance (ASCUS) (48%) followed by low-grade squamous intraepithelial lesion (LSIL) 39%, and high-grade squamous intraepithelial lesion (HSIL) 13%; on the contrary, in 2013 the most frequent diagnostic category was LSIL (63%), followed by ASCUS (30%), HSIL (4%), and atypical squamous cells-high-grade not excluded (ASCH) 3% (Table 2). The hr-HPV positive rate among women aged 25-34 with diagnosis of ASCUS was 83.6% and the positive predic-

Table 1. — *Results of the screening test performance of the HPV screening program.*

	2012	%	2013	%	Overall %	Reference value	Min	Max
Proportion of positive hr-HPV test								
35-64 years	240 / 4752	5.1	852 / 16216	5.3	5.2	6%	5%	7%
Proportion of positive Pap test								
25-34 years	23 / 678	3.4	147 / 3564	4.1	4	3.3%	1%	4.4%
35-64 years (among HPV+)	67 / 240	27.9	181 / 852	21.2	22.7		25%	35%
Referral rate to colposcopy								
25-34 years	21 / 678	3.1	140 / 3564	3.9	3.8	3.3%	1%	4.4%
35-64 years	67 / 4752	1.4	181 / 16216	1.1	1.2	2.4%		
Overall	88 / 5430	1.62	321 / 19780	1.63				
Compliance with referral to colposcopy								
25-34 years	20 / 21	95.2	134 / 140	95.7	96	>90%		
35-64 years	62 / 67	92.5	172 / 181	95.0	94	>90%		
Overall	82 / 88	93.2	306 / 321	95.3				
PPV for CIN2+								
25-34 years	3 / 20	15	9 / 134	6.7	7.8			
35-64 years	6 / 62	9.7	19 / 172	11.0	10.7			
Overall	9 / 82	10.9	28 / 306	9.15				
Detection rate CIN2+								
25-34 years	3 / 678	4.42	9 / 3564	2.53	2.83	0.29%	0.04%	0.57%
35-64 years	6 / 4752	1.26	19 / 16216	1.17	1.19	0.60%	0.35%	1.0%
Overall	9 / 5430	1.66	28 / 19780	1.41				
Referral rate at one year								
35-64 years	171/4752	3.6	667 / 16216	4.1	4.0			

Table 2. — *Results of cytological diagnosis.*

Cytological diagnosis	ASCUS	LSIL	ASCH	HSIL	AC
2012					
25-34	11 (48%)	9 (39%)	0	3 (13%)	0
35-64	17 (25%)	42 (63%)	1 (1.5%)	7 (10.5%)	0
2013					
25-34	44 (30%)	93 (63%)	4 (3%)	6 (4%)	0
35-64	42 (23%)	118 (65%)	5 (3%)	14 (8%)	2 (1%)

Table 3. — *Results of histological diagnosis.*

Histological diagnosis		CIN1	CIN2	CIN3	Invasive CA
2012	25-34	11 (61%)	1 (6%)	2 (11%)	0
	35-64	43 (84%)	1 (2%)	5 (10%)	0
2013	25-34	75 (81%)	3 (3%)	6 (6%)	0
	35-64	92 (74%)	8 (6%)	11 (9%)	0

tive value (PPV) for CIN2+ in ASCUS category was 1.2% and 2.2% in 2012 and 2013, respectively.

The overall positivity rate at cytology among women aged 35-64 who were hr-HPV+ was 22.7% (248/1092); particularly, this rate decreased from 2012 to 2013 (27.9% to 21.2%). Among women aged 35-64, the frequency of diagnostic categories was similar in 2012 and 2013; indeed, the most frequent diagnostic category was LSIL (63% *vs* 65%), then ASCUS (25% *vs* 23%), HSIL (10.5% *vs* 8%), ASCH (1.5% *vs* 3%) and, only in 2013 the authors found two adenocarcinoma (AC) (Table 2). The inadequate PAP test rate was 0.35 and 0 in women aged 25-34 and 35-64, respectively. The referral rate to colposcopy was higher in 25-34 group (3.8%) than in women aged 35-64 (1.2%), while it was the same in both years (1.6%) and the adhesion to colposcopy increased from 2012 to 2013 in both age-classes. The PPV for CIN2+ at colposcopy was similar in 2012 and in 2013 (10.9% to 9.1%) while it was lower among women aged 25-34 than among women aged 35-64 (7.8% to 10.7%). The detection rate (DR) for CIN2+ was higher among women aged 25-34 than among women aged 35-64 (2.8% to 1.1%), while it was similar in 2012 and in 2013 (1.6% to 1.4%) (Table 1). The most frequent histologic category among women aged 25-34 and 35-64 in 2012 and 2013 was CIN1 followed by CIN3 and CIN2 (Table 3). The referral rate to one year for the women aged 35-64 hr-HPV+/PAP- was 3.6% in 2012 and 4.1% in 2013 (Table 1). The specificity values of hr-HPV test were 96.1% and 95.5% in 2012 and 2013, respectively.

Discussion

This study presents the results of the first two years of HPV screening program of Latina district; it allowed the authors to understand the effects of the introduction of a molecular test in screening for cervical cancer. First of all,

the most encouraging result was the increase in the uptake of hr-HPV test in the screening program. The introduction of this molecular test in place of PAP test was a large change that has not worried the women and it was probably due to the success of information campaign carried out in the district; indeed the present data showed that acceptance to perform the new test was higher than in the group of women aged 25-34 that underwent only PAP test. Moreover the present result was higher than the regional average (29.3%) reported in the previous three years [22]. The hr-HPV positive rate in both years was equal to the value observed in NTCC study where the hr-HPV test was performed on women aged 35-60 [7, 22]. An interesting aspect was the cytological triage of hr-HPV positive cases, not only because the present results were similar to those reported in the literature [7], but mainly because the authors noted that in 2013 there was a decrease in the PAP test positive rate, corresponding to a decrease in the percentage of ASCUS diagnosis. These data were probably due to the gained experience of the operators involved in the reporting of PAP test after hr-HPV test, and these results were expected and suggested by GISCi [3]. The decrease of ASCUS cases is important because minor cytological lesion often regress spontaneously, hence referring all women with ASCUS diagnosis to further examination results in a growth of over-diagnosis, colposcopy, and overtreatment [22]. The present results regarding the inadequate PAP test rate showed that there was a decrease in number from 2012 to 2013 and that the rate was close to zero and lower than the regional average [3]. This has led to a considerable saving in terms of time and costs.

With the introduction of hr-HPV test and cytological triage, it was assumed that there would be an increase in PAP test positive rate and consequently an increase in the number of women referred to colposcopy [3]; in the present study, not only did the authors not observe an increase of PAP test positive rate but moreover, among women aged 35-64, they found that referral rate to colposcopy was lowest than the regional and national average (2.5% to 2.4%) [21, 23]. In the present study this rate in women aged 35-64 was lower in 2013 than 2012, reflecting the decrease of PAP test positive rate and ASCUS rate.

The PPV is an indicator that measures the sensitivity of the test and is calculated as the proportion of women with histological cervical intraepithelial neoplasia grade 2 or worse (CIN2 +). In Italy the PPV differs from 2.8% to 52.7% among screening programs [21, 24]. In the present study, PPV values were lower than the regional and national averages (13.5% to 15.3%), however the PPV value of hr-HPV test was higher than PPV value of PAP test. Generally, the screening program with hr-HPV test and cytological triage have a DR for CIN2+ higher than PAP test alone [7, 23]; in the present study the DR for CIN2+ in women aged 35-64 was lower (1.1) compared to the national reference range (2.1 to 3.6) calculated on women with age of 25-64 over the last three years. This difference could be due to the higher age of women undergoing to hr-HPV test and to the different period in which the different screening programs were commenced; indeed, the screening program in Latina district began earlier than the others.

The present authors obtained good results in terms of test specificity using hr-HPV test followed by cytological triage in the screening program; indeed, both in 2012 and 2013 they obtained specificity values near to 96%.

In conclusion, the present data confirm that the early detection of HPV infection using hr-HPV test does not involve an increase in over-diagnosis and consequently an increase in treatments; instead the combination hr-HPV test and cytological triage defines high levels of specificity and sensitivity. Moreover, the new algorithm allows to stratify the population in three groups: women with a very low risk of disease (HPV-/PAP-), women at high risk of disease (HPV+/PAP+), and women with average risk of disease (HPV+/PAP-), to reduce the costs related to referral colposcopy and overall reduce cancer incidence.

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