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Expression of E6/E7 HPV-DNA, HPV-mRNA and colposcopic features in management of CIN2/3 during pregnancy

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Abstract. – OBJECTIVE: The incidence of abnormal cervical cytology in pregnancy is similar to that reported for non-pregnant women. Furthermore, 1% of pregnant women annually screened for cervical cancer will be diagnosed with cervical intraepithelial neoplasia (CIN) of various degrees. For this reason, Pap smear should be performed in the first trimester of pregnancy. The persistence of HR-HPV infection is related to the development of CIN. However, the relationship between CIN and HR-HPV infection during pregnancy and postpartum can hardly be found. The aim of this work was to assess the proper management of abnormal cytology during and after pregnancy evaluating regression rate, persistence rate and risk of progression and the predictive role of HPV molecular tests.

PATIENTS AND METHODS: Patients with abnormal cervical cytology were followed-up using colposcopy and colposcopy-directed biopsies every 12 weeks. Molecular tests were performed at the moment of the cytological diagnosis. Patients not treated in pregnancy were re-evaluated with cytology, colposcopy, biopsies, HPV-DNA test and HPV-mRNA test for a final diagnosis 8 weeks postpartum. Women with a persistent CIN 2-3 lesion at this follow-up check, underwent an excisional procedure by LEEP and then re-evaluated every 6 months for a year.

RESULTS: HPV-DNA test showed a sensitivity of 90.5% and a negative predictive value of 96.4%. Specificity and positive predictive values were 67.9% and 43.2%, respectively. For HPV-mRNA test, a sensitivity of 76.2% and a NPV of 93.9% were found; specificity and PPV were 98.7% and 94.1% respectively.

CONCLUSIONS: An observational management based on the use of molecular test and particularly HPV-mRNA test for its higher specificity, is a reasonable possibility in the follow-up of CIN2/3 lesions during pregnancy.

Key Words:
CIN, Colposcopy, HPV, HPV DNA test, HPV mRNA test, Pregnancy.

Introduction

Cervical cancer is the most common gynecologic neoplasia encountered during pregnancy, with an incidence ranging from 1.6 to 10.6/10 000 pregnancies. Approximately 30% of women diagnosed with cervical cancer is in the reproductive age, and an overall 3% of cases is diagnosed in pregnancy[1]. The occurrence of abnormal cervical cytology in pregnancy is similar to that reported for non-pregnant women (5-8%)[2]; however, the majority of pregnancies occurs in the age range 18-25, that is also the age range associated with the higher prevalence of cervical intraepithelial neoplasia, estimated to be 0.08-5.0%[3,4]. For a long time, pregnancy was considered as a condition at higher risk of complications, such as heavy bleeding, infections, and miscarriage, discouraging any procedure to be performed on the uterine cervix[5].

The identification of HPV as *causa sine qua non* for the onset of CIN, and consequently of invasive cervical cancer, has promoted the development of some molecular tests, aimed to reveal the presence of cervical HPV infection and the oncogenic activity of the virus. HPV-mRNA test

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is a progression index, indicating the persistent expression of viral proteins that are integrated into the cellular genome and responsible for the cellular transformation. HPV-DNA test is a risk parameter because the presence of the virus causes a 100-folds increase of the risk of developing a preneoplastic or neoplastic lesion[6].

Some studies have shown that persistent HR-HPV infection is closely related to CIN occurrence and development. In a recent one, He et al[7] evaluated the role of HPV-DNA test in pregnant women with abnormal Pap smear, reporting two interesting results: during pregnancy as the CIN grade increased, the HR-HPV infection rates increased ($p = 0.002$), while 3-6 months postpartum as the CIN grade increased, the natural negative rate of HR-HPV decreased ($p = 0.000$). The aim of this study was to assess the proper management of low and high squamous intraepithelial lesion (LSILs and HSILs) during and after pregnancy evaluating regression rate, persistence rate and risk of progression and the predictive role of HPV molecular tests.

Patients and Methods

From January 2009 to December 2014, 500 pregnant women attended at the Department of Surgical and Medical Science and Translational Medicine, Faculty of Medicine and Psychology, Sapienza University of Rome and to Section of Gynecology and Obstetrics, Department of Surgical Sciences, Tor Vergata University Hospital, Rome. All patients recruited were properly elucidated and signed an informed consent (Prot. CE 1591/13). The study was conducted in accordance to the Helsinki Declaration. Patients, who had not received a Pap smear for more than one year, were submitted to cervical cytological screening. Cytological findings were reported in agreement with the 2001 Bethesda Reporting System[8]. Patients with low and high squamous intraepithelial lesions (LSIL, HSIL) were evaluated with colposcopy. A standard OM50 Zeiss colposcope (Carl Zeiss, Inc., Oberkochen, Germany) was used and the examination was performed after the application of a 5% acetic acid solution, followed by a Schiller test. Colposcopy was considered satisfactory when the squamous-columnar junction (SCJ) was entirely detected. Colposcopic findings were formulated according to the International Nomenclature IFCPC 2012[9]. In the areas showing the higher grade of atypia, colposcopy-directed biopsies were executed. The excised specimens were fixed with a 10% formalin solution and microscopically examined. In order to assess the most frequently represented HPV genotype, the DNA genotyping was performed with the LiPA (Innogenetics, Gent, Belgium) using biotinylated SPF10 PCR primers for the amplification of a 65-bpregion of the L1 gene of a broad spectrum of HPV types (AmpliTaq Gold DNA polymerase; Applied Biosystems, Foster City, CA, USA). LiPA is capable of detecting 26 HPV types: HR-HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 70; low-risk HPV genotypes 6, 11, 34, 40, 42, 43, 44, 53, 54, and 74. The assay was performed according to the manufacturer's protocol. HPV-DNA test was performed using Hybrid Capture 2 (HC2 Digene Corporation, Gaithersburg, MD, USA), that is a semi-quantitative signal-amplified hybridization assay for the chemiluminescent detection of the 13 most common HR-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). Digene Hybrid CaptureII hybridization assay (HC2 Digene Corporation, Gaithersburg, MD, USA) was performed in agreement with the manufacturer's instructions. The PreTect HPV-Proofer Kit (referred to as the mRNA test) (Norchip, Klokkarstua, Norway) was used for the detection of E6/E7 mRNA of HPV types16, 18, 31, 33 and 45, according to the manufacturer's instructions. Five milliliters of PreservCyt sample were processed for the extraction of HPV-mRNA using the RNeasy Mini Kit (QIAGEN, Milan, Italy). The PreTect HPV-Proofer utilizes an isothermal nucleic acid sequence-based amplification (NASBA), which amplifies mRNA in a DNA background, detecting and genotyping HPV transcripts in the same reaction. The amplified products were detected in real time using fluorescent-labelled molecular beacon probes directed against full-length E6/E7 mRNA. Accumulated mRNA fluorescent profiles were analyzed and assigned a positive or negative status, for each type included, by the supplied PreTect analysis software. During pregnancy, patients with abnormal cervical cytology were followed up using colposcopy and, if necessary, colposcopy-directed biopsies every 12 weeks. Furthermore, molecular tests were performed at the moment of the cytological diagnosis. Women with cytological HSIL and colposcopic abnormalities suggestive of microinvasion or cytological HSIL with endocervical involvement were submitted to an excisional procedure

by LEEP, within 16-18 weeks of pregnancy. These patients were excluded from the study. Patients not treated in pregnancy were reevaluated with cytology, colposcopy, biopsies, HPV-DNA test and HPV-mRNA test for a final diagnosis 8 weeks after delivery. Those women, who showed a persistent CIN 2-3 lesion at this follow-up check, were submitted to an excisional procedure by LEEP and then re-evaluated every 6 months for a year.

Statistical Analysis

The collected data were analyzed using the statistical package SPSS (v. 21) and MedCalc (v. 15.2.1), the latter of which is specifically dedicated to the processing of biomedical data. Moreover, sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) have been calculated to assess the accuracy of both molecular tests, HPV-DNA and HPV-mRNA test. The significance level was set at 0.05 (p-value, Sig < 0.05).

Results

Out of 500 pregnant patients screened with cervical smear, 116 reported a cytological diagnosis of squamous intraepithelial lesions. Two patients were lost after the first prenatal check, six were lost after delivery, and four were lost after the first postpartum check, so that the final study population was composed of 104 patients (Figure 1). The clinical characteristics of the study population are summarized in Table I.

The genotyping showed that HPV-16 was most commonly detected (65.4%). HPV-18 was the second most common type (15.6%). Other common genotypes detected were HPV-33 (12%) and HPV-31 (7%).

During pregnancy, the HR-HPV-DNA test was positive in 73/99 (73.7%) cases, of whom 26 (61.9%) CIN 1, 22 (73.3%) CIN 2 and 25 (92.6%) CIN 3. On the contrary, the test was negative in 16 (38.1%) CIN 1, 8 (26.7%) CIN 2 and 2 (7.4%) CIN 3.

At the 8 weeks postpartum check, the HPV-DNA test was negative in 29/73 (39.7%) cases: particularly, it was negative in 15 (61.5%) out of 26 CIN 1 cases, in 11 (50%) out of 22 CIN 2 cases and in 3 (12%) out of 25 CIN 3 cases.

During pregnancy, the HPV-mRNA test was positive for HR-HPV in 43/99 cases (43.4%), of whom 17 (40.5%) CIN 1, 13 (43.3%) CIN 2 and 13 CIN 3 (48.1%); on the contrary, it was negative in 25 (59.5%) CIN 1, 17 (56.7%) CIN 2 and 14 CIN 3 (51.9%).

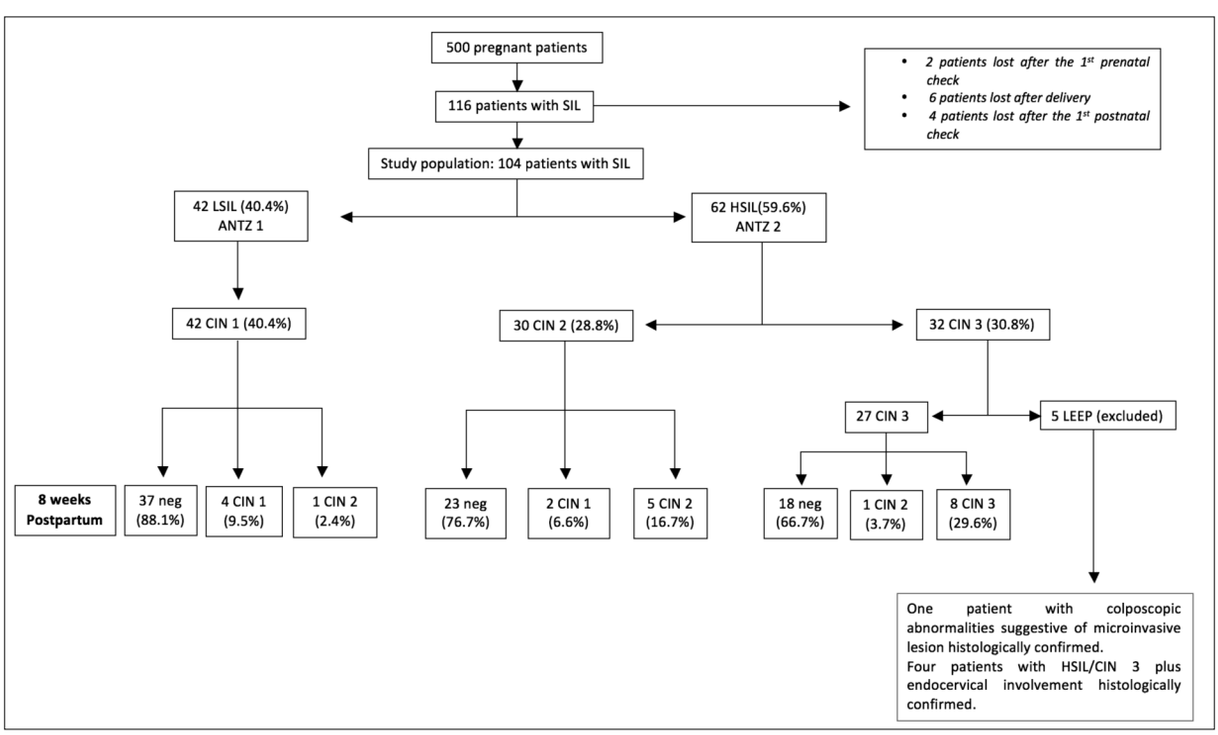

Figure 1. Flow-chart.

Table I. Clinical characteristics of the study population.

Clinical characteristics	
Mean gestational age at diagnosis	10 weeks
Meanage	27 years (21-37)
Mean parity	1.7 (0-4)
Mean age at first sexual intercourse	18 years (14-27)
Mean number of sexual partners	4 (1-8)
Tobacco use (current or past)	34.3%
Mean BMI	25.3 (20-31)

At the 8 weeks postpartum check, the HPV-mRNA test resulted negative in 26/43 (60.5%) cases: in particular, it was negative in 11 (64.7%) out of 17 cases of CIN 1, in 8 (61.5%) out of 13 CIN 2 and in 7 (53.8%) out of 12 CIN 3 (Tables II and III).

The accuracy of both molecular tests was also assessed. For HPV-DNA test, a sensitivity of 90.5% (95% CI, 0.7-1) was found and a negative predictive value (NPV) of 96.4% (95% CI, 0.9-1). Moreover, specificity and positive predictive value (PPV) were 67.9% (95% CI, 0.6-0.8) and 43.2% (95% CI, 0.3-0.6), respectively. As regard to HPV-mRNA test, a sensitivity of 76.2% (95% CI, 0.6-0.9) and a NPV of 93.9% (95% CI, 0.7-1) were found; specificity and PPV were 98.7% (95% CI, 0.9-1) and 94.1% (95% CI, 0.7-0.9), respectively.

Patients diagnosed with high-grade dysplasia at the 8 weeks postpartum check (one CIN 1 progressed to CIN 2, 5 persistent CIN 2, 1 CIN 3 regressed to CIN 2, 8 persistent CIN 3) were submitted to an excisional procedure by LEEP. Patients with low-grade dysplasia (four persistent CIN 1, 2 CIN 2 regressed to CIN 1) were followed-up. All the study population (99 pts) was reevaluated every 6 months for a year using cytology, colposcopy, histology and molecular tests.

Six months after LEEP, Pap test resulted positive in 17 (17.2%) out of 99 patients: 4 (23.5%) LSIL and 13 (76.5%) HSIL. At the colposcopic examination, 4 (23.5%) ANTZ resulted in grade I of abnormality and 13 (76.5%) ANTZ were grade II. The histologic analysis revealed the following results: 4 (23.5%) CIN 1, 7 (41.2%) CIN 2 and 6 (35.3%) CIN 3. The remaining 82 (82.8%) patients reported negative cytocolposcopic and histologic results.

Table II. HPV-DNA test and mRNA test results in pregnancy and 8 weeks postpartum. Number of patients (%).

	HR-HPV DNA test				HR-HPV mRNA test			
	Pregnancy		8 weeks postpartum		Pregnancy		8 weeks postpartum	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
CIN 1	26 (61.9%)	16 (38.1%)	11/26 (42.3%)	15/26 (61.5%)	17 (40.5%)	25 (59.5%)	6/17 (35.3%)	11/17 (64.7%)
CIN 2	22 (73.3%)	8 (26.7%)	11/22 (50%)	11/22 (50%)	13 (43.3%)	17 (56.7%)	5/13 (38.5%)	8/13 (61.5%)
CIN 3	25 (92.6%)	2 (7.4%)	22/25 (88%)	3/25 (12%)	13 (48.1%)	14 (51.9%)	6/13 (46.2%)	7/12 (53.8%)
Total	73 (73.7%)	26 (26.3%)	44 (60.3%)	29 (39.7%)	43 (43.4%)	57 (56%)	17 (39.5%)	26 (60.5%)

Table III. Results of HPV-DNA test and HPV-mRNA test in relationship to histological findings at 8 weeks postpartum.

		HPV-DNA test		HPV-mRNA test	
	8 weeks postpartum histology	Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)
CIN 1	Regressed to normal 37 (88.1%)	6 (14.3%)	31 (73.8%)	1 (2.4%)	36 (85.7%)
	Persistent/progressed dysplasia 5 (11.9%)	5 (11.9%)	0 (0%)	5 (11.9%)	0 (%)
CIN 2	Regressed to normal 23 (76.7%)	6 (20%)	17 (56.7%)	0 (0%)	23 (76.7%)
	Persistent/progressed dysplasia 7 (23.3%)	5 (16.7%)	2 (6.6%)	5 (16.7%)	2 (6.6%)
CIN 3	Regressed to normal 18 (66.7%)	13 (48.1%)	5 (18.6%)	0 (0%)	18 (66.7%)
	Persistent/progressed dysplasia 9 (33.3%)	9 (33.3%)	0 (0%)	6 (22.2%)	3 (11.1%)

Both molecular tests were repeated in all the study population (99 pts). The HPV-DNA test was positive in 27 (27.3%) out of 99 cases, of whom 6 (14.3%) out of 42 CIN 1, 8 (26.7%) out of 30 CIN 2 and 13 (48.1%) out of 27 CIN 3. The HPV-mRNA test resulted positive in 14/99 (12.8%) cases: in particular, it was positive in 4 (9.5%) out of 42 CIN 1, 5 (16.7%) out of 30 CIN 2 and 5 (22.2%) out of 27 CIN 3.

One year after LEEP, Pap test was positive in 11 (11.1%) out of 99 patients: 2 (18.2%) LSIL and 9 (81.8%) HSIL. At the colposcopic examination, 2 (18.2%) ANTZ resulted in grade I of abnormality and 9 (81.8%) ANTZ were grade II. The histologic analysis revealed the following results: 2 (18.2%) CIN 1, 5 (45.4%) CIN 2 and 4 (36.4%) CIN 3. The remaining 88 (88.9%) patients reported negative cytocolposcopic and histologic results.

All the study population (99 pts) was reevaluated using both molecular tests. The HPV-DNA test was positive in 18 (18.2%) out of 99 cases, of whom 3 (7.1%) out of 42 CIN 1, 5 (16.7%) out of 30 CIN 2 and 10 (37.0%) out of 27 CIN 3. The HPV-mRNA test resulted positive in 9/99 (9.1%) cases: in particular, it was positive in 2 (4.8%) out of 42 CIN 1, 3 (10%) out of 30 CIN 2 and 4(14.8%) out of 27 CIN 3.

Discussion

All pregnant women, in the absence of a recent Pap smear, should be submitted to a cytological screening examination[10]. Pap smear should be performed in the first trimester of pregnancy[10]. However, in the interpretation of Pap smear during pregnancy, physicians should be aware of the physiologic pregnancy-related cellular changes that could mimic the cytological appearance of a high-grade SIL and cause a false positive diagnosis: degenerated decidual cells (Arias-Stella reaction) and trophoblastic cells with variable cytoplasmic staining and enlarged nuclei[11]. Also, the colposcopic examination could be more difficult due to the pregnancy: the active immature metaplasia can create a large thin aceto-white areas, with fine punctuation or mosaicism that could mimic a low-grade intraepithelial lesion[5]. The late stromal decidualization may appear as dense acetowhite lesions with spidery superficial blood vessels that appear similar to a high-grade lesion; a thin ring of decidualized aceto-white stroma may surround normal capillaries, causing a "starry-sky" appearance[12].

The main purpose of colposcopy in pregnancy is to exclude invasive disease[2]: only the colposcopic impression of invasive cancer requires a more accurate evaluation and a prompt decision making, while the management of preinvasive lesions may be conservative and treatment can be safely postponed until the postpartum period[13].

If at colposcopy a high-grade lesion is seen or suspected, targeted biopsies should be performed. Despite the increased vascularization of the cervix, cervical biopsy in pregnancy is a safe procedure, with a hemorrhage risk of only 0.6%, which is similar to that reported in non-pregnant women[10].

Based on the 2006 guidelines of the American Society of Colposcopy and Cervical Pathology (ASCCP) in pregnant women with ASC variant or LSIL cytology, colposcopy and subsequent management could be postponed until 6 weeks postpartum, because these abnormalities are likely to regress spontaneously with expectant management and unlikely to harbor an invasive occult neoplasia[13]. Indeed, Fader et al[13] reported a postpartum regression of 86% of low-grade lesions with no cases of invasive cancer. Moreover, pregnant women with ASC-US or LSIL cytology rarely have a colposcopic impression of CIN 2-3 at their initial colposcopy[14].

In pregnant patients diagnosed with HSIL cytology, the ASCCP guidelines support that cervical biopsies and serial colposcopies during pregnancy are acceptable if the colposcopic impression is CIN 2-3 or invasive cancer. However, Fader et al[13] suggest that biopsies and repeated colposcopies may not be necessary during pregnancy if the colposcopic impression is of CIN 2-3, as these lesions are unlikely to progress and likely to regress during or within the first year after pregnancy (35%), with a low risk of invasive malignancy (0.45-1/1000 live births). However, in women with previously untreated cervical dysplasia or with risk factors for persistent or progressive disease (such as smoking or immunosuppression) or noncompliance, antepartum cervical biopsies and serial colposcopies are reasonable.

The use of molecular test (HPV-DNA and HPV-mRNA test) is well known in the management of HPV infections. Dockter et al[15] showed in their study how HPV-mRNA (APTIMA) had a similar sensitivity to the HC2 test for detection of CIN2+ and CIN3+ but the clinical specificity of APTIMA HPV assay for detection of CIN2+ and CIN3+ was significantly higher than that of

the HC2 test. Our results, concerning the accuracy of both molecular testing the detection of CIN2/3 lesions in pregnant women, are in agreement with previously mentioned study (HPV DNA: sensibility 90.5% and specificity 67.9%; HPV mRNA: sensibility 76.2% and specificity 98.7%) so, in this regard, pregnancy does not seem to influence HPV detection[16].

Compared to the HPV DNA test, the higher specificity and the lower sensibility of HPV mRNA test could be explained by the limited number of genotypes targeted by the mRNA test[17]. Moreover, CIN2-3 lesions linked with those oncogenic types not targeted by Proofer will regress spontaneously[18]. The HPV-mRNA test was not positive in all patients who experienced a recurrent disease. Finally, during pregnancy HPV-mRNA test designed to detect transcriptionally active infection with the five most common oncogenic HPV types (16, 18, 31, 33 and 45) might still identify the majority of women whose lesions are likely to progress.

Conclusions

An observational management based on the use of molecular test and particularly HPV-mRNA test for its higher specificity is a reasonable possibility in the follow-up of CIN2/3 lesions during pregnancy. HPV mRNA test may be considered as a tool in risk stratification. Moreover, the management of preinvasive lesions should be observational, looking for the balance between the risk of progression of the disease and the overtreatment that could set negatively the pregnancy outcomes.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Nguyen C, Montz FJ, Bristow RE. Management of stage I cervical cancer in pregnancy. Obstet Gynecol Surv 2000; 55: 633-643.
2) Frega A, Scirpa P, Corosu R, Verrico M, Scarciglia ML, Primieri MR Primieri MR, Palazzo A, Iacovelli R, Moscarini M. Clinical management and follow-up of squamous intraepithelial cervical lesions during pregnancy and postpartum. Anticancer Res 2007; 27: 2743-2746.
3) Kaplan KJ, Dainty LA, Dolinsky B, Rose GS, Carlson J, McHale M, Elkas JC. Prognosis and recurrence risk for patients with cervical squamous intraepithelial lesions diagnosed during pregnancy. Cancer 2004; 102: 228-232.
4) Penna C, Fallani MG, Maggiorelli M, Zipoli E, Cardelli A, Marchinni M. High-grade cervical intraepithelial neoplasia (CIN) in pregnancy: clinical therapeutic management. Tumori 1998; 84: 567-570.
5) Origoni M, Salvatore S, Perino A, Cucinella G, Candiani M. Cervical Intraepithelial Neoplasia (CIN) in pregnancy: the state of the art. Eur Rev Med Pharmacol Sci 2014; 18: 851-860.
6) Frega A, Sesti F, Sopracordevole F, Biamonti A, Votano S, Catalano A, Milazzo GN, Gentile M, Ricciardi E, French D, Moscarini M. Multiple intraepithelial neoplasias of the lower female genital tract: the reliability of HPV mRNA test. J Low Genit Tract Dis 2014; 18: 174-181.
7) He Y, Wu YM, Zhao Q, Wang T, Song F, Zhu L. High-risk human papilloma virus management in pregnancy with cervical intraepithelial neoplasia during pregnancy and postpartum in China. J Obstet Gynaecol Res 2014; 40: 538-544.
8) Solomon D, Davey D, Kurman R, Moriarty A, O'Connor D, Prey M, Raab S, Sherman M, Wilbur D, Wright T Jr, Young N; Forum Group Members; Bethesda 2001 Workshop. The 2001 Bethesda System: terminology for reporting results of cervical cytology. JAMA, Bethesda 2001 Workshop 2001; 287: 2114-2119.
9) Tatti S, Bornstein J, Prendiville W. Colposcopy: a global perspective: introduction of the new IFCPC colposcopy terminology. Obstet Gynecol Clin North Am 2013; 40: 235-250.
10) Italian Society of Colposcopy and CervicoVaginal Pathology. Guidelines for the management of abnormal pap smear. www.Colposcopiaitaliana.it 2006; 1: 1-25.
11) Han SN, Mhallem Gziri M, Van Calsteren K, Amant F. Cervical cancer in pregnant women: treat, wait or interrupt? Assessment of current clinical guidelines, innovations and controversies. Ther Adv Med Oncol 2013; 5: 211-219.
12) Waxman A, Diaz ML. Obstetrics and Gynecology Clinics of North America. HPV, colposcopy, and prevention of squamous anogenital tract malignancy. Preface. Obstet Gynecol Clin North Am 2013; 40: XV-XVI.
13) Fader AN, Alward EK, Niederhauser A, Chirico C, Lesnock JL, Zwiesler DJ, Guido RS, Lofgren DJ, Gold MA, Moore KN. Cervical dysplasia in pregnancy: a multi-institutional evaluation. Am J Obstet Gynecol 2010; 203: 113.e1-6.
14) Wetta LA, Matthews KS, Kemper ML, Whitworth JM, Fain ET, Huh WK, Kendrick JE, Straughn JM Jr. The management of cervical intraepithelial neoplasia during pregnancy: is colposcopy necessary? J Low Genit Tract Dis 2009; 13: 182-185.
15) Dockter J1, Schroder A, Hill C, Guzenski L, Monsonego J, Giachetti C. Clinical performance of the

APTIMA HPV Assay for the detection of high-risk HPV and high-grade cervical lesions. J Clin Virol 2009; 45(Suppl 1): 55-61.

16) Schmeink CE, Melchers WJ, Hendriks JC, Quint WG, Massuger LF, Bekkers RL. Human papillomavirus detection in pregnant women: a prospective matched cohort study. J Womens Health (Larchmt) 2012; 21: 1295-1301.

17) Ratnam S, Coutlee F, Fontaine D, Bentley J, Escott N, Ghatage P, Gadag V, Holloway G, Bartellas E, Kum N, Giede C, Lear A. Clinical performance of the PreTect HPV-Proofer E6/E7 mRNA assay in comparison with that of the Hybrid Capture 2 test for identification of women at risk of cervical cancer. J Clin Microbiol 2010; 48: 2779-2785.

18) Smith JS, Lindsay L, Hoots B, Keys J, Franceschi S, Winer R, Clifford GM. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesion: a meta-analysis update. Int J Cancer 2007; 121: 621-632.