Acta Biomed 2024; Vol. 95, N. 3: e2024037 DOI: 10.23750/abm.v95i3.15352 

Original article

# Prevalence of hr-HPV genotypes among vaccinated and unvaccinated women in central Italy: a retrospective study

*Angela Serafini*[1], *Mauro De Santi*[2], *Giuditta Fiorella Schiavano*[3], *Michele De Nictolis*[1], *Giorgio Brandi*[2]

[1]Biolab - Laboratori Analisi Cliniche Convenzionati, Istituto di Ricerca, Pesaro, Italy; [2] Unit Hygiene, Department of Biomolecular Sciences, University of Urbino Carlo Bo, Urbino, Italy; [3]Department of Humanities, University of Urbino Carlo Bo, Urbino, Italy

**Abstract.** *Background and aim: The* Human papillomavirus (HPV) is the most common sexually transmitted infection worldwide. Data on the prevalence of genital HPV infection are heterogeneous since the risk of infection and the severity of diseases are related to the geographic region and population, socioeconomic conditions, and the assessed population. In the present study, the prevalence of high-risk HPV genotypes was evaluated in women who have attended at a diagnostic laboratory in the Marche region (Italy) for cervical cancer tests. *Methods:* Pap tests and biomolecular analyses were conducted in 875 women for the identification of hr-HPV genotypes and common concomitant infections of the genital tract. Multiplex Real-time PCR was used for the simultaneous identification of hr-HPV 16, 18, and non-specified pooled detection of HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68. *Results:* Of 875 women tested, 228 (26%) resulted hr-HPV positive, with a higher prevalence (28%) in the range of 35-44 years. In the unvaccinated group, the prevalence of infection was about 5 times higher *vs.* vaccinated women. The hr-HPV16 was the most diagnosed genotype, followed by hr-HPV18. The high-grade cytological abnormalities were identified only in unvaccinated women. Moreover, 71 % of hr-HPV infections were concomitant with other infections of the genital tract. *Conclusions*: A meaningful diffusion of hr-HPV, prevalently genotypes HPV-16, was observed in women > 25 years, frequently associated with other sexually transmitted infections, and a substantial difference in the risk of cervical cancer in unvaccinated compared to vaccinated women. The enhancement of primary and secondary prevention interventions must be further incentivized. (www.actabiomedica.it)

**Key words:** HPV genotypes, cervical cancer, real-time PCR, pap test, anti-HPV vaccination

## Introduction

The Human papillomavirus (HPV) is the most common sexually transmitted infection (STI) in the world (1). Generally, most of HPV infections are benign; however, a persistent infection with one of the carcinogenic HPV types can lead to the development of cervical cancer (CC) (2,3). Currently, more than 200 HPV genotypes are known, but only 20 genotypes are considered oncogenic (1,4-6); based on their risk of causing cancer, HPVs are classified as high-risk HPV (hr-HPV) or low-risk HPV (lr-HPV). The hr-HPV genotypes are the causative agents of almost all cases of CC, with about 340000 deaths in 2020 (7-9). In particular, the hr-HPV types 16 and 18 are the two most common genotypes related to CC development (10).

Most women acquire the HPV infection after their first sexual experience, and the disease develops slowly, and it usually takes 10–20 years for HPV infection-driven lesions to progress to malignancy (6, 11-16).

Data on the prevalence of genital HPV infection are heterogeneous, since the risk of HPV infection and the severity of diseases is related to the geographic region and population, socioeconomic conditions, the assessed population, as well as the availability and the quality of services for CC prevention and diagnosis (1). Worldwide, CC is the fourth most common type in women and it represents the seventh cancer type for incidence, which is variably distributed between developed and less-resourced countries (1).

The main strategies to fight HPV infections are represented by screening and vaccination. With the Global Strategy to Accelerate the Elimination of Cervical Cancer as a Public Health Problem, the WHO advice, among other measures, highlight the necessity of implementing population-based screening programs to reduce the incidence of cervical cancer globally (17).

The cervical cytology has been used for years as the standard test for CC screening (18); currently, the most diffused screening and diagnostic tools are the Papanicolaou test and the more sensitive HPV-DNA test. The aim of the HPV-DNA test is the early identification of HPV strains with high oncogenic risk. Furthermore, the CC screening in Italy has been incorporated into the National Prevention Plan 2020-2025 (19). The Italian board on health recommends screening every three years to women from 25 to 30 years of age, followed by HPV-based screening every five years, to women from 30 to 65 years of age (Exhibit 3) (20). The screening adherence in Italy is higher than 80%, although with some regional differences. The second line of prevention that can significantly reduce the risk of cervical cancer is the HPV vaccination. In 2006–2007 the Food and Drug Administration (FDA) approved the bivalent and quadrivalent HPV vaccines (including HPV 16/18 and HPV 6/11/16/18, respectively) (21), offered in Italy since 2008; then, in 2014 the FDA approved the 9-valent vaccine (HPV 6/11/16/18/ 31/33/45/52/58) (22), licensed in Italy in the 2015. This vaccine protects against 7 hr-HPV types (HPV 16/18/ 31/33/45/52/58) and 2 lr-HPV types (HPV6/11). Unfortunately, the vaccination coverage in Italy is below the optimal threshold set by the National Vaccination Prevention Plan (95% in the 12th year of life). To date, vaccine status does not affect CC screening programs (23).

The aim of the present retrospective study was to evaluate the prevalence of hr-HPV genotypes in women resident in the Marche region (Italy) who attended to the CC test in a diagnostic laboratory, and to understand if the assessed frequency of infection is related to the vaccination status (vaccinated *vs.* unvaccinated women). Moreover, concomitant infections with HPV and other infections affecting the genital tract were also evaluated.

## Methods

### *Subjects*

This retrospective study included medical reports from 875 female patients aged between 15 and 84, residing in the province of Pesaro-Urbino (PU), Marche region, Italy, who went to the Biolab Clinical Analysis Laboratory of Vallefoglia (PU, Italy) for the CC test; the considered period was between January 2020 and February 2023.

Inclusion criteria: In women of childbearing age, the cervical sampling had to be performed at least five days after the end of the menstrual cycle, no more than five days before the expected menstrual start date, and at least two days after the last sexual intercourse. Women were excluded from the study if they have used vaginal creams, ovules, douches or foams in the previous 48 hours from the pap test.

The Marche Territorial Ethics Committee approved the work on 10/19/2023, protocol number 2023/303.

### *Microbiological analyses*

A pap test and a complete cervicovaginal swab were conducted to identify simultaneously, by Real-Time PCR, the presence of hr-HPV, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Gardnerella vaginalis*, *Trichomonas vaginalis*, *Mycoplasma hominis*, *Mycoplasma genitalium*, *Ureaplasma urealitycum*, and *Ureaplasma parvum.*

The biomolecular analysis aimed at determining the presence of hr-HPV and other pathogens was conducted using QIAsymphny SP/AS and Rotor Gene Q Mdx 5plex HRM (QIAGEN) instruments.

The microbial genome was extracted and purified using the QIAsymphony DSP Virus/Pathogen Midi Kit; the QIAsymphony SP module was used for DNA purification and extraction. The HPV genome was analyzed using the GeneNavTM HPV One qPCR Kit (GenomeMe- CANADA). This *in vitro* diagnostic kit enables the detection and specific discrimination between HPV 16, HPV 18 and non-specific pooled detection of the other 12 hr-HPV genotypes (HPV 31, HPV 33, HPV 35, HPV 39, HPV 45, HPV 51, HPV 52, HPV 56, HPV 58, HPV 59, HPV 66 and HPV 68) (Table 1). The primers used are universal oligonucleotides designed on the genomic region encoding the E6/E7 proteins. Human B-actin was used as the internal control.

The detection of Chlamydia trachomatis, Neisseria gonorrhoeae, Gardnerella vaginalis, Trichomonas vaginalis, Mycoplasma hominis, Mycoplasma genitalium, Ureaplasma urealitycum, and Ureaplasma parvum was conducted using the REALQUALITY RQ-SevenSTI kit (AB Analitica).

All biomolecular analyses were conducted following the manufacturer's instructions.

*Statistical analysis*

The chi-square test was used to find differences in vaccination status and HPV positivity within age groups. Differences were considered significant at P: <0.05. To calculate the sample size, the latest national data released by the Istituto Superiore di Sanità regarding HPV prevalence by age group were considered (24). The required number of samples positive for HPV (total sample size) was calculated considering a significance level (α) of 0.05 and a power (1-β) of 0.9, resulting in 67 positive samples (G*Power software). Then, taking into account that the HPV prevalence of HPV in healthy populations is 8% (24), the total number of samples needed is at least 838.

## Results

A total of 875 women (age range 15-84 years) were included in this study and tested for the positivity to hr-HPV infection, in the period January 2020 - February 2023. Among these women, 213 (24.3%) declared anti-HPV vaccination. About 58% of vaccinated were aged between 15 and 24 years; the percentage of vaccinated women decreased with age (Figure 1; P: <0.001).

A total of 228 women (26%) resulted hr-HPV positive, with a prevalence significantly different in the various age groups (p <0.001). In detail, 14% resulted positive in the range of 15-24 years, 31% in the range of 25-34 years, 36% in the range of 35-44 years, 28% in the range of 44-65 years, and 13% in the women with 65 years or older (Figure 2).

Furthermore, the prevalence of infection in the vaccinated women was 7%, compared to 32% of the unvaccinated group (Figure 3). Interestingly, the prevalence of hr-HPV types sorted by the age groups reported in Figure 3 shows that most of the infections occurred in women aged 25-65 years, in comparison to women under 25 or over 65 years. The distribution of hr-HPV genotypes in the 228 positive women showed that 88 (38,6%) were positive for HPV 16, and 25 (10.9%) were positive for HPV 18; HPVs 31, 33,

**Table 1.** Detection scheme of the multiplex real-time PCR analysis.

| Channel | Excitation (nm) | Emission (nm) | Fluorophore | Genotyperevealed |
|---|---|---|---|---|
| Green | 470 ± 10 | 510 ± 5 | FAM (Fluorescein) | HPV 31,33,35,39,45,51,52,56,58,59,66,68 |
| Yellow | 530 ± 5 | 557 ± 5 | HEX (Hexachloro-fluorescein) | HPV 16 |
| Orange | 585 ± 5 | 610 ± 5 | ROX (X-Rhodamine) | HPV 18 |
| Red | 625 ± 5 | 660 ± 10 | Cy5 (Cyanine 5) | Human B-actin |

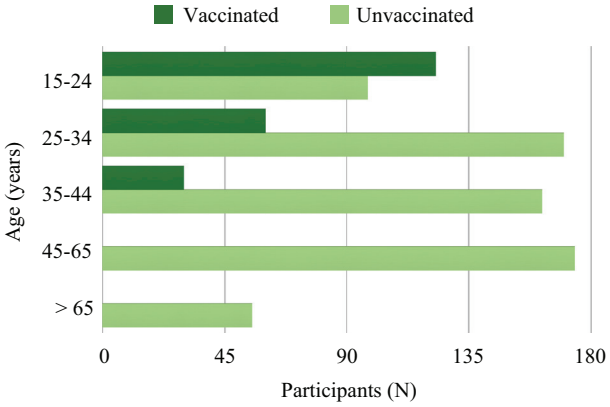


**Figure 1.** Vaccination status of women by age groups. P: <0.001; chi-squared test.

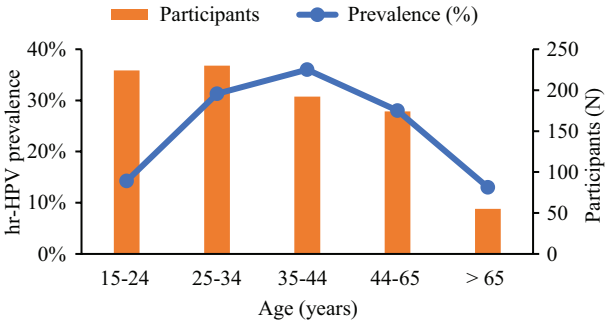


**Figure 2.** Prevalence of hr-HPV infection among women by age groups.

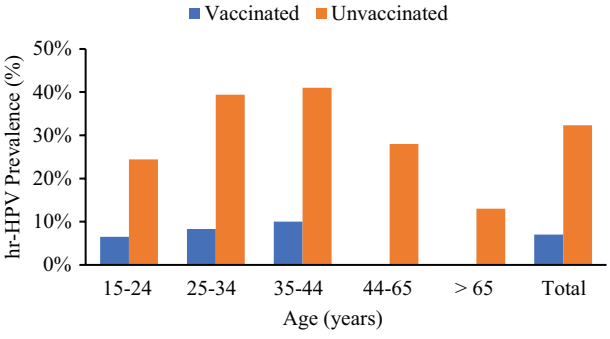


**Figure 3.** Prevalence of hr-HPV in vaccinated and unvaccinated women by age groups.

35, 39, 45, 51, 52, 56, 58, 59, 66, 68 were found in 115 (50.4%) of women. Of the 213 women vaccinated, 15 (7%) were found positive for hr-HPV, but none for genotypes 16 or 18; on the other hand, 213 (32%)

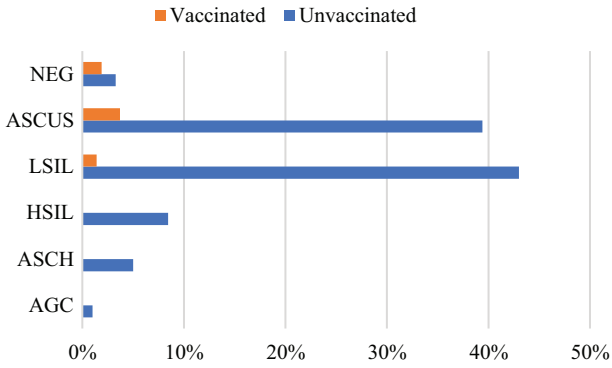


**Figure 4.** Cytological diagnosis in vaccinated and unvaccinated women positive for hr-HPV. NEG, no lesions; ASCUS, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesions; HSIL, high-grade squamous intraepithelial lesions; ASCH, atypical squamous cells; AGC, atypical glandular cells.

of 662 unvaccinated women were found hr-HPV infected, in particular with hr-HPV16 and hr-HPV 18 genotypes.

The correlation between the hr-HPV positivity and the cervical cytology result (normal/pathological) was also studied. Of the fifteen infected women with hr-HPV who have received the vaccination, none developed lesions greater than *low-grade squamous intraepithelial lesions* (LSIL). In particular, eight displayed *atypical squamous cells of undetermined significance* (ASCUS), three presented LSIL, whereas four did not display lesions (Figure 4). The cytological analysis performed in the 213 unvaccinated patients, instead, identified high-grade cytological abnormalities: 18 of them presented *high grade squamous intraepithelial lesions* (HSIL), 72% of which are related to genotype 16 (aged 28-59, median 42); 11 presented *atypical squamous cells* (ASCH); moreover, a endocervical *atypical glandular cell* (AGC) was detected in one case (Figure 4).

The distribution of concomitant infections of hr-HPV with other infections of the genital tract was found in 162 women (71%); particularly 51% with *Gardnerella vaginalis*, 16% with *Ureaplasma urealitycum*, 14% with *Mycoplasma hominis*, 7% with *Ureaplasma parvum*, 5% with *Mycoplasma genitaluim,* 4% with *Trichomonas vaginalis*, and 3% with *Chlamydia trachomatis.*

## Conclusions

The HPV leads to the most common viral infection of the reproductive tract and hr-HPV genotypes are responsible for more than 95% of CC (7). More than 90% of the HPV infected populations eventually clear the infection, and most pre-cancerous lesions resolve spontaneously. However, persistent infection may become chronic and cause cancerous lesions, to then progress to invasive CC (25).

The overall HPV prevalence in a population of women who requested the CC test at a diagnostic laboratory in the Marche region, was 26%, a value that falls within the wide range of prevalence reported in other studies (26). The HPV-16 was the most prevalent genotype (38.6%) detected, followed by HPV-18 (10.9%) and our results are in agreement with other reports (27-29). Our results show that HPV prevalence significantly differs based on age. The highest prevalence of infection (>30%) was found in women aged 35-44 years, followed by women aged 25-34 years (about 30%), whereas it was 14% in the 15-14 age group. This data could be explained, at least in part, considering the higher vaccination coverage in the age group 15-24 years *vs.* other years groups (Figure 1).

There are currently three vaccines available, all protecting against HPV types 16 and 18. The 9-valent vaccine protects against five additional oncogenic HPV types, which cause a further 20% of CC. Since 2008, the HPV vaccination has been offered free of charge to 12-year-old girls in Italy, and 70.6% of eligible people belonging to the first vaccinated cohort (females born in 1997) have received at least one vaccine dose (29). Subsequently, in 2017, the strategy was extended to males aged 11–12 years. Therefore, in our study, women aged more than 30 years were unlikely to be protected by vaccination.

In countries where HPV vaccination has been implemented with high coverage, HPV types 16 and 18 have significantly decreased (30); on the other hands, at least 90% of the new cases and deaths worldwide in 2020 occurred in low- and middle-income countries, in which the HPV vaccine have been introduced in less than 25% of their national immunization schedules (17).

In Italy, the vaccination coverage is far below the optimal threshold set by the National Vaccine Prevention Plan (95%) and shows significant differences between regions. Furthermore, during these years of the COVID-19 pandemic, anti-HPV vaccination has suffered a further decrease. To overcome this problem, and in agreement with the WHO strategy, launched in 2020 to accelerate the elimination of CC within 2030 (17), the Italian Ministry of Health will enforce the administration of the HPV vaccine in the new 2023-2025 vaccination plan (19). Currently, in the Marche Region, the anti-HPV vaccination is offered free of charge to girls up to the age of 26, to women born after 1996 who have not been vaccinated previously, and also to women who have undergone documentable treatment for HPV-related lesions (31).

Moreover, in the last twenty years, the secondary prevention represented a second line of intervention that has contributed to progressively decrease the incidence of CC in developed countries. It is based on the screening tests, that allow pre-cancerous lesions to be identified at stages in which they can easily be treated.

In this study, the diagnosis combined liquid-based cytology Papanicolaou stain (Pap test) and HPV-DNA testing. This procedure is minimally invasive, and detects hr-HPV, allowing a better risk stratification and evaluation. The HPV-DNA test was proposed several years ago by WHO (32) and by the European Guidelines for Quality Assurance in Cervical Cancer Screening (33) and proved to be cost-effective compared to the conventional Pap test in women from low- and middle-income countries (34). Conversely, in postmenopausal women, the HPV testing added to Pap test is an optimal and safe practice (35).

The cytological results reported in this study show that most of cytological lesions, e.g. HSIL, ASCH and AGC, were present only in unvaccinated women and that HSIL was mainly related to genotypes 16; conversely, in the fifteen hr-HPV-infected women that have received the vaccination, the lesions were of low grade or absent.

Furthermore, 71% of women HPV infected resulted positive for one or more of the eight sexually transmitted infections (STI) tested with the biomolecular analysis. Of these, *Gardnerella vaginalis* was the most associated infection (51%), followed by

*Ureaplasma urealitycum* (16%), *Mycoplasma hominis* (14%*), Ureaplasma parvum (7%), Mycoplasma genitaluim* (5%), *Trichomonas vaginalis (4%)* and *Chlamydia trachomatis (3%)*.

Interactions between HPV and other pathogens in cervical mucosa could enhance HPV replication and infection persistence; there is growing evidence about the role of other STI as co-factors for the development of CC in HPV-positive women (36-37).

Martinelli et al. reported a positivity of 49.2 % for one or more of the seven STI analyzed, with *Ureaplasma parvum* found to be the most frequently identified pathogen, followed by *Ureaplasma urealyticum* (38). Furthermore, Roeters et al. (39) showed an association between *Trichomonas vaginalis*, *Mycoplasma hominis* and *Ureaplasma urealyticum* with cervical inflammatory processes, suggesting that this situation may facilitate the entrance of HPV (39). Moreover, other studies (36-40) have reported that there is an increased risk of CC in women co-infected with HPV and *Chlamydia trachomatis*. This hypothesis is reinforced by Castle et al. (41), which sustains that *Chlamydia trachomatis* infection determines an increased inflammatory response that can facilitate HPV's entrance and persistence of infection into the cervix basal membrane. A recent systematic review and meta-analysis that have investigated the association between female genital mycoplasmas and HPV infection, cervical cytopathology, and CC concluded that *Ureaplasma urealyticum* and *Ureaplasma parvum* were associated with a significantly increased risk of overall HPV infection (42).

This study also has some limitations. The type of vaccine used for vaccination was not collected as well as the motivation that led the women to request a complete cervico-vaginal swab and a Pap test. Despite these limitations, we believe that our results have important clinical value because indicate the importance of the test for the diagnosis of high-risk HPV genotypes and highlights the importance of HPV vaccination in the prevention of high-grade cervical cytological abnormalities.

In conclusion, we have found a meaningful diffusion of hr-HPV, prevalently of genotype HPV-16, in women aged more than 25 years in Marche Region; it was frequently associated with STI, and a substantial difference in the risk of CC, higher in unvaccinated than vaccinated women. Despite the development of effective strategies for prevention, such as vaccination and screening, CC is still one of the major public health problems for middle-aged women; thus, the enhancement of primary and secondary prevention interventions must be further incentivized.

**Ethics Committee**: The study was conducted in accordance with the Marche Territorial Ethics Committee approved the work on 10/19/2023, protocol number 2023/303.

**Conflict of Interest**: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement, etc.) that might pose a conflict of interest in connection with the submitted article.

**Authors Contribution**: GB conceptualization; GB, AS methodology; AS software; GB, MDS, GFS validation; GB formal analysis; AS, investigation; GB, MDS, GFS data curation and original draft preparation: GB, MDS, GFS, AS review and editing. All authors have read and agreed to the published version of the manuscript.

## References

1. Kombe AJK, Li B, Zahid A, et al. Epidemiology and Burden of Human Papillomavirus and Related Diseases, Molecular Pathogenesis, and Vaccine Evaluation. Front Public Health. 2021; 8:552028. doi: 10.3389/fpubh.2020.552028.
2. World Health Organization. Human papillomavirus vaccines: WHO position paper. May 2017-Recommendations. Vaccine.2017; 35:5753–55. doi. org/ 10. 1016/j.
3. Walboomers JM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol. 1999; 189:12– 9. doi.org/10.1002 /(SICI) 1096-9896(199909)189:1%3C12::AID-PATH431 %3E3.0.CO;2-F.
4. Plummer M, de Martel C, Vignat J, Ferlay J, Bray F, Franceschi S. Global Burden of Cancers Attributable to Infections in 2012: A Synthetic Analysis. Lancet Global Health. 2016:4:e609–16. doi: 10.1016/s2214-109x(16)30143-7.
5. Serrano B, Brotons M, Bosch FX, Bruni L. Epidemiology and Burden of HPV-Related Disease. Best Pract Res Clin Obstet Gynaecol. 2018: 47:14–26.doi: 10.1016/j.bpobgyn. 2017. 08.006.
6. de Martel C, Plummer M, Vignat J, Franceschi S. Worldwide Burden of Cancer Attributable to HPV by Site, Country and HPV Type. Int J Cancer. 2017: 141:664–70. doi: 10 .1002/ ijc.30716.

7. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71: 209–249.doi: 10.3322/caac.21660
8. Arbyn M, Weiderpass E, Bruni L, et al. Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis. Lancet Glob Health. 2020; 8:e191–e203. doi. org /10. 1016/ S2214- 109X(19) 30482-6.
9. Crosbie EJ, Einstein MH, Franceschi S, Kitchener HC. Human papillomavirus and cervical cancer.Lancet.2013; 382: 889–99. doi. org/ 10. 1016/ S0140- 6736(13) 60022-7.
10. Burd EM. Human papillomavirus and cervical cancer. Clin Microbiol Rev. 2003; 16:1–17.doi:10.1128/CMR.16.1.1-17.2003.
11. Burd EM. Human papillomavirus laboratory testing: The changing paradigm. Clin. Microbiol. Rev. 2016; 29: 291–319. doi. org/10.1128/ CMR.00013-15.
12. Bharti AC, Singh T, Bhat A, Pande D, Jadli M. Therapeutic startegies for human papillomavirus infection and associated cancers. Front Biosci. (Elite Ed). 2018; 10:15–73. doi. org/ 10. 2741/ e808.
13. Doorbar J, Egawa N, Griffin H, Kranjec C, Murakami I. Human papillomavirus molecular biology and disease association. Rev Med Virol. 2015; 25:2–23. doi.org/10.1002 /rmv.1822.
14. Palefsky JM. Epidemiology of human papillomavirus infections. In: UpToDate, Post TW (Ed), 2016; UpToDate, Waltham, MA.
15. Schiffman M, Clifford G, Buonaguro FM. Classification of weakly carcinogenic human papillomavirus types: Addressing the limits of epidemiology at the borderline. Infect Agent Cancer. 2009; 4:8. doi:10.1186/1750-9378-4-8.
16. Petca A, Borislavschi A, Zvanca ME, Petca RC, Sandru F, Dumitrascu MC. Non-sexual HPV transmission and role of vaccination for a better future (Review). Exp Ther Med. 2020; 20:186. doi: 10.3892/etm.2020.9316.
17. World Health Organization. Global strategy to accelerate the elimination of cervical cancer as a public health problem. 2020. www.who.int/publications/i/item/9789240014107.
18. Arbyn M, Anttila A, Jordan J, et al. European guidelines for quality assurance in cervical cancer screening second edition—summary document. Ann Oncol. 2010;21: 448–58. doi.org/ 10.1093/ anno nc/ mdp471.
19. Ministero della Salute. Piano Nazionale della Prevenzione 2020-2025. 2020.www.salute. gov.it.
20. Ministero della Salute. Screening per il tumore del collo dell'utero. 2019. www.salute.gov.it.
21. Ciavattini A, Giannella L, De Vincenzo R, et al. HPV vaccination: the position paper of the Italian society of colposcopy and cervico-vaginal pathology (SICPCV). Vaccines (Basel). 2020;8(3):E354. doi:10.3390/ vaccines8030354.
22. Food and Drug Administration. FDA approves gardasil 9 for prevention of certain cancers caused by five additional types of HPV. Available from: https://www.esmo.org/oncology -news/archive/fda-approves-gardasil-9-for-prevention-of -certain-cancers-caused-by-five-additional-types-of-hpv. Accessed September 20, 2023.
23. Perkins RB, Guido RS, Castle PE, et al. ASCCP risk-based management consensus guidelines for abnormal cervical cancer screening tests and cancer precursors. J Low Genit Tract Dis. 2020;24(2):102–31. doi:10.1097/LGT.0000000000000525.
24. Giorgi Rossi P, Chini F, Borgia P, et al. Epidemiologia del Papillomavirus umano (HPV), incidenza del cancro della cervice uterina e diffusione dello screening: differenze fra macro aree in Italia. Epidemiol Prev. 2012; 36(2):108-19. PMID: 22706361.
25. Lei J, Ploner A, Elfström KM, et al. HPV Vaccination and the Risk of Invasive Cervical Cancer. N Engl J Med. 2020; 383:1340-8. doi: 10.1056/NEJMoa1917338.
26. Adams AR, Nortey PA, Dortey BA, Asmah RH, Wiredu EK. Cervical human papillomavirus prevalence, genotypes, and associated risk factors among female sex workers in greater Accra, Ghana. J Oncol. 2019;2019:8062176. doi: 10.1155 /2019/8062176.
27. Piana A, Sotgiu G, Castiglia P, et al. Prevalence and type distribution of human papillomavirus infection in women from North Sardinia, Italy. BMC Public Health. 2011; 11:785.doi: 10.1186/1471-2458-11-785.
28. Piana A, Sotgiu G, Cocuzza C, et al.High HPV-51 prevalence in invasive cervical cancers: Results of a pre-immunization survey in North Sardinia, Italy. PLoS ONE. 2013; 8: e63395. doi: 10.1371/journal.pone.0063395.
29. Chan CK, Aimagambetova G, Ukybassova T, Kongrtay K, Azizan A. Human Papillomavirus Infection and Cervical Cancer: Epidemiology, Screening, and Vaccination—Review of Current Perspectives. J Oncol. 2019;10:2019:3257939. doi: 10.1155/2019/3257939.
30. Kavanagh K, Pollock KG, Potts A, et al. Introduction and sustained high coverage of the HPV bivalent vaccine leads to a reduction in prevalence of HPV 16/18 and closely related HPV types. Br J Cancer. 2014; 110:2804–11.doi: 10.1038 /bjc.2014.198.
31. Delibera della Giunta Regionale, seduta del 3/02/2020 delibera 74. Modifica della DGR 458/2017 concernente "Adeguamento dell'offerta vaccinale nella Regione Marche in relazione al recepimento del nuovo piano Nazionale Prevenzione Vaccinale 2017-2019-Direttive alle Aziende del S.S.R".
32. Sichero L, Picconi MA, Villa LL. The contribution of Latin American research to HPV epidemiology and natural history knowledge. Braz J Med Biol Res 2020;53(2):e9560. doi: 10.1590/1414-431X20199560.
33. Schiffman M, Doorbar J, Wentzensen N, et al. Carcinogenic human papillomavirus infection. Nat Rev Dis Prim 2016;1(2):16086.doi: 10.1038/nrdp.2016.86.
34. Casas CPR, Albuquerque RCR, Loureiro RB, et al. Cervical cancer screening in low- and middle-income countries: A systematic review of economic evaluation studies. Clinics (Sao Paulo). 2022;77:100080. doi: 10.1016/j.clinsp.2022.100080.
35. Salibay C, Chen Z, Ma B, et al. High-risk HPV testing improves accuracy in detection of CIN2+ lesions in ASC-H postmenopausal women? An academic hospital experiences. J Am Soc Cytopathol. 2023;12(1):58-65. doi: 10.1016/j .jasc.2022.08.004.

36. Zhu H, Shen Z, Luo H, Zhang W, Zhu X. Chlamydia Trachomatis Infection-Associated Risk of Cervical Cancer: A Meta-Analysis. Medicine 2016; 95: e3077. doi: 10.1097 /MD.0000000000003077.
37. Wang L, Zhu L, Li H, et al. Association between asymptomatic sexually transmitted infections and high-risk human papillomavirus in cervical lesions. J Int Med. Res. 2019; 47: 5548–89. doi: 10.1177/0300060519865633.
38. Martinelli M, Musumeci R, Sechi I, et al. Prevalence of Human Papillomavirus (HPV) and Other Sexually Transmitted Infections (STIs) among Italian Women Referred for a Colposcopy. Int J Environ Res Public Health. 2019 Dec 9;16(24):5000. doi: 10.3390/ijerph16245000.
39. Roeters AM, Boon ME, van Haaften M, Vernooij F, Bontekoe TR, Heintz AP. Inflammatory events as detected in cervical smears and squamous intraepithelial lesions. Diagn Cytopathol. 2010; 38:85–93. doi: 10.1002/dc.21169.
40. Mancini F, Vescio F, Mochi S, Accardi L, di Bonito P, Ciervo A. HPV and Chlamydia trachomatis coinfection in women with Pap smear abnormality: Baseline data of the HPV Pathogen ISS study. Infez. Med. 2018; 26;139–44. PIMD299332086.
41. Castle PE, Giuliano AR. Chapter 4: Genital tract infections, cervical inflammation, and antioxidant nutrients–assessing their roles as human papillomavirus cofactors. J Natl Cancer Inst Monogr. 2003; 31: 29–3. doi: 10.1093/oxfordjournals. jncimonographs.a003478.
42. Ye H, Song T, Zeng X, Li L, Hou M, Xi M. Association between genital mycoplasmas infection and human papillomavirus infection, abnormal cervical cytopathology, and cervical cancer: A systematic review and meta-analysis. Arch Gynecol Obstet. 2018; 297: 1377–87. doi: 10.1007 /s00404-018-4733-5.

**Correspondence:**
Received: 25 October 2023
Accepted: 8 December 2023
Giorgio Brandi PhD
Unit of Hygiene, Department of Biomolecular Sciences,
University of Urbino "Carlo Bo"
Via S. Chiara, 27 61029 Urbino (PU) Italy
Phone: 0722303542
E-mail: giorgio.brandi@uniurb.it