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Sensitivity and specificity values of high-risk HPV DNA, p16/ki-67 and HPV mRNA in young women with atypical squamous cells of undetermined significance (ASCUS) or low-grade squamous intraepithelial lesion (LSIL)

A. FREGA[1], M. PAVONE[1], F. SESTI[2], C. LEONE[1], P. BIANCHI[1], G. COZZA[1], C. COLOMBRINO[1], A. LUKIC[1], R. MARZIANI[1], L. DE SANCTIS[3], G. DELLI CARPINI[4], D. CASERTA[1], A. CIAVATTINI[4]

[1]Department of Surgery, Medicine and Translational Medicine, Sant'Andrea Hospital, Sapienza University of Rome, Rome, Italy
[2]Department of Biomedicine and Prevention, University of Rome Tor Vergata, Rome, Italy
[3]Obstetrical and Gynecological Unit, "Valli del Noce" Hospital, Cles, Trento, Italy
[4]Woman's Health Sciences Department, Gynecologic Section, Polytechnic University of Marche, Ancona, Italy

Abstract. – OBJECTIVE: The aim of the study was to evaluate the sensitivity and specificity values of high risk HPV DNA test, p16/ki-67, and HPV mRNA in histologically high-grade cervical intraepithelial lesions (CIN2-CIN3) in women aged 21-24 years with diagnosis of atypical squamous cells of undetermined significance (ASCUS) or low-grade squamous intraepithelial lesion (LSIL) at pap smear.

PATIENTS AND METHODS: 342 patients between 21-24 years old, attending spontaneously our clinics, 118 with ASCUS and 224 with LSIL, were enrolled in the study. All patients underwent colposcopy and biopsies were performed in the areas with major changes. All patients were tested at the same time for p16/ki-67, high risk HPV DNA and HPV mRNA.

RESULTS: Nineteen out of 118 women with ASCUS showed a high-grade cervical intraepithelial lesion, 11 out of 118 (9.32%) CIN2, and 8 out of 118 (6.78%) CIN3. The sensitivity of high-risk HPV DNA was 99.9%, and the specificity 23.2%; p16/ki-67 pointed out a sensitivity of 90.9%, and a specificity of 81.8%; HPV mRNA showed a sensitivity of 81.8%, and specificity of 87.9% in CIN2 lesions. In CIN3 lesions, the sensitivity of high-risk HPV DNA was 99.9%, while the specificity was 19.1%; p16/ki-67 showed a sensitivity of 99.9%, and a specificity of 73.7%; HPV mRNA relived a sensitivity of 87.5%, and a specificity of 80.8%. In women with LSIL, a total of 42/224 (18.75%) of CIN2 were found at the histopathological examination, while 17/224 (7.59%) women presented a CIN3. No case of invasive cancer was identified. High-risk HPV DNA was positive in 190/224 (84.8%), p16/ki-67 in 119/224 (53.1%), and HPV mRNA in 104/224 (46.4%). In women with CIN2, the sensitivity of high-risk HPV DNA was of 92.8%, and the specificity 17.5%, the sensitivity of p16/ki-67 was 95.2%, and specificity 61.8%. HPV mRNA showed a sensitivity of 88.8% and a specificity of 87.8%. In women with CIN3, the sensitivity of high-risk HPV DNA was 88.2%, and the specificity 29.7%; p16/ki-67 pointed out a sensitivity of 94.1%, and a specificity of 49%; HPV mRNA showed a sensitivity of 88.2% and a specificity of 80.6.

CONCLUSIONS: Taking into account the high rate of spontaneous regression of high-grade lesions in young women, these tests, in particular the HPV mRNA test, used as a triage test for ASCUS or LSIL, can modify follow-up triage strategy. In fact, this biomarker, due to its high specificity, could lead to a cytology repetition instead of an immediate colposcopy, avoiding over diagnosis and potential overtreatment in this category of women.

Key Words:
Young women, Human papilloma virus, Low-grade squamous intra-epithelial lesions, Atypical squamous cells of undetermined significance, High-risk HPV DNA test, HPV mRNA test, p16/Ki-67, Colposcopy.

Introduction

It is well known how HPV infection is the most common sexual transmitted infection worldwide

Corresponding Author: Antonio Frega, MD; e-mail: a.frega@tin.it

with a prevalence of 30% in women younger than 25 years old. Different studies from several research groups have demonstrated the same trend with a peak of infection in girls under 25 years old and an abiding wane hereafter. This virus appears quickly afterwards the first sexual activity and more than 50% of young women has a positive HPV test in 3-4 years from the exposition[1]. The reason for this remarkable prevalence is due to their sexual behavior and/or immunological vulnerability. The risk for adolescents to be infected more than adult women can be also find in the number of sexual partners or in the wrong use of the condom. Moreover, in adolescent's cervix the area of columnar and metaplastic epithelium is more represented than in the adult's one. All these reasons contribute to the higher risk of HPV infection in this age, in addition to the physiological immune immaturity[2].There is strong evidence in the literature that persistent high-risk HPV infection is a necessary cause for the development of high-grade cervical intraepithelial lesion (CIN2-3), which could progress to invasive cervical carcinoma in an estimated period of 10-20 years[3]. The prevention of cervical carcinoma is strongly based on the use of HPV vaccination, and the early diagnosis of pre-neoplastic lesions made by cervical cytology and colposcopy. In the last decades, the great improvement in prevention strategies has brought to a reduction of mortality but not to a decrease of the incidence for this neoplasia[4,5], even in young patients. Indeed, in Europe and America there were 38,704 new cases of cervical cancer among women 15-39 years old, and the UK Cancer Research has collected 450 new cases of cervical cancer in the United Kingdom in 2014 among women between 25 and 29 years old[4]. Actually, the management of cervical pre-neoplastic lesions in young patients is as much conservative as possible, considering the reported high rate of regression in this period of life, and the increased risk of prematurity, neonatal mortality, and morbidity in subsequent pregnancies after cervical excisional treatments[6,7]. More specifically, CIN1 lesions do not need any kind of treatment, CIN2 lesions require observation, and CIN3 lesions require an immediate intervention[8], since they present a significantly lower regression rate[9]. Therefore, it becomes of crucial importance to identify correct strategies to carefully select women that may develop histologically advanced lesions, in order to reduce the gap between the cytological finding of a dubious lesion and the execution of a biopsy under colposcopy guidance, decreasing the risk of unnecessary treatments. It is well known the better efficacy to predict HSIL using more specific molecular tests, like HPV mRNA test and p16/ki-67 respect on HR HPV DNA in adult population taking part in the screening programs[10].

The aim of the study was to evaluate the performance of high-risk HPV DNA test, p16/ki-67 (Dual stain test) and HPV mRNA test in histologically high-grade lesions (CIN2-CIN3) in women aged 21-24 years with diagnosis of atypical squamous cells of undetermined significance (ASCUS) or low-grade squamous intraepithelial lesion (LSIL) at pap smear.

Patients and Methods

An observational cohort study was conducted between January 2009 and September 2017 on women aged 21-24 years old, who attended spontaneously to our clinics, with cytological diagnosis of atypical squamous cells of undetermined significance (ASCUS) or low-grade squamous intraepithelial lesion (LSIL). All women underwent colposcopy and were tested at the same time for p16/ki-67 dual stain cytology (Cintec PLUS Cytology kit; Roche laboratories, Mannheim, Germany), HC2 HPV test (Digene HC2 HPV DNA Test, Digene Corporation, Gaithersburg, MA, USA), and HPV mRNA Test (PreTect HPV Proofer Kit NorChip, Klokkarstua, Norway). The exclusion criteria were: oral contraceptive treatment longer than 5 years, previous ablative or excisional cervical treatments, systemic disease (e.g., metabolic disorders, immunodepression, infections), pregnancy, smoke, alcohol or substance abuse.

The cytological findings were classified according to Bethesda system terminology (Bethesda, 2001). The colposcopy was performed by Zeiss Colposcope T 50 (Carl Zeiss, Inc., Jena, Germany). The colposcopy findings were reported according to the International Federation for Cervical Pathology and Colposcopy (IFCPC) 2011 colposcopic terminology. The colposcopies performed before the introduction of the 2011 IFCPC nomenclature were revised accordingly through the revision of colposcopic finding. Colposcopy was considered satisfactory if the squamous-columnar junction (SCJ) was entirely identified. All biopsies were performed under colposcopy guidance in the areas with major changes. The histopathological findings were classified as

negative, cervical intraepithelial neoplasia grade 1 (CIN1), cervical intraepithelial neoplasia grade 2 (CIN2), cervical intraepithelial neoplasia grade 3 (CIN3), or suspicious for invasive cancer.

The values of sensitivity and specificity in detection of CIN2 or CIN3 lesions for p16/ki-67 dual stain cytology, HC2 HPV test, and HPV mRNA test were performed according to the referral cervical cytology results (ASCUS or LSIL).

A written informed consent was obtained by all the included women. The study was reviewed and approved by the Institutional Review Board.

Statistical sample size considerations were based on estimation of the sensitivity and specificity with widths of 95% confidence intervals (95% CIs). The diagnostic performance results are reported without correction for verification bias.

Results

Women with ASCUS Cervical Cytology

19 out of 118 women with ASCUS presented a high-grade cervical intraepithelial lesion at colposcopy directed biopsy, 11 out of 118 (9.32%) women presented a CIN2, and 8 out of 118 (6.78%) a CIN3. No case of invasive cancer was found. High risk HPV DNA test was positive in 96/118 (83.9%) women, p16/ki-67 in 62 /118 (52.5%), and HPV mRNA test in 47/118 (40.3%). In women with a diagnosis of CIN2, the sensitivity of high-risk HPV DNA was 99.9%, and the specificity 23.2%; p16/ki-67 pointed out a sensitivity of 90.9% and a specificity of 81.8%; HPV mRNA showed a sensitivity of 81.8% and specificity of 87.9%.

In women with a diagnosis of CIN3, the sensitivity of high-risk HPV DNA was 99.9%, while the specificity was 19.1%; p16/ki-67 showed a sensitivity of 99.9% and a specificity of 73.7%; HPV mRNA showed a sensitivity of 87.5%, and a specificity of 80.8% (Table I).

Women with LSIL Cervical Cytology

In women with LSIL, a total of 42/224 (18.75%) of CIN2 were found at the histopathological examination, while 17/224 (7.59%) women presented a CIN3. No case of invasive cancer was identified. High risk HPV DNA was positive in 190/224 (84.8%), p16/ki-67 in 119/224 (53.1%) and HPV mRNA in 104/224 (46.4%). In women with CIN2, the sensitivity of high-risk HPV DNA was of 92.8%, and the specificity 17.5%, the sensitivity of p16/ki-67 was 95.2% and specificity 61.8%. HPV mRNA showed a sensitivity of 88.8%, and a specificity of 87.8%. In women with CIN3, the sensitivity of high-risk HPV DNA was 88.2%, and the specificity 29.7%; p16/ki-67 pointed out a sensitivity of 94.1%, and a specificity of 49%; HPV mRNA showed a sensitivity of 88.2% and a specificity of 80.6 (Table II).

Discussion

Literature databases[11,12] are plenty of information regarding how to triage ASCUS or LSIL lesions in women older than 30 years. One of the most important innovation was the co-test based on Pap test and high-risk HPV DNA test in women with suspicious lesions. However, this protocol is not applicable to patients under the age of 30 due to an excessive rate of exposure for high-risk HPV strains that compromise the specificity of the examination[13]. According to the guidelines, the finding of ASCUS or LSIL lesions in young women between 21 and 24 years old requires cytology repetition after 12 months. If the lesion of the second cytology check is lower than ASC-H or HSIL, a second pap test is performed after further 12 months; if greater or equal to ASC-H or HSIL the patient is sent to colposcopy. Colposcopy and cytological control will continue for 2 years with a six-month interval for ASC-H or HSIL lesions. If these lesions persist for 12 months, biopsy is recommended; if HSIL persist for 24 months excision is recommended; if they are negative in two controls, patients can return to routine screening[14]. It is important to emphasize how during this period the patient is subjected to numerous checks with high costs in terms of resources and psychological stress.

We have turned our attention to girls between 21-24 years for whom the guidelines are currently very restrictive regarding the treatment. Our research has taken in consideration the possibility of using different markers to identify histologically advanced lesions in young patients and was conducted on 342 patients between 21-24 years old diagnosed with ASCUS or LSIL. It allowed us to highlight and compare the specificity and sensitivity values of lesion markers that could help in the early diagnosis of cytologically low-grade and histologically CIN2-3 lesions. Our study showed a prevalence of high-grade histological lesions similar to what is reported in the scientific literature[15]. Co-testing with high-

Table I. Sensitivity and specificity of p16/ki-67, HR HPV DNA, and HPV mRNA in women with ASCUS.

	CIN 2					CIN 3				
	N. positive results	Sensitivity	CI 95%	Specificity	CI 95%	N. positive results	Sensibility	CI 95%	Specificity	CI 95%
p16/ki-67	62/118 52.5%	10/11 90.09%	(89.4-92.4%)	81.8%	(74.2-89.4%)	8/8	99.9%	(92.2-99.9%)	73.7%	(65.0-82.4%)
HR HPV DNA	96/118 83.9%	11/11 99.9%	(93.1-99.9%)	23.2%	(14.9-31.5%)	8/8	99.9%	(92.2-99.9%)	19.1%	(8.2-30.0%)
HPV mRNA	47/118 40.3%	9/11 81.8%	(59.0-99.9%)	87.9%	(81.5-94.3%)	7/8	87.5%	(64.6-99.9%)	80.8%	(73.1-88.5%)

N. of women with ASCUS: 118 (11 CIN 2 (9.32%) 8 CIN3 (6.78%)).

Table II. Sensitivity and specificity of p16/ki-67, HR HPV DNA and HPV mRNA in women with LSIL.

	CIN 2					CIN 3				
	N. positive results	Sensitivity	CI 95%	Specificity	CI 95%	N. positive results	Sensibility	CI 95%	Specificity	CI 95%
p16/ki-67	119/224 53.1%	40/42 95.2%	(88.7-99.9%)	61.8%	(54.4-69.2%)	16/17	94.1%	(82.9-99.9%)	49%	(41.4-56.6%)
HR HPV DNA	190/224 84.8%	39/42 92.8%	(85.0-99.9%)	17.5%	(2.2-32.8%)	15/17	88.2%	(72.9-99.9%)	29.7%	(22.7-36.7%)
HPV mRNA	104/224 46.4%	37/42 88.8%	(79.3-98.3%)	87.8%	(82.5-93.1%)	15/17	88.2%	(72.9-99.9%)	80.6%	(73.9-87.9%)

N. of women with LSIL: 224 (42 CIN 2 (18.75%) 17 CIN3 (7.59%)).

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risk HPV DNA test and Pap test in triage of both ASCUS and LSIL lesions seems to not be useful for diagnostic purposes in this age group. Although the sensitivity of high-risk HPV DNA test is extremely high (99.9% for ASCUS and 93% for LSIL), the specificity is too low to be useful for diagnosis (23% for ASCUS and 17% for LSIL).

The role of the evaluation of the p16/ki-67 is different. The overexpression of this marker is extremely useful in the framing of lesions. The specificity of the tests based on the research of the p16 is considerably higher than that offered by the HPV test, especially under 25 years, due to the enormous spread of the virus among young women. It is known that the co-expression of p16 and Ki-67 in the same cell is strongly associated with established high-grade disease. In spite of that, the dual stained cytology showed 95.1% of sensitivity, and 72.4% specificity values for histologically CIN lesions after an ASCUS cervical cytology, and 96.7% and 62.1% from a LSIL cervical cytology[16]. The increase of p16 expression is directly related to the interruption of the negative feedback mediated by the Rb protein. Alterations of these physiological mechanisms are implications of the integration of the viral genome into the host cell[17]; the increase in Ki-67 levels is also a sign of de-regulation of the cell cycle. Looking for an ideal high-grade lesion marker, the study of the expression of the E6 and E7 oncoproteins mRNA may play an important role[18]. In fact, the expression of E6 and E7 proteins is ubiquitous in all cases of cervical cancer. This is decisive for the high specificity that this marker assumes[19,20]. Furthermore, all studies[21,22] conducted over the years have shown that once viral DNA has been integrated and the oncoproteins have been expressed, the biological behavior will no longer depend on the genotype.

Conclusions

These data revealed the importance of the mRNA test to define how severe is a lesion. The specificity of the HPV mRNA test in defining CIN2 lesions in women with ASCUS was 88.0%. It is much higher than what the HPV test has shown for 23% and higher than what the p16/ki-67 did for 72.4%. The same evidence was found in patients with LSIL, where the specificity of the test was 88.0%, respect to 62.1% for the p16/ki-67 and 17% for the HPV test. It was shown that the specificity of high-risk HPV DNA test is too low in women aged 21-24 years[23]. For this reason, this test cannot be used in the triage of patients with ASCUS or LSIL in this age group. On the contrary, between 21-24 years the HPV mRNA test and even more, the p167/ki-67 have demonstrated specificity and sensitivity values that seem to be optimal for the early detection of histologically high-grade cervical intraepithelial lesions. The introduction of these tests for the follow-up of young women with ASCUS and LSIL could lead to improve the management of these lesions in young women. Taking into account the high rate of spontaneous regression of high-grade lesions in young women, these tests, in particular the HPV mRNA test, used as a triage test for ASCUS or LSIL, can modify follow-up triage strategy. In fact, this biomarker, due to the high specificity values, could lead to a cytology repetition instead of an immediate colposcopy, avoiding over diagnosis and potential overtreatment in this category of patients.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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