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The role of human papillomavirus in cervical pre-neoplastic lesions: the relationship between virus genotype and persistence or clearance of the infection

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Summary

Human papilloma virus (HPV) infection persistence is responsible for modifications that will lead to progression to a pre-neoplastic cervical lesion. This study examined persistence or clearance of HPV infection related to genotypes and to risk factors, like patients' age, smoking habit, and use of oral contraceptives. The data of 90 patients, who had a positive high risk-HPV (HR-HPV) test for more than 18 months irrespective of the genotype, were taken from the pathology database of the Colposcopy Clinic and the presence of HPV was assessed by the linear array test which utilized amplification of target DNA by polymerase chain reaction (PCR) and nucleic acid hybridization. During the first visit a careful anamnesis was taken, with information about lifestyle. Clearance was observed in 25% of infections by low risk genotypes and in 43% of which by high risk and, among high oncogenic risk genotypes, HPV 16, 31, 33, 35, 39, 45, 51, and 59 were mostly associated to persistence of infection. Among persistent HPV infections, progression occurred with HPV16 in 50% of cases, with HPV18 in 44.4% of cases, with HPV31 and HPV35 in 33.3% of cases, with HPV33 in 28.5% of cases, with HPV45 in 41.7% of cases, with HPV51 in 25% of cases, and with HPV59 in 20%. There was no statistically significant difference between persistence of high risk and low risk genotypes and a significant association with the cited risk factors. A long period of follow-up, HPV-DNA detection by PCR, and the study of risk factors could lead to improve the diagnosis of persistent HPV infections.

Key words: HPV infection persistence; HPV infection clearance; Squamous intraepithelial lesion; Cervical intraepithelial neoplasia; HPV infection progression.

Introduction

Human papilloma viruses (HPV) are small, non-enveloped DNA viruses (Family: Papovaviridae; Genus: alphapapillomavirus; Species: human papillomaviruses). HPV infection represents an argument of remarkable gynecological interest for its tight link with cervical cancer. This neoplasia is the one type for which the World Health Organization (WHO) recognized a viral etiology. It has been estimated that HPV infection is the most diffused and diagnosed sexually transmitted disease in the world [1-3]. HPV infection is contracted at least once in a lifetime by 90% of women, but in 80% of cases, it is transient and regresses in 18-24 months, so it persists only in 20% of cases. No efficient antiviral has been found to fight this infection, so its clearance is due to the immune system, even if it seems that the use of oral and topical products containing carboxymethyl-betaglucan and other natural substances, could increase the rate of viral negativity [4, 5]. Persistence of infection over five years, mostly related to high risk genotypes, has been found in older women [6]. This condition is probably due to immunological senescence effects which interfere with the normal viral clearance [7]. Persistence of infection is also due to many factors which cause immune-depression, like cigarettes smoke, multiparity, co-infections, recurrent cervical infections, long term therapy with hormonal contraceptives, and it is strongly related to incidence of low and high grade pre-neoplastic lesions [8-10]. Indeed, persistence of infection represents one of the principal pathogenetic moments for determination of cytological modifications that will lead to progression to a pre-neoplastic cervical lesion. At this point it is clear that the long the duration of infection, the higher the probability to develop high grade cervical intraepithelial neoplasia (CIN) 2-3 / high-squamous intraepithelial lesion (HSIL). Furthermore a longer persistence of HPV cervical infection is tightly associated to progression of pre-neoplastic lesions to cervical cancer, so the detection of HPV DNA in the uterine cervix could be an early marker for cancer evolution [11]. In the last years, has shown a higher specificity in detection of HPV-DNA than HC2 which may give false negative results also when there is a histological lesion. In fact some high risk HPV (HR-HPV) genotypes were not in-

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cluded in the panel of HR types of the HC2 detection kit [12]. The main objective of the study was to examine persistence or clearance of HPV infection related to genotypes. Observations came to light regarding genotypes mostly responsible of infections in the examined population and about the association of persistence and clearance of HPV infection with patients' age, smoking habit, and use of oral contraceptives.

Materials and Methods

This study was approved by the Ethics Committee of Department of Woman, Child and of General and Specialized Surgery, Second University of Naples, in accordance with the ethical standards of the Declaration of Helsinki. The data were taken from the pathology database of the Colposcopy Clinic of the Second University of Naples, at the Department of Women, Children and General and Specialized Surgery and at the Sant'Anna and San Sebastiano" Civil Hospital of Caserta, for all patients with a positive HR-HPV test for more than 18 months, from 2010 to 2014, and assessed the risk of progression of CIN.

Inclusion criteria were free will to participate in the study in anonymity, no age limits, and a first positive HPV DNA test irrespective of genotype. Exclusion criteria were previous pre-neoplastic lesions or cancer already treated with negative HPV test at the first examination after the surgery and pregnancy.

During the first visit a careful anamnesis was taken, in particular searching for information about lifestyle (smoking, age of first intercourse, the number of sexual partners in their lifetime, condom use, duration of oral contraceptive use, numbers of pregnancies, and a history of sexually transmitted diseases to be considered as confounding factors within HPV infection).

The study finally included the following: patients with a negative or ASC-US diagnosis at papanicolaou cytology and a positive HR-HPV for more than 18 months, patients with low squamous intraepithelial lesion (LSIL) diagnosis at Papanicolaou cytology and/or CIN1, and a positive HR-HPV for more than 18 months, and patients in follow-up after surgery for HSIL diagnosis at Papanicolaou cytology and/or CIN2-3, and a positive HR-HPV, previously and thereafter to surgery for more than 18 months.

The aim of the study was to assess whether there are other genotypes, other than HPV16 or HPV18, which can significantly predict the progression to neoplastic lesions; therefore complete documentation for each patient in follow-up at the present Department was collected and recorded in the pathology database of the Colposcopy Clinic which was been analyzed. In the data review it was considered a positive cytological progression when the follow-up cytological diagnosis was one of the following: atypical squamous cells (ASC-H), cannot exclude a high-grade squamous intraepithelial lesion, LSIL and HSIL), squamous cell carcinoma or adenocarcinoma. Positive histological progression was defined as colposcopic biopsy showing mild (CIN1), moderate (CIN2), or severe squamous dysplasia (CIN3) after the previous negative or ASC-US cytological diagnosis. A concordance between the cytological and histological progression was confirmed for each patient; therefore, the squamous intraepithelial lesion was always confirmed by cytological and histological examinations. The time of progression was calculated as the number of months intervening between the first diagnosis and the follow-up. The first follow-up after the diagnosis was not made before 180 days. Cytological and surgical pathology follow-up reports were reviewed for all specimens available, including Pap smears, biopsies and resections, and follow-up documentation.

The presence of HPV was assessed by the linear array test. This test is a qualitative in vitro test for the detection of HPV in clinical specimens. The test utilizes amplification of target DNA by polymerase chain reaction (PCR) and nucleic acid hybridization, and detects 37 anogenital HPV DNA genotypes [6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73 (MM9), 81, 82 (MM4), 83 (MM7), 84 (MM8), IS39, and CP6108] in cervical cells collected in PreservCyt solution.

Fisher's Exact Test was used to verify statistical significance of the various examined variables. A p-value of <0.05 was considered statistically significant. GraphPadPrism, a statistical computer program, was used for statistical analyses.

Table 1. — *Charateristics of patients included in the study.*

	Total	Percent
Age		
< 30 years	28	31%
> 30 years	62	69%
Smokers		
Yes	36	40%
No	54	60%
Oral contraceptives use		
Yes	42	47%
No	48	53%
Infections		
Single	80	89%
Multiple	10	11%
Oncogenic risk		
Low	16	18%
High	74	82%

Results

The mean age of the 90 examined women was 30.8 (20-49) years. The group was constituted by women with the following characteristics: mean age >30 years (69%), non-smokers (60%), and not using oral contraceptives (53%) (Table 1).

At first instance clearance was evaluated irrespective of the grade of cytological lesions. Single infections, meaning infections with only one viral genotype, were the most frequent (89%), whereas multiple infections occurred only in a few cases (11%). In the examined sample, infections by high risk genotypes were found in most of cases (82%), whereas infections by low risk genotypes were found in 18% of cases.

After a 18-month follow-up, patients repeated HPV DNA testing and found persistence of infection in 54 of them (60%) and its clearance in 36 of them (40%).

Table 2 shows viral clearance or persistence related to single or multiple infections and to oncogenic risk.

Clearance occurred in 43% of single infections and in 20% of multiple ones. Eighty percent of women with multiple infections showed viral persistence after 18 months, whereas only 58% of ones with single infections had persistence; nevertheless multiple infections were not significantly associated to persistence of infection (Table 2).

Table 2. — *HPV infection evolution related to single or multiple infections and to oncogenic risk.*

	Total, n	Clearance, n (%)	Persistence, n (%)	*p*
Infection				
Single	80	34.(43)	46 (58)	0,3
Multiple	10	2(20)	8 (80)	
Oncogenic risk				
Low	16	4 (25)	12.(75)	0,26
High	74	32 (43)	42 (57)	

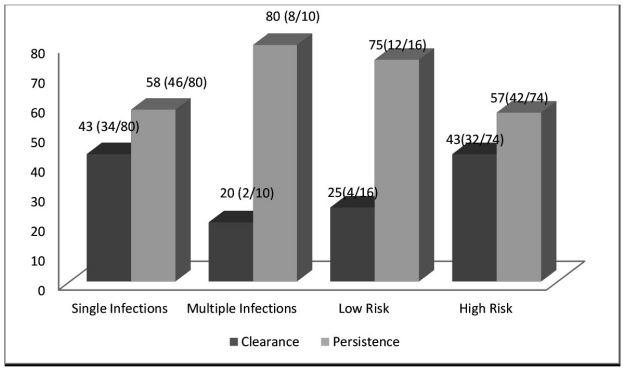

Figure 1. — Clearance and persistence in single and multiple HPV infections and by low and high risk HPV after 18 months.

Figure 1 shows clearance and persistence in single and multiple HPV infections and by low and high risk HPV after 18 months. Considering oncogenic risk, there was clearance in 25% of infections by low risk genotypes and in 43% of which by high risk. Furthermore there was no statistically significant difference between persistence of high risk and low risk genotypes, indeed with a *p*-value of 0.26. HPV infection persisted in 57% of which caused by high risk genotypes, in 75% of those caused by low risk genotypes (Figure 1).

Among high oncogenic risk genotypes, HPV 16, 31, 33, 35, 39, 45, 51, and 59, most were associated to persistence of infection. HPV18 and 52 were the less persistent and HPV 56 and 58 showed a\ higher clearance (Table 3).

The cytological and surgical pathology follow-up showed among persistent HPV infections, a progression with HPV16 in 50% of cases, of which 18% were ASC-US that evolved to LSIL; 64% were LSIL which became HSIL and 18% were LSIL that underwent surgical treatment and relapse. There was progression of the infection also by HPV18 in 44.4% of cases, by HPV31 and HPV35 in 33.3% of cases, in 28.5% by HPV33, in 41.7 by HPV45, in 25% by HPV51, and in 20% by HPV59 (Table 4).

For low risk genotypes, HPV6 showed a higher clearance (66.7%). Genotypes 11 and 72 persisted in 100% of cases and HPV 61 in 85.7% (Table 3).

In this study, HPV infection evolution related to patient's characteristics fas finally evaluated (Table 5).

Table 3. — *Persistence and clearance of infections related to genotype after an 18-month follow-up.*

High risk genotypes			
Genotypes	Clearance, n (%)	Persistence, n (%)	Total, n
16	12 (35.3)	22 (64.7)	34
18	9 (50)	9 (50)	18
31	3 (25)	9 (75)	12
33	0 (0)	7 (100)	7
35	0 (0)	6(100)	6
39	0 (0)	2 (100)	2
45	0(0)	12 (100)	12
51	0 (0)	8 (100)	8
52	1 (50)	1 (50)	2
56	1 (100)	0 (0)	1
58	1 (100)	0 (0)	1
59	0 (0)	10 (100)	10
Low Risk Genotypes			
6	8 (66.7)	4 (33.3)	12
11	0 (0)	4 (100)	4
61	2 (14.3)	12(85.7)	14
72	0 (0)	2 (100)	2

Table 4. — HPV infection progression related to HR-HPV.

	Progression (%)	ASC-US to LSIL (%)	LSIL to HSIL (%)	Relapse of LSIL (%)
HPV16	50	18	64	18
HPV18	44.4	25	50	25
HPV31	33.3	33.3	66.7	0
HPV33	28.5	50	50	0
HPV35	33.3	0	100	0
HPV45	41.7	25	75	0
HPV51	25	50	50	0
HPV59	20	50	50	0

In the age group of patients < 30 years, clearance of infection occurred in 43% of cases, whereas in the one of >30 years in 39%. Persistence was of 57% in women < 30 years and of 61% in >30 years. In both cases there was no considerable difference and the *p*-value showed a relationship that was not statistically significant.

Regarding use of oral contraceptives, there was a clearance in 43% of women who used them and in 42% of those that did not, whereas 57% of treated women and 58% of ones who never used oral contraceptives had persistence of infection. Values of clearance and persistence are similar, without a significant difference.

Finally, 33% of smokers and 44% of not smokers showed clearance of infection, whereas persistence occurred in 67% of smokers and 56% of women who never smoked. Even this time there was no significant association. Therefore the authors observed an absence of statistically significant association among persistence of infection and some risk factors like age, smoking habit, and oral contraceptives use (Table 5).

Table 5. — *HPV infection evolution related to patient's characteristics.*

	Total, n	Clearance, n (%)	Persistence, n (%)	*p*
Age				
< 30 years	28	12 (43)	16 (57)	0.82
> 30 years	62	24 (39)	38 (61)	
Oral contraceptives use				
Yes	42	18 (43)	24 (57)	1.00
No	48	20 (42)	28 (58)	
Smokers				
Yes	36	12 (33)	24 (67)	0.38
No	54	24 (44)	30 (56)	

Discussion

The prevalence of HPV in world's population and its proven association with the development of cervical cancer generated in recent decades great attention by the entire international scientific community [13). The knowledge of viral transmission and mechanism of replication appears to be essential for the construction of appropriate therapeutic strategies. However, to date, there is no effective treatment that can eliminate HPV infections and the only possibility to overcome the infection is represented by the immune system. This consideration provides exactly the impetus for further study on persistence and clearance of HPV infection, in relation to specific genotypes. Of particular interest is the evidence that HPV infection is eradicated in two years by about 80% of women, without any therapy [8, 14-16). In the present case the rate of clearance of HPV infection was lower compared to the expected value. It can be assumed that this result is due in part to the small size of the examined sample and to the free constancy of sexual intercourses with an infected partner that could lead to persistent infection by the same genotype. In fact, more than half of the patients enrolled, reported to have intercourse with a steady partner. Without considering that in any case the recorded values are in agreement with those reported by Banura *et al.* 2010 [17]. In particular the study of Banura *et al.* showed clearance in 31.2% of HPV infections, a result probably due even to the short duration of the observation period, as the median follow-up time was 18.5 months, although the incidence of pre-neoplastic lesions was much lower compared to infections. The short duration of follow-up was definitely also a limitation of the present study, being only of 18 months.

Considering only infections by high-risk genotypes, persistence was 62% and clearance 38 %, in agreement with values reported by other authors [18-20). On the contrary, in the case of low-risk virus infections, the recorded result, which provides a persistence in 75% of cases and a clearance only in 25% of those, is in contrast to what is reported in literature, since it provides a persistence even higher than that registered for high oncogenic risk viruses. Probably this result is due to the small size of the examined sample of patients with LR-HPV in comparison to sample of patients with HR-HPV, but also to the which does not allow to come to a certain conclusion. Banura *et al.* reported a clearance for low-risk genotypes between 50% and 100% [17). Perhaps the clearance of infections by HR-HPV was higher than the ones by LR-HPV because of an increase of lymphocytic population selected by the HR-HPV which guarantees a more efficient and faster reaction of the immune system.

The study also allowed to evaluate the association between infection persistence and the presence of risk co-factors, such as the use of oral contraceptives and smoking habit. It came to light that there is no statistically significant correlation between these two risk factors and persistence of HPV infection. However, it has to be considered that the duration and amount of smoking of each patient were not known, and that the lack of association between use of hormonal contraceptives and persistence of infection could be due to short periods of treatment, as evidenced by other authors [18, 19). The persistence of HPV infection in women aged more than 30 years was also analyzed, but even in this case, there is no statistically significant association. In future studies must assess the relationship between persistence of infection and causes of immune depression like the smoking habit, age of first intercourse, many sexual partners, multiparity, autoimmune diseases, alcohol abuse, use of oral contraceptives, and age >30 years.

According to the findings, the presence of multiple infections is mostly associated with virus persistence after 18 months of follow-up. This result is in line with the hypothesis that presence of multiple infections could be due to a decreased ability of the immune system to overcome them, and then also to eliminate them from body [21-24). A lower clearance in multiple infections could be caused by an increase of sensitization by the immune system which produces antibodies targeted for homologous sequences of HPV with a consequent loss of lymphocytic load for cross-reaction among genotypes. A future target could be to find specific lymphocytic populations for each HPV genotype analyzing blood samples.

This study confirms that the genotype most responsible of persistence is represented by HPV16. This result is widely reflected in the literature [17, 21).

Results showed a higher progression of the infection by HPV16, HPV18, HPV31, and HPV45, in fact they are phylogenetically linked to HPV16 and 18 and associated to a higher risk of incidence of pre-neoplastic lesions and cervical cancer [25, 26].

In particular, an increased incidence of onset of lesions greater than or equal to LSIL was noticed in the case of persistence of HPV31 and HPV45 infections, or rather genotypes phylogenetically related to HPV16 or HPV18. The presented data are certainly preliminary; further investigations and a larger sample size are required to validate the findings. However, if confirmed, the implications would be certainly significant, since 30% of cases of cervical cancer,

which are not HPV16 or HPV18 related, may be involved. Furthermore, the present data can be extremely useful for the purposes of primary prophylaxis by vaccination, in case of patients who are already positive for HPV16 or HPV18. It has been demonstrated that the vaccine formulations available in the market, in particular the bivalent form, indeed offer a cross-protection for the HPV genotypes -31 and -45 that are phylogenetically similar to -16 and -18, respectively. In particular, it is assumed that this type of gene concordance is found in specific transcription factors: capsid proteins and E6eE7 proteins (responsible for the immortality of cells); therefore validate the use of HPV persistence as a surrogate endpoint in clinical trials and potentially in cervical cancer screening, with standardization of definitions and testing, detection of persistent HPV DNA can become a valuable marker of CIN-2e3 and cancer for clinical and research applications.

In conclusion, in light of this study findings, the association of most persistent forms of HPV infection with development of pre-neoplastic lesions is clear, therefore a long period of follow-up and to carry out HPV-DNA testing with screening of first and second level, may be helpful in identifying these kind of infections and to prevent the evolution in LSIL and HSIL. At the same time, it is essential to understand epidemiological and biological features of natural history of HPV infection to lead the development of new strategies for the prevention and control of cervical cancer and to improve those already used.

References

[1] zur Hausen H.: "Papillomaviruses and cancer: from basic studies to clinical application". *Nat. Rev. Cancer*, 2002, *2*, 342.

[2] Steben M., Duarte-Franco E.: "Human papillomavirus infection: epidemiology and pathophysiology. Gynecol Oncol. 2007. 107: S2–S5.

[3] zur Hausen H. Human papillomavirus and cervical cancer". *Indian J. Med. Res.*, 2009, *130*, 209.

[4] Scardamaglia P., Carraro C., Mancino P., Stentella P.: "Effectiveness of the treatment with beta-glucan in the HPV-CIN 1 lesions". *Minerva Ginecol.*, 2010, *62*, 389.

[5] Schettino M.T., Ammaturo F.P., Labriola D, De Franciscis P., Colacurci N., Torella M.: "Betulinic acid and possible influence on the clearance of Human Papilloma Virus: cytological and virological follow-up". *Minerva Ginecol.*, 2013, *65*, 661.

[6] Castle P.E., Schiffman M., Herrero R., Hildesheim A., Rodriguez A.C., Bratti M.C., *et al.*: "A prospective study of age trends in cervical human papillomavirus acquisition and persistence in Guanacaste, Costa Rica". *J. Infect Dis.*, 2005. *191*, 1808.

[7] Colacurci N., De Franciscis P., Atlante M., Mancino P., Monti M., Volpini G., et al.: "Endometrial, breast and liver safety of soy isoflavones plus Lactobacillus sporogenes in post-menopausal women". *Gynecol. Endocrinol.*, 2013, *29*, 209.

[8] Gravitt P.E.: "The known unknowns of HPV natural history". J. Clinical Invest., 2011, *121*, 4593.

[9] Arnheim Dahlström L., Andersson K., Luostarinen T., Thoresen S., Ögmundsdottír H., Tryggvadottír L., *et al.*: "Prospective seroepidemiologic study of human papillomavirus and other risk factors in cervical cancer". *Cancer Epidemiol Biomarkers Prev.*, 2011, *20*, 2541.

[10] Yetimalar H., Kasap B., Cukurova K., Yildiz A., Keklik A., Soylu F.: "Cofactors in human papillomavirus infection and cervical carcinogenesis". *Arch, Gynecol, Obstet.*, 2012., *285*, 805.

[11] Koshiol J., Lindsay L., Pimenta J.M., Poole C., Jenkins D., Smith J.S.: "Persistent human papillomavirus infection and cervical neoplasia: a systematic review and meta-analysis". Am. J. Epidemiol., 2008, *168*, 123.

[12] Igidbashian S., Schettino M.T., Boveri S., Barberis M., Sandri M.T., Carinelli S., *et al.*: "Tissue genotyping of 37 in situ and invasive cervical cancer with a concomitant negative HC2 HPV DNA test". *J. Low Genit. Tract Dis.*, 2014, *18*, 87.

[13] Bosch F.X., Lorincz A., Muñoz N., Meijer C.J., Shah K.V.: "The causal relation between human papillomavirus and cervical cancer". *J. Clin. Pathol*., 2002, *55*, 244.

[14] Franco E.L., Villa L.L., Sobrinho J.P., Prado J.M., Rousseau M.C., Désy M., *et al.*: "Epidemiology of acquisition and clearance of cervical human papillomavirus infection in women from a high-risk area for cervical cancer". *J. Infect. Dis.*, 1999, *180*, 1415.

[15] Woodman C.B.J., Collins S., Winter H., et al.: "Natural history of cervical human papillomavirus infection in young women: a longitudinal cohort study". *Lancet*, 2001, *357*, 1831.

[16] Muñoz N., Méndez F., Posso H., Molano M., van den Brule A.J., Ronderos M., *et al.*: "Incidence, duration, and determinants of cervical human papillomavirus infection in a cohort of Colombian women with normal cytological results". *J. Infect. Dis.*, 2004, 190, 2077.

[17] Banura C., Sandin S., van Doorn L.J., Quint W., Kleter B., Wabwire-Mangen F., et al.: "Type-specific incidence, clearance and predictors of cervical human papillomavirus infections (HPV) among young women: a prospective study in Uganda". *Infect. Agents Cancer*, 2010, *5*, 7.

[18] Sammarco M.L., Del Riccio I., Tamburro M., Grasso M.G., Ripabelli G.: "Type-specific persistence and associated risk factors of human papillomavirus infections in women living in central Italy". *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 2013, *168*, 222.

[19] Howe E.R., Li Z., McGlennen R.C., Hellerstedt W., Downs L.S.: "Type-specific prevalence and persistence of human papillomavirus in women in the United States who are referred for typing as a component of cervical cancer screening". *Am. J. Obstet. Gynecol.*, 2009, *200*, 245.

[20] Carcopino X., Bolger N., Henry M., Mancini J., Boubli L., Olive D., *et al.*: "Evaluation of type-specific HPV persistence and high-risk HPV viral load quantitation in HPV positive women under 30 with normal cervical cytology". *J. Medical Virol.*, 2011, *83*, 637.

[21] Nielsen A., Kjaer S.K., Munk C., Osler M., Iftner T.: "Persistence of high-risk human papillomavirus infection in a population-based cohort of Danish women". *J. Med. Virol.*, 2010, *82*, 616.

[22] Trottier H., Mahmud S., Prado J.C., Sobrinho J.S., Costa M.C., Rohan T.E., *et al.*: "Type-specific duration of human papillomavirus infection: implications for human papillomavirus screening and vaccination". J Infect Dis 2008. 197: 1436–1447.

[23] Castle P.E., Rodríguez A.C., Burk R.D., Herrero R., Wacholder S., Hildesheim A., *et al.*: "Long term persistence of prevalently detected human papillomavirus infections in the absence of detectable cervical precancer and cancer". *J. Infect. Dis.*, 2011, *203*, 814.

[24] Schmeink C.E., Massuger L.F., Lenselink C.H., Quint W.G., Witte B.I., Berkhof J., et al.: "Prospective follow-up of 2065 young unscreened women to study human papillomavirus incidence and clearance". *Int. J. Cancer*, 2013, *133*, 172.

[25] Schettino M.T., Ammaturo F. P., Grimaldi E., Legnante A., Marcello A., Donnarumma G., *et al.*: "Persistent papillomavirus type-31 and type-45 infections predict the progression to squamous intraepithelial lesion". *Taiwan J. Obstet. Ginecol.*, 2014, *53*, 494.

[26] Jensen K.E., Schmiedel S., Norrild B., Frederiksen K., Iftner T., Kjaer S.K.: "Parity as a cofactor for high-grade cervical disease among women with persistent human papillomavirus infection: a 13-year follow-up". *Br. J. Cancer*, 2013, *108*, 234.

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