[Frontiers In Bioscience, Landmark, 23, 1587-1611, March 1, 2018]

Genital human papillomavirus infections

Vanja Kaliterna[1,2], Zvonimir Barisic[1]

[1]Clinical Microbiology Department, Teaching Institute of Public Health of Split and Dalmatia County, Vukovarska 46, 21000 Split, Croatia, [2]University of Split School of Medicine, Soltanska 2, 21000 Split,Croatia

TABLE OF CONTENTS

1. ABSTRACT

Human papillomavirus (HPV) infections can have clinical presentations from self-limited benign growth in the skin and mucosal epithelia to malignant growth. HPV infects basal epithelial cells (undifferentiated keratinocytes) of the squamous-columnar junction, especially of the cervix. Although today we understand HPV oncogenesis very well, we have very powerful methods of diagnosis, treatment and prevention of HPV related precancerous lesions, however, more than 270,000 women annually die from cervical cancer worldwide. Integrating HPV vaccination with new, more sensitive, cervical screening assays as part of routine preventive care will improve healthcare for all women. The availability of prophylactic HPV vaccines has provided powerful tools for primary prevention of cervical cancer and other HPV-associated diseases. Secondary prevention through primary high-risk HPV (hr-HPV) testing has the potential to further reduce morbidity and mortality of cervical cancer. However, to achieve the maximum benefit of screening, there is need to continue to identify women who are either unscreened or under-screened. Synergies between HPV vaccination and HPV screening is recommended to improve the effectiveness and cost-effectiveness of prevention HPV-related disease.

2. INTRODUCTION

Papilloma viruses (PV) are small non-enveloped double-stranded DNA viruses that belong to the Papillomaviridae family that infect warm-blooded vertebrates, mammals and birds (1-3). Virtually all humans are simultaneously colonized by several human papilloma viruses, causing asymptomatic infections in skin and mucosa (2). Human papillomavirus (HPV) infections can have clinical presentations from self-limited benign growth in the skin and mucosal epithelia (hand or plantar warts), to malignant growth (cervical or anal cancer) (2, 3).

Skin and genital warts were well known among ancient Greek and Romans. Particularly genital warts were considered as the result of sexual promiscuity and thus, regarded as potentially infectious (4). Also, viral DNA similar to HPV-18 and to HPV-91 was retrieved from a genital lesion in a female XVI-century mummy (2). An Italian physician, Rigoni-Stern (1842), analyzed death certificates of women in Verona during the period 1760-1839 and noted of a high frequency of cervical cancer in married women, widows and prostitutes, but their rare occurrence in virgins and nuns. He concluded that the development of this type of cancer should be related to sexual contacts (4).

HPV, an epitheliotropic virus, infects basal epithelial cells (undifferentiated keratinocytes) of the squamous-columnar junction, especially of the cervix. The virus makes its entry into the basal epithelial cells through micro-wounds or micro-abrasions (2, 5). Heparan sulfate proteoglycans found in the extracellular matrix on the cell surface are thought to be the initial receptors of HPV. Alpha 6-integrin and laminin-5 play an important role as co-receptors for an efficient viral infection (6-8).

Papilloma viruses have circular double-stranded DNA genomes with sizes close to 8 kb. It encodes early genes: three oncogenes, E5, E6, and E7, which modulate the transformation process and two regulatory proteins, E1 and E2, which modulate transcription and replication, E4 that allows viral assembly and late genes encoding two structural proteins, L1 and L2, which compose the viral capsid (3, 9). There is also an upstream regulatory region (URR) harbouring transcription factor-binding sites and controlling gene expression (2).

Classification of HPVs is based on the nucleotide sequence of the open reading frame (ORF) coding for the capsid protein L1 (1). A new papillomavirus isolate is recognized as such if the complete genome has been cloned and the DNA sequence of the L1 ORF differs by more than 10% from the closest known PV type. Differences between 2% and 10% homology define a subtype and less than 2% a variant (3). Among 184 different HPV genotypes, only 40 diverse types can infect anogenital region which can be classified into 3 classes based on their oncogenic potential. HPV-16, -18, -31, -33,

-35, -39, -45, -51, -52, -56, -58, -59, -68, -73 and -82 are included in high-risk group (hr-HPV) while HPV-6, -11, -40, -42, -43, -44, -54, -61, -70, -72 and -81 are included in low-risk group (lr-HPV) whereas HPV-26, -53 and -66 belong to the group of intermediate risk (10-12). Authors also found sublineages of some types of HPV. So, among HPV-16 were described European (Eu), Asian (As), Asian-American (AA), North American (NA), African-1 (AF1), and African-2 (AF2) variants, in HPV-18 Asian-American (A1 and A2), European (A3 to A5) and African variants (B and C) and in case of HPV-52 A, B and C variant lineages was reported (13-21).

3. VIRAL ONCOGENESIS

It seems that papillomaviruses, like many other DNA tumour viruses, cause cancers when their regulated pattern of gene expression is disturbed (22).

High-grade neoplasia represents an abortive infection in which viral gene expression becomes deregulated, and the normal life cycle of the virus cannot be completed. Most cervical cancers arise within the cervical transformation zone at the squamous/columnar junction, and it has been suggested that this is a site where productive infection may be inefficiently supported (22). Complex formation between the products of oncogenes and tumor suppressor genes is believed to be important in cellular transformation, providing a mechanism to disrupt the normal physiological functions of the specific tumor suppressor gene products (23).

Although integration is not a part of the normal HPV life cycle, hr-HPV DNA is often integrated into the human genome in cervical squamous cell carcinoma (SCC) tissue sample. It has been proposed that integration can be an early event associated with progression from low-grade squamous intraepithelial lesion (LSIL) to high-grade squamous intraepithelial lesion (HSIL), so expected to be a biomarker in cancer progression (10). The site of integration is distributed throughout the genome as chromosomal fragile sites where DNA double strand breaks are failed to repair. DNA damage is often induced by oxidative molecules and HPV proteins E1, E6 and E7 (10).

Although variable portions of the hr-HPV genome are deleted in viral integrants, consistent features are loss of the viral E2 gene, which can inhibit transcription from the integrated viral promoter. Integration is detected in almost 90% of cervical carcinomas, in a very high percentage of HPV-16 and HPV-18 positive cervical cancers, and at substantially lower frequencies in high-grade cervical precancerous lesions, however, almost never in early HPV-induced lesions (24, 25). In general, integration leads to increased expression and stability of transcripts encoding the E6 and E7 proteins, which bind and disrupt the function of a number of key cellular proteins such as p53 and pRb. Such effects are restricted to high-risk HPV types, providing a biological explanation for the difference in cancer risk associated with hr-HPV and lr-HPV types (25).

The oncogenic activity of the E6 proteins of the high-risk HPVs has been correlated with their ability to interact with and inactivate the cellular p53 protein (26). E6 proteins stimulate the ubiquitin-dependent degradation of p53 *in vitro*. This suggested that E6 might function in immortalization by stimulating the degradation of p53 (26).The p53 gene has tumor suppressor properties and it is a target for several of the oncoproteins encoded by DNA tumor viruses (27).

The E7 proteins of different HPVs were assessed for their ability to form complexes with the retinoblastoma tumor suppressor gene product (p105-RB) (23). One consequence of this interaction is disruption of the complex that pRB can form with the E2F transcription factor (26). The E7-mediated release of E2F from these complexes is thought to influence the expression of genes involved in cell cycle progression (26). Deletions or mutations of the retinoblastoma gene, RB1, are common features of many tumors and tumor cell lines (28).

So, an important occurrence in cervical carcinogenesis is deregulated expression of the hr-HPV oncogenes E6 and E7. Several risk factors for cervical neoplastic progression are likely to contribute to viral oncogene deregulation, particularly integration of hr-HPV into the host genome (25).

4. PATHOGENESIS AND TRANSMISION OF INFECTION CAUSED BY HUMAN PAPILLOMAVIRUS

After a successful binding to the receptor, virus is internalized into the cell by endocytosis (29, 30). Then viral genome enters into the nucleus. The E6 and E7 HPV proteins hijack the checkpoint mechanisms ensuring that the different cell cycle steps are completed properly. That allows the differentiating keratinocyte to enter uncontrolled proliferation (2). Viral genome replication switches to support productive viral genome amplification concomitant with increased levels of the E1, E2, E4 and E5 proteins (2). As a result, viral copy number amplifies to thousands of copies per cell. In the terminally differentiated layer of epithelium L1 and L2 capsid proteins are expressed and viral particles are assembled. The virions are sloughed off with the dead squamous cells of the host epithelium for further transmission (2, 10).

Women acquire HPV through sexual intercourse with an infected partner and thus HPV prevalence is high around the age of sexual debut, when

exposure is high in the absence of immunity. Infections "clear" within 2 years in more than 90% of individuals (31). Therefore conservative management of adolescent girls with high-grade cytological results is a good therapeutic option based on the latest knowledge about the natural history of HPV infection (32). The infections that persist have a higher risk of progression to true cervical cancer precursor lesions as cervical intraepithelial neoplasia (CIN 3), and these lesions are likely to progress to cervical cancer over a period of several years if left untreated (2, 31). Progression of precursor lesions to invasive cancer usually requires more than one decade, which allows time for the cancer screening programs, identification and treatment (33). Spanish study included women aged 25 years or younger with high-grade cytological lesions which were followed up at 15 months. During follow-up, 63% of high-grade cytological lesions and all high-grade histological lesions were cleared. HPV was eliminated from 23% of patients with one HPV serotype and 27% with multiple HPV serotypes without any treatment (32).

Some clinical and epidemiological observations have documented that genital HPVs can also be transmitted in other ways, especially from mother to child. Indirect transmission via HPV-contaminated fomites (clothing, sheets, towels, objects and instruments) has also been suggested by some studies, but its impact in passing and inducing active infections is most likely small if any (34). High risk HPVs were identified in milk samples of 15% normal lactating women and it suggests the possibility of milk transmission of these viruses (35).

5. IMMUNOLOGY AND RISK FACTORS FOR HPV INFECTION

Natural infection by HPVs causes specific immune response in most cases but only a limited number of individuals develop high antibody titres that provide protection against reinfection with the same type (36-38). It is still unclear why natural immunity does not always generate protective immune responses (2). Possible explanation is that during infection, L1 protein will usually be exposed to the immune system at very low dose and predominately in a noninflammatory setting, a situation that would seem unlikely to induce a long lived germinal center reaction. With intramuscular vaccination, the antigen is delivered at high dose to the systemic immune system in a pro-inflammatory context, due to the presence of the adjuvant. This type of exposure of a repetitive particulate antigen generally induces a strong germinal center reaction in the draining lymph nodes (39).

Persistence of the virus is essential for development of high-grade CIN and cervical cancer and factors that correlate with higher persistence rates include age, immunodeficiency, cigarette smoking, long-duration oral contraceptive use, *Chlamydia trachomatis* infection and multiple live births (31, 40). Japanese study conducted among female college students shows that 125 (16.2.%) of them were positive for hr-HPV. They showed that HPV infection was associated with smoking history, total number of partners, number of partners in the past 6 months, improper use of condoms, and chlamydial infection (41). Study performed in Brazil try to find link between hr-HPV and other sexually transmitted diseases in the risk of developing cervical cancer. Authors found that *C. trachomatis* and *Neisseria gonorrhoeae* were the primary pathogens associated with hr-HPV for the increased risk for all grades of cervical abnormalities but mainly for HSIL, suggesting a possible synergistic action in cervical lesions progression (42). Latent infection with Epstein-Barr Virus (EBV) can act as a carcinogenic co-factor. Presence of EBV co-infection is associated with a five-fold higher risk of integration of concurrent hr-HPV into the human genome which is an important step in the progression to invasive carcinoma (43).

The vaginal microbiota plays a significant role in health and disease of the female reproductive tract (44). The investigators observed that HPV-positive women having higher species diversity and significantly less *Lactobacillus* spp. Presence, compared to their uninfected twin (45). Bacterial vaginosis, often associated with strict anaerobic species including *Gardnerella*, *Megasphera*, *Sneathia* and *Prevotella* has previously been correlated with higher incidence, prevalence and persistence of HPV infection and with development of CIN (44, 46-48). In the case of the female reproductive tract, health is more commonly associated with low microbial diversity and dominance by only one or a few species of *Lactobacillus* (40, 44). Brotman and colleagues also suggested that vaginal microbial community CST II (community state type II), dominated by *Lactobacillus gasseri*, may be associated with the most rapid clearance of acute HPV infection (46). Rational selection of probiotics would be most effective for women's health and protection from sexually transmitted diseases (46). Probiotics have also been suggested as an intervention to promote HPV clearance, and *in vitro* and *in vivo* evidence exist to support this technique (40). Results from the Ludwig-McGill cohort study provide novel insights on reproductive health and vaginal hygiene factors in HPV infection. This study shows that use of menstrual cloths had a slightly protective effect on HPV infections, while use of hygienic tampons had an adverse effect. Authors speculate that tampon use can lead to dryness and irritation in the vagina and cervix, thereby increasing susceptibility to HPV infections through possible tearing or microabrasions (49).

The International Agency for Research on Cancer (IARC) has classified both HPV and human

immunodeficiency virus type 1 (HIV) as carcinogens: HPV is a direct carcinogen and HIV-1 is an indirect carcinogen through immune suppression (50). HPV prevalence is very high in HIV-infected people (50). The meta-analysis performed on 113 publications showed that prevalence of cervical high-risk HPV infection among persons living with HIV/AIDS was 46% versus 29% in US females (51). Studies performed in Cape Town, South Africa on 1,371 HIV-positive women and 8,050 HIV-negative women, aged 17-65 years, showed that HPV prevalence was higher among HIV-positive women (52.4.%) than among HIV-negative women (20.8.%) (52). HIV-positive women were more likely to have CIN 2 or 3 than HIV-negative women and infections with multiple high-risk HPV types were more common in HIV-positive than HIV-negative women (52, 53).

Immunosuppressed allograft recipients are at a high risk of certain infections such as HPV and its related malignancies (54). In Iranian study, performed on 58 female kidney transplant recipients, the incidence of HPV infection was zero before transplant surgery, but it increased to 6.9.% one year later. Authors suggested that HPV and Pap test screening should start before planned transplant surgery, and they should be repeated at regular intervals in order to avoid irreversible situations such as high-grade SILs that are difficult to treat (53). On the other side Polish studies showing no correlation between hr-HPV presence and immunosuppressive regimen, underlying disease, graft function, or time interval from transplantation (55, 56).

The natural history of CIN and HPV infection during pregnancy was studied among pregnant and non-pregnant women. Authors compared the rates of persistence, progression and regression of CIN by colposcopically guided biopsy during pregnancy with outcome in non-pregnant-women. The postpartum histopathologic evaluation of the pregnant cohort revealed a significantly higher tendency to spontaneous regression (56.9.% versus 31.4.%) and a considerably, but not significantly higher complete remission rate (41.2.% versus 27.5.%) when compared to the non-pregnant cohort. Authors observed a significantly lower CIN persistence rate than in the non-pregnant cohort (39.2. versus 58.8.%). The progression rate was notably low in the pregnant cohort (3.9. %) and no progression to invasive cancer was observed. This study suggests that a conservative management of CIN in pregnancy is safe because of high regression rates and low progression rates after delivery (57).

6. EPIDEMIOLOGY OF HUMAN PAPILLOMA-VIRUS

Every year more than 270,000 women die from cervical cancer and the majority of these deaths are in low and middle income countries (58, 59). The worldwide prevalence of infection with HPV in women without cervical abnormalities is 11-12% (59). Cervical cancer ranks 3rd place amongst cancers affecting women worldwide and 2nd in developing countries (60). Women in developing countries account for 85% of the global incidence of cervical cancer. Incidence rates are nearly double in developing compared to developed countries, 17.8.% and 9.0.%, respectively. This difference is thought to be largely due to the implementation of early diagnostic screening methods, which have reduced the risk of cervical cancer associated with persistent HPV infection (58, 60). The peak age for infection in girls is around 20 years (40). It is estimated that 80% of sexually active women will have been infected at some point by age 50 (40). To estimate of the prevalence and characteristics of HPV genomes, authors examinated tissue from the cervices of 99 women undergoing hysterectomy for reasons unrelated to epithelial abnormality. *In situ* hibridization detected hr-HPV in 42% of study population (61). HPVs-16 and -18 are the most oncogenic and prevalent viruses and are responsible for around 70% of cases worldwide (40, 62), although the estimated HPV-16/18 fraction is slightly higher in more developed (72-77%) than in less developed (65-72%) regions (63). The eight most common types (HPV-16, -18, -33, -45, -31, -58, -52 and -35) accounted for 90% of cases (63). Different studies noticed regional differences among prevalence of HPV types in populations. HPV types 16 or 18 (HPV 16/18) were identified in 93.5. % of HPV-positive invasive cervical cancers (ICC) from the Indianapolis, Indiana, USA, in 93.8. % from Kenya, and in 61.8. % from Botswana (64). Because HPV vaccines are available, this malignancy is theoretically preventable, but the vaccines are largely type-specific in protection against infection. It is important to understand differences in the HPV types causing ICC in different regions of the world (64).

Many studies have examined the knowledge and opinions about HPV. Argentinean study showed that women, single people, workers, the better educated, those who have had a STDs or HPV and receiving information through medical or educational establishments had greater knowledge of the topic (65). In Greece, senior students, students of the health sciences, and students with a working mother, had more often a higher total level of knowledge about HPV (59). In studies conducted on adolescents and university students in Scotland, Portugal, and Italy, it was reported that percentage of respondents who knew that HPV causes cervical cancer was above 90 % (65). The most significant reasons for ignorance of having vaccination (39.4.% answers) were “Vaccination is expensive” and “Worried about side effects” (66). A prospective survey of women 18–26 years of age was conducted at an urban university student health clinic (University of Missouri, Kansas City) and regardless

of the woman's vaccination status, women had significantly higher (strongly agree/agree) preferences for the male partner being vaccinated with quadrivalent HPV vaccine than not caring if he was vaccinated (67).

7. CLINICAL MANIFESTATIONS OF HUMAN PAPILLOMAVIRUS INFECTION

The vast majority of HPV infections at all sites are subclinical and asymptomatic. These infections are characteristically noninflammatory; therefore, most individuals who acquire HPV never know that they have been infected (68).

Female LR-HPV infection of the anus, cervix, vagina, and vulva can result in benign warts caused predominantly by HPV types 6 and 11 (68).

About 90% of hr-HPV infections are self-limited and regress spontaneously within several months (5, 6). In about 10% of the cases, however, the infection persists and may progress to a transforming hr-HPV infection that induces outgrowth of high grade preneoplastic lesions or invasive cancers (24). The precancerous lesions, caused by HPV, which progress to squamous cell carcinoma (SCC) are called cervical intraepithelial neoplasia (CIN) or squamous intraepithelial lesion (SIL) which is classified according to the grade of the lesion. A productive hr-HPV infection may develop into low-grade SILs (LSILs) which are nonmalignant bearing the low risk of progression to malignancy and corresponding to CIN 1. The high-grade SILs (HSILs) comprise abortive virus infections in which there is deregulated expression of HPV early genes in basal epithelial cells, with a greater risk of progression to invasive disease and corresponding to CIN 2/3. Squamous cell carcinoma (SCC) is the most common type of cervical cancer (10, 69, 70). hr-HPV infection can cause both high-grade squamous intraepithelial lesions (HSILs), CIN, VIN (vulvar intraepithelial neoplasia), VAIN (vaginal intraepithelial neoplasia), and AIN (anal intraepithelial neoplasia) as well as invasive cancer at all female anogenital sites, though hr-HPV cancers occur at a much higher frequency at the cervix than at the other sites (68).

The glandular epithelium is also vulnerable to HPV infections, especially by HPV-18, which may cause adenocarcinoma (ADC), while HPV-16 is being identified more often in SCC than in ADC (63). The adenocarcinoma of the uterine cervix accounts for 10 to 20% of the premalignant and malignant lesions and is different from the cervical intraepithelial neoplasia and invasive squamous cell carcinoma (71).

HPV infection in the anogenital tracts of men is more likely to remain undetected. Benign warts and flat lesions can occur in all areas of the male lower genital tract, and HR-HPV infection may rarely result in the development of anal and penile intraepithelial neoplasia (AIN and PIN) and cancers (68). Anal infection with oncogenic genotypes of HPVs is a key causal precursor of anal cancer via the same mechanism as for cervical cancer. Similar to cervical cancer, anal cancer is suspected of progressing from AIN. The spectrum of AIN includes low-grade lesions (AIN 1) and high-grade lesions (AIN 2 and AIN 3) (72).

8. DIAGNOSIS OF HUMAN PAPILLOMAVIRUS INFECTION

Molecular understandings of malignant transformations of the lower genital tract caused by Human papillomavirus and epidemiologic information have led to development of many strategies for timely detection and early intervention. Newer tests for oncogenic HPV genotypes have made it possible to predict the risk of future development of cervical cancer (73). Selection of a screening assay involves a balance between accuracy, reproducibility, cost, and easy integration into screening programs (coverage, acceptability, technology requirements) (74).

8.1. Direct visual inspection with acetic acid of the cervix (VIA)

VIA is the simplest and cheapest method that is used in many screening programs for cervical cancer in low income countries. It involves examination of the cervix with naked eye, using a bright light source, after application of 3-5% dilute solution of acetic acid to the cervix using cotton swab or spray. Detection of well-defined aceto-white areas close to the squamo-columnar junction indicates positive test. In neoplasia there is higher concentration of intracellular proteins that with acetic acid produce aceto-whitening (due to a reversible coagulation of intracellular proteins with acetic acid). Advantages of VIA are: it is inexpensive, it can be carried out using modest equipment without the need of laboratory infrastructure, health workers can be rapidly trained to perform VIA in short courses, it yields an immediate result and it is possible to treat abnormal lesions at the same visit "screen and treat" The test has a sensitivity to detect high-grade precursor lesions and cervical cancer of 84% (range 66-96%) and specificity of 82% (range 64-98%). VIA done alone is not sufficient to diagnose cervical lesions, but if it is followed by HPV DNA test could be cost effective cervical cancer screening strategy in low resource areas. Visual inspection with Lugol's iodine (VILI) instead of acetic acid can be used too (identification of mustard-yellow lesions in the cervix immediately after application) (73, 75-78).

8.2. Cervical cytology using either conventional or liquid based cytology (LBC)

Cervical cytology as a standard for secondary prevention has been conducted in developed healthcare systems for many years. Organized and

quality assured cytology-based screening programs have substantially reduced cervical cancer incidence in many developed countries, where adequate resources exist to ensure high quality and good coverage of the population at risk. The key of this success lies in a systematic program where every woman in the screening age range received regular invitations (79).

8.2.1. Conventional cytology or Pap smear

Conventional cytology or Pap smear has been the main method of cervical screening for 60 years. It was the first cervical screening test and its use significantly reduces the incidence of cervical cancer. After taking sample with spatula, brush or plastic broom, exfoliate is rolled immediately onto a glass slide, then fixing and staining (76).

8.2.2. Liquid based cytology

Liquid based cytology (LBC) is more sensitive than conventional cytology. After taking sample with brush, exfoliate is stirred immediately into a pot containing a preservative fluid (ThinPrep, Hologic, Bedford, MA, USA) and, on receipt at the laboratory, the cells are aspirated onto a filter and stained on a glass slide. Slide has a more homogenously spread preparation in compare to Pap smear, and LBC was shown to reduce inadequate slides by 80%, thereby decreasing a need to re-testing and increasing laboratory throughput. The liquid residue of sample can be used for further testing, such as HPV (79).

According “The 2014 Bethesda System” for reporting cervical cytology, squamous epithelial cell abnormalities are classified as: 1. Atypical squamous cells (ASC): - of undetermined significance (ASC-US), or - cannot exclude HSIL (ASC-H), 2. Low-grade squamous intraepithelial lesion (LSIL) (HPV mild dysplasia, CIN 1), 3. High-grade squamous intraepithelial lesion (HSIL) (moderate and severe dysplasia; CIN 2 and CIN 3), 4. Squamous cell carcinoma (SCC). The dichotomous reporting terminology for LSIL and HSIL is maintained and reflects our current understanding of the natural history of HPV-related infections - low-grade changes represent productive, largely transient HPV infection, and high-grade morphology represents a precancerous lesion. The focus of cervical cancer screening is primarily aimed at detection and treatment of HSIL (80).

The sensitivity of cytology to detect high-grade lesions ranges from 30% to 87% with an average specificity of 62% (61-94%), depending on the laboratory, the experience of the cytologists, the adequacy of the sample and the fixation technique (73). Cytology has a lot of limitations: it depends of specimen quality, it has limited sensitivity and poor reproducibility, and it is subjective method, labour intensive with highly trained personnel and some specialized equipment. Advantages of cytology are: its simplicity, relative low-cost of one test but because of low sensitivity it needs to be repeated in short intervals (it requires a lot of interventions in a lifetime, at least every three years). Probably on the end cytology is not the most cost-effective option for screening (75, 79). Unfortunately, the benefits of cytology screening have not been available to countries in the developing world due to lack of resources and infrastructure, with 80% of overall cervical cancer incidence and mortality in these countries. Therefore, the new approaches have been introduced, such as a strategy of primary prevention offered by vaccination, and such as new cost-effective strategies of secondary prevention offered by HPV DNA testing, which are feasible in low-resources settings (79). A switch from cytology to molecular approach integrated into cervical cancer screening is the most likely solution to the goals of improved screening in both developed and developing world (74).

8.3. High risk (hr) Human papilloma virus (HPV) testing

HPV testing was originally used as reflex testing after cytology to help triage atypical Pap smears to colposcopy or to close follow-up. HPV test has better sensitivity to detect high-grade cervical lesions (about 90%) than the cytological examination, it helps to resolve uncertain cytological diagnosis (ASCUS, LSIL), but this test lacks specificity (about 60%) due to the fact that it cannot separate transient from persistent infection, and only the latter are associated with an increased risk of high-grade CIN and cancer (73, 78). Adding cytology to HPV testing increased the sensitivity to 96.7.% (81).

The recognition of the strong causal relationship between persistent infection of the genital tract with high-risk HPV types and occurrence of cervical cancer has resulted in the development of series of HPV-DNA or -RNA detection systems (82).

8.3.1. HPV DNA testing

Due to the important implication of HPV infection in the development of gynecological malignancies, routine HPV diagnostics has become a standard of care in the triage of borderline and low-grade abnormal cytology to evaluate treatment efficacy, and as an adjunct to cytology in women above 30 years. In some countries, the use of HPV testing is starting to replace cytology, as a primary screening. HPV can be detected as a contamination, it can be present in productive as well as in transforming infections, and its detection does not primarily correlate with the clinical importance. A certain amount of virus has to be present for a certain time in order to induce

Table 1. Food and drug administration approved HPV tests

HPV test (manufacturer)	FDA approved	Method	References
Hybride Capture 2 HR HPV DNA test (Qiagen)	2003	hr HPV DNA screening test	84-86, 87, 100
Cervista HPV HR test (Hologic)	2009	hr HPV DNA screening test	84-86, 100
Cervista HPV 16/18 test (Hologic)	2009	hr HPV DNA screening test with reflex individual genotyping for HPV-16 and -18	84-86, 100
Cobas 4800 HPV test (Roche)	2011	hr HPV DNA screening test with concurrent individual genotyping for HPV-16 and -18	84-86, 100
Aptima HPV Assay (Gen Probe)	2011	hr HPV E6/E7 mRNA test	84-86, 100
Aptima 16,18/45 Genotype Assay (Gen Probe)	2012	hr HPV E6/E7 mRNA test for HPV-16,-18 and -45	84, 85, 100

cervical neoplasia. Therefore, transient infection with HPV and minimal viral load are clinically irrelevant. Hence routine HPV testing requires a detection system with a clinically defined cut-off value for viral load to avoid HPV-positive results which compromise specificity and cause unnecessary further diagnostic procedures and treatments (83).

All HPV tests currently in diagnostic use (some of them listed below) rely on the detection of HPV nucleic acids in clinical specimens, and they can be divided in several groups (84-86).

8.3.1.1. High-risk HPV-DNA-based screening assays

They represent a group of qualitative or semi-quantitative multiplex assays in which the DNA of the targeted HPV types is detected using mixtures of probes (probe cocktails) for several HPV types with similar clinical characteristics. The results are reported as positive or negative for the targeted HPV types without determination of the exact HPV type (84, 85).

8.3.1.1.1. Hybid capture 2 high-risk HPV DNA test

Hybrid Capture 2 High-Risk HPV DNA Test (hc2; Qiagen, Hilden, Germany) was approved by the FDA (*Food and Drug* Administration) in 2003 for the routine detection of hr-HPV infection for triage in cases of equivocal cytology results (ASC-US), to determine which patients should be referred for a colposcopy, and as a screening test for use in addition to cytology screening for women 30 years of age and older (83, 84, 87) (Table 1). This test, using Hybrid Capture 2 technology, is a nucleic acid hybridization assay with signal amplification that utilizes microplate chemiluminescent detection. Specimens containing the target DNA hybridize with a specific HPV RNA probe cocktail for 13 HPVs: HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59 and -68. The resultant RNA:DNA hybrids are captured onto the surface of a microplate well coated with monoclonal antibodies specific for RNA:DNA hybrids. Immobilized hybrids are detected by the addition of an alkaline phosphatase marked antibody to hybrids, followed by the addition of a chemiluminescent substrate. The intensity of the light emitted denotes the presence or absence of target DNA in the specimen (84). Women who are hc2 hr-HPV negative at screening have a lower risk of developing CIN 3+ lesions in the next 3–6 years compared with women with a negative cervical cytology result. Primary screening for hr-HPV types using hc2 is more sensitive but less specific for identifying underlying CIN 2+ and CIN 3+ compared with cytology. The main problems of hc2 test are: lack of internal control to evaluate specimen adequacy (for the presence of human cellular material) or the presence of potentially interfering substances, and analytical inaccuracy due to the cross-reactivity of its probe cocktails with untargeted HPV types (false-positive results may result in unnecessary colposcopy procedures) (83, 84). The potential for cross-reaction with certain low-risk HPV types is 7.8.% of all positive hc2 results (84, 88). The test has a sensitivity to detect high-grade precursor lesions and cervical cancer ranging between 91.3.% and 97.3.% and specificity from 57% to 93.2.% (73, 78, 83, 87).

8.3.1.1.2. Cervista HPV HR test

Cervista HPV HR Test (Hologic, Madison, WI, USA) is another FDA-approved (since 2009) signal amplification-based qualitative test for the routine detection of 14 HPVs: HPV-51, -56, -66, -18, -39, -45, -59, -68, -16, -31, -33, -35, -52 and -58 (Table 1). Test is approved for screening patients with ASC-US cervical cytology results to determine the need for referral to colposcopy; and to be used adjunctively with cervical cytology to screen women 30 years of age and older to assess the presence or absence of hr-HPV types. It has internal control to evaluate specimen adequacy (for presence of cellular DNA in the sample). The assay showed no

cross-reactivity with DNA from 7 lr-HPV types and 17 different microorganisms (84). The test has a sensitivity to detect high-grade precursor lesions and cervical cancer ranging between 89% and 98% and specificity from 85.2.% to 91.2.% (89).

8.3.1.1.3. Amplicor HPV test

Amplicor HPV Test (Roche Molecular Systems, Pleasanton, CA, USA) is a qualitative PCR-based test designed to detect the same 13 HPV types as hc2: HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59 and -68. Similarly to hc2 and Cervista, Amplicor expresses the results of the tested group of hr-HPV types as positive or negative. Amplicor is based on standard PCR amplification and detection of PCR products on microwell plates, with a human beta-globin as internal control. Amplicor is analytically more specific than hc2 for detecting targeted hr-HPV types, mainly due to hc2 cross-reactivity with nontargeted lr-HPVs. However, because of the higher analytical sensitivity, the clinical specificity of Amplicor was significantly lower in comparison with hc2 (84).

8.3.1.1.4. CareHPV test

CareHPV Test (Qiagen, Gaithersburg, MD, USA), based on simplified hc2 technology, with support from the Bill and Melinda Gates Foundation, has been recently developed to detect the 13 HPV types included in the original hc2 plus HPV-66, in approximately 3 h. Such rapid, this test is promising as a primary screening method for prevention of cervical cancer in low-resource countries, due to the ability to obtain accurate HPV results in a few hours, allowing the treatment of high-grade CIN during the same visit ("screen and treat strategy"). Test showed sensitivity and specificity for CIN 2+ of 90% and 84.2.%, respectively (84, 90).

8.3.1.2. hr-HPV-DNA-based screening assays with concurrent or reflex testing

Numerous studies showed a significantly higher risk for the development of a CIN 2+ among women positive for HPV-16 and HPV-18 compared to positivity for other high-risk types. Therefore, HPV screening that distinguishes HPV-16 and HPV-18 from other hr-HPV types may identify women at greatest risk of CIN 3 and may permit less aggressive management of women with other hr-HPV infections (84, 91). Based on these data, which clearly demonstrated the exceptionally high oncogenic potential of HPV-16 and HPV-18 compared with other hr-HPV types, the ASCCP (*American Society for Colposcopy and Cevical Pathology*) consensus guidelines for the management of women with abnormal cervical cancer screening tests included a recommendation that women could benefit from HPV-16/HPV-18 genotyping. High-risk HPV-DNA-based screening assays with individual or pooled HPV-16 and HPV-18 genotyping are a group of HPV assays in which qualitative detection of 13–14 HPV types is combined with concurrent or reflex HPV-16 and HPV-18 genotyping (84, 92). Four commercially available assays have the potential to be used to separate cytology negative/hrHPV-positive women at high risk for CIN 3+. Currently, two of the four available assays are FDA-approved for this indication (Table 1). Two of the available assays allow concurrent detection of 14 HPVs and individual typing for HPV-16 and HPV-18, and two assays are designed to be used for HPV-16 and HPV-18 reflex testing after HPV positivity is determined by corresponding HPV DNA-based screening assays (84).

8.3.1.2.1. Abbott realtime high risk HPV test

Abbott RealTime High Risk HPV test (Abbott Molecular, Wiesbaden, Germany) is a real-time PCR (*Polymerase Chain Reaction*) assay based on concurrent individual genotyping for HPV-16 and HPV-18 and pooled detection of 12 other HPVs: HPV-31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66 and -68. Amplification of human beta-globin is used as an internal control. The fully automated high-throughput instrument m2000sp or smaller m24sp instrument can be used for DNA extraction or, alternatively, DNA can be prepared manually. RealTime has similar analytical sensitivity but better analytical specificity than hc2 for detecting targeted hr-HPVs, mainly due to hc2 cross-reactivity with non-targeted lr-HPV types. The test has a sensitivity to detect high-grade precursor lesions and cervical cancer ranging between 95.6.% and 100% and specificity from 93% to 93.3.% (93, 94).

8.3.1.2.2. Cobas 4800 HPV test

Cobas 4800 HPV Test (Roche Molecular Diagnostics, Pleasanton, CA, USA) is a real-time PCR assay, FDA approved since 2011, based on concurrent individual genotyping for HPV-16 and HPV-18 and pooled detection of 12 other HPVs: HPV-31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66 and -68 (Table 1). Amplification of human beta-globin is used as an internal control (84). This test is performed using the cobas 4800 System, which consists of two separate instruments. The cobas x480 instrument is an automated multichannel pipetting instrument used to extract purify and prepare target nucleic acid, which then automatically sets up the PCR in a microwell plate. The microwell plate with the PCR-ready samples is then manually unloaded, sealed and transferred to another instrument cobas z480 analyzer, a rapid thermal block cycler for amplification and detection using real-time PCR. The cobas 4800 System has software that integrates sample preparation, amplification and detection, and result management (84, 95, 96). The Roche Cobas HPV

test is the only one that is approved by the FDA (since 2014) as a primary screening test (95, 96). This was based on the results of ATHENA (*Addressing the Need for Advanced HPV diagnostics*) trial, which showed that the Cobas 4800 system was more accurate than HC2 (73). The test has a sensitivity to detect high-grade precursor lesions and cervical cancer ranging between 90% and 98.3.% and specificity from 86.2.% to 94.6.5% (89).

8.3.1.2.3. Cervista HPV 16/18 test

Cervista hpv 16/18 test (hologic, madison, wi, usa) is a signal amplification qualitative test, which separately detect HPV-16 and HPV-18. It is intended to be used as a reflex test after hr-HPV positivity is detected using Cervista. Test has an internal control, producing a signal from cellular DNA present in the sample. This test is FDA-approved (since 2009) to assess the presence or absence of specific hr-HPV types for two clinical indications: adjunctively with Cervista in patients with ASC-US cervical cytology results, and, second, in women 30 years of age and older the test may be used adjunctively with Cervista in combination with cervical cytology (Table 1) (84).

8.3.1.2.4. HR-HPV 16/18/45 probe set test

HR-HPV 16/18/45 Probe Set Test (PST; QIAGEN, Hilden, Germany) is a signal amplification qualitative test based on standard hc2 chemistry specifically designed to detect HPV-16, HPV-18 and HPV-45. It is intended to be used as a reflex test after hr-HPV positivity is detected using standard hc2. Similarly to standard hc2, PST does not identify specific HPV types but expresses the test result as negative or positive for three targeted HPV types (84, 97).

8.3.1.3. HPV DNA-based full genotyping assays

HPV DNA-based genotyping assays allow exact determination of several HPV types present in a clinical sample. As the use of prophylactic HPV vaccines becomes more widespread, surveillance for population level effectiveness will become an increasingly important activity, which will require the use of a HPV genotyping method (74, 84). The most frequently used HPV genotyping assays today utilize the principle of reverse line-blot hybridization. In these assays a fragment of the HPV genome is first PCR-amplified using biotinylated HPV-specific primers and the resulting amplicons are then denatured and hybridized with HPV-specific oligonucleotide probes immobilized as parallel lines on nylon or a nitrocellulose membrane strip. The genotyping strip is then read and interpreted visually by comparing the pattern of HPV-positive probes to the test reference guide for each of the targeted HPV types (84, 87).

8.3.1.3.1. INNO-LiPA HPV genotyping

INNO-LiPA HPV Genotyping (Innogenetics, Gent, Belgium) is one of the most widely used HPV genotyping tests. INNO-LiPA Extra allows simultaneous identification of 28 different HPV types: HPV-6, -11, -16, -18, -26, -31, -33, -35, -39, -40, -43–45, -51–54, -56, -58, -59, -66, -68, -69, -70, 71, -73, 74 and -82. This test showed a significantly higher sensitivity for the detection of multiple infections (84). The test has a sensitivity to detect high-grade precursor lesions and cervical cancer of 95.8.% and specificity of 96.7.% (89).

8.3.1.3.2. Linear array HPV genotyping test

Linear Array HPV Genotyping Test (Roche Molecular Systems, Pleasanton, CA) is one of the most commonly used HPV genotyping assays, which combines PCR amplification and reverse line-blot hybridization for the identification of 36 alpha-HPV types: HPV-6, -11, -16, -18, -26, -31, -33, -35, - 39, -40, -42, -44, -45, -51–54, -56, -58, -59, -61, -62, -64, -66–73, -81–84 and 89, and one subtype (subHPV-82 or IS39). It has internal control. Linear Array was able to detect more multiple HPV infections and a greater number of HPV types per multiple infections. Linear Array has a sensitivity of 98.2.% for detection of CIN 2+ lesions (84, 98).

8.3.1.3.3. PapilloCheck HPV-screening test

PapilloCheck HPV-Screening Test (Greiner Bio-One GmbH, Frickenhausen, Germany) is one of the most frequently used PCR-microarray-based assays. The assay allows identification of 24 HPV types: HPV-6, -11, 16, -18, -31, -33, 35, -39, -40, -42–45, -51–53, -56, -58, -59, -66, -68, -70, -73 and -82. Microarray-based HPV genotyping assays employ the principle of reverse hybridization. Following PCR amplification of a fragment of a viral genome with HPV-specific primers, the resulting amplicons are denatured and hybridized with a number of HPV-specific oligonucleotide probes attached on the surface of an insoluble supporter or DNA chip (also known as microchip). After hybridization, fluorescence light from the bound PCR amplicon is detected by excitation with monochromatic light (84). The test has a sensitivity to detect high-grade precursor lesions and cervical cancer of 95.8.% and specificity of 96.7.% (89).

8.3.2. HPV mRNA testing

Although hr-HPV types are associated with any grade of CIN, hr-HPV DNA can be detected in a significant proportion of women without disease. E6 and E7 are over-expressed in the cervical epithelial cells in high-grade lesions and cancer. HPV messenger RNA (mRNA) is detected in more than 90% of women with

CIN 3 and cancer. Thus, detection of mRNA from E6 and E7 genes of hr genotypes is strongly associated with severity of histological diagnosis, and it allows clinicians to differentiate women with transient or non-significant infection from those who already have pre-cancer or cancerous change (74). HPV screening tests that detect DNA have a high negative predictive value but less than a 50% positive predictive value for the determination of CIN 2 and greater. The addition of tests for E6 and E7 mRNA improves the positive predictive value to 78% (73). The development of highly specific molecular tests, as HPV mRNA test, opens the possibility for detecting women at the highest risk for cancer but with a decreased number of colposcopies or biopsies compared to that required for HPV DNA or cytology-based approaches. HPV mRNA test can be used as a primary screening test, as well as a triage tool for women after a positive HPV-DNA result (78). RNA testing is very complicated, due to general instability of mRNA. It can be degraded by RNases in biological sample; therefore sample should be taken only in special media for its preservation, such as PreservCyt (Cytyc Corp., Marlborough, MA, USA) or SurePath (TriPath Imaging Inc., Burlington, NC, USA) (74).

8.3.2.1. APTIMA HPV assay

The mRNA based APTIMA HPV Assay (Gen Probe Inc., San Diego, CA, USA) is the first FDA approved assay (since 2011) that detects the messenger RNA of two HPV viral oncogenes, E6 and E7. It is transcription mediated amplification which allows the detection of E6 or E7 mRNA transcripts of 14 high-risk HPV types: HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66 and -68 (Table 1). The assay generates a qualitative result for the presence/absence of 14 targeted HPVs and does not allow the exact determination of HPV type(s) present in a clinical specimen. It does not cross-react with any tested lr-HPVs, nor with normal flora and opportunistic organisms that may be found in cervical samples. An internal control transcript is added to each reaction to verify the performance of each step of the assay (84). The APTIMA assay is sensitive (95.5.%) in detection of CIN 2+ as HC2 and it is more specific (94.5.%) than HC2 for excluding cervical pre-cancer. Negative APTIMA test has low longitudinal risk of CIN 3+ similar to the risk after negative HC2 test, which means that screening at 3-year intervals after a negative test is acceptable (89, 99, 100).

8.3.2.2. APTIMA HPV 16, 18/45 genotype assay

APTIMA HPV 16, 18/45 Genotype Assay (Gen Probe Inc., San Diego, CA, USA) is FDA approved test since 2012 for detection HPV RNA from high-risk genital HPV genotypes 16, 18 and/or 45 (Table 1). Patients who are HPV -16, -18 and -45 positive should be monitored carefully for the development of high-grade CIN. This test is more specific than HC2 without showing a loss in sensitivity for CIN 2 (99, 101).

8.3.2.3. PreTect HPV-Proofer

PreTect HPV-Proofer (NorChip, Klokkarstua, Norway) is an assay based on nucleic acid sequence-based amplification (NASBA) technology, which allows qualitative determination of E6/E7 mRNA transcripts of the five most frequently identified hr-HPV types in cervical cancer worldwide: HPV-16, -18, -31, -33 and -45. The assay utilizes multiple primer sets to amplify hr-HPV mRNAs together with mRNA of human U1 as control of the sample quality, mRNA extraction and integrity, and amplification inhibition. HPV-Proofer has a lower clinical sensitivity for the detection of CIN 2+ lesions than DNA-based assays, but a significantly higher clinical specificity. It is believed that the lower sensitivity of HPV-Proofer is mainly due to the detection of only five hr-HPVs, rather than the 13–14 types detected by DNA based screening assays (102).

8.3.3. Notes for sampling for HPV testing

Specimens for HPV tests are obtained using a cervical brush, which is then placed in appropriate HPV transport test medium, depending on the method (73). When compares with cytology; the requirements for a good sample are less rigorous for HPV testing (75).

Self-collection of samples for HPV testing has developed, in an attempt to make high-risk HPV testing more efficient, less invasive, and less costly. This method also has the advantage of reaching patients in remote locations or settings with limited resources. Thus far, self-collection has shown good concordance with physician collected samples (75, 78, 81, 100, 102, 103).

Routine *male partner HPV testing* is not recommended, because there is no treatment for asymptomatic HPV infections in men. There is no FDA approved use of the HPV DNA test in men and there are no current guidelines for the management of partners of women who are HPV positive. Unlike the importance of partner notification in treatable infections such as HIV and syphilis, recommendations are less clear for HPV infections. Many practitioners still recommend that their patients notify their partners of HPV positivity as it may affect sexual activity and condom use, which may decrease rates of transmission (81).

8.4. Biomarkers

Different biomarkers have been proposed for use in triaging women with cervical dysplasia to increase diagnostic accuracy, akin to incorporating

HPV testing into clinical practice as a triage of women with equivocal cytologic abnormalities to determine which women are in need of immediate colposcopy. The main suggested markers of increased cell proliferation are Ki-67 and p16 ^{INK4a}. The *p16*^{INK4a} represents the most promising candidate to be used for triage after a positive HPV test. It is a cell cycle regulation protein which accumulates in abnormal epithelial cells infected with high-risk HPV as a result of a loss of negative regulation by the retinoblastoma (pRB) protein induced by E7 expression. p16 ^{INK4a} has demonstrated potential as an immune-histochemical biomarker of activated gene expression and virus-induced deregulation of the cell cycle in both cervical histopathology specimens and LBC specimens (102). The studies consistently revealed that the majority of p16 ^{INK4a}-negative CIN 1 lesions almost inevitably regress, whereas most of the p16 ^{INK4a}-positive CIN 1 lesions progress to high-grade CIN. The p16 ^{INK4a} test showed sensitivity less than HPV-DNA tests but much better specificity (78). The *Ki-67 immunostaining* proved to be predictive for high-risk HPV infection, and it can differentiate reactive lesions from cervical dysplasias. Ki-67 quantitative analysis in 3 epithelial layers is a sensitive and specific method of differentiation between CIN 1 and CIN 2/CIN 3 grades and can be a valuable adjunctive method for more accurate CIN grading (104).

A combined stain for p16 and Ki-67 (CINtec plus; mtm Laboratories, Heidelberg, Germany) is the only test that uses dual biomarker technology to simultaneously detect p16 and Ki-67 to provide a strong indicator of the presence of transforming HPV infection. The stain has substantially higher sensitivity than cytology to detect high-grade lesions (CIN 2+) and comparable specificity. In women more than 30 years of age, HPV testing is more sensitive than p16/Ki67 dual-stain, but significantly less specific. If CINtec plus proves to be reliable and not costly for routine screening, it could be used as triage of HPV positive women. The test has a sensitivity to detect high-grade precursor lesions and cervical cancer of 88% and specificity of 96% (78).

8.5. *In situ* hybridization (ISH)

ISH is the only molecular method allowing reliable detection and identification of HPVs in topographical relation to their pathological lesions. Unlike other molecular methods, in ISH the whole HPV detection procedure occurs within the nuclei of infected cells and not on solid supports or in solutions. The result of the hybridization reaction is evaluated microscopically and the appearance of an appropriate precipitate within the nuclei of epithelial cells is indicative of the presence of HPVs in the specimen being tested (84). ISH has low sensitivity and lacks suitability for high-throughput; therefore it is not in routine use (83).

8.6. Colposcopy

Colposcopy is used to evaluate women who have abnormal cytology. There are no data to support the use of colposcopy as a primary screening test and this is not generally recommended (76). Colposcopy is performed if suspicious results occurs, with the colposcopic hallmark of CIN being sharply delineated acetowhite epithelium (due to the dehydration of cells via acetic acid), and abnormal vascular patterns like punctation and mosaics. If such an appearance occurs a punch biopsy should be taken (105).

It is widely accepted that women with HSIL require colposcopic assessment and possibly treatment. The optimum way of managing women with minor abnormalities remains uncertain and the resources allocated to manage these are frequently disproportionate to their clinical significance. These lesions are particularly common in young women and decisions on national recommendations on their management should often balance the benefits and risks of each approach together with cost, affordability and availability of more advanced tests such as the HPV DNA test (106). Reflex HPV-testing should be an option for the management of women with ASC-H to decrease unnecessary colposcopic biopsies, which are expensive and invasive (106, 107). In the absence of HPV DNA test, a general policy could be immediate colposcopy after a single low-grade or borderline cervical smear when poor compliance is anticipated. Clinicians should be cautious and intervene only on women that are found at colposcopy to have high-grade disease in order to minimize the risk of over-treatment (106).

9. TREATMENT OF HUMAN PAPILLOMAVIRUS INFECTIONS AND RELATED DISEASES

Lower genital tract neoplasia comprises cervical (CIN), vaginal (VAIN), and vulvar intraepithelial neoplasia (VIN), which in a small proportion of cases, progresses to invasive cancer. Virtually 100% of cervical, ~43% of vulvar, and ~70% of vaginal tumors are attributable to human papillomavirus infection. Treatment standards for HPV-associated anogenital lesions have primarily been by surgical excision (108).

9.1. Treatment of cervical intraepithelial neoplasia (CIN)

Current treatment strategies focus on eliminating the abnormal HPV-infected precancerous cells while minimizing harm to the cervical integrity (108). The treatment of choice is cone biopsy, which means the excision of a cervical cone including the area of suspicion, performed either by laser, electrical sling or cold knife (105).

Common procedures for CIN treatment include a laser cone biopsy that produces a small cone, but it is expensive and assessment of margins is difficult. Loop electrosurgical excision procedure (LEEP) is one of the most commonly used procedures, due to the relatively inexpensive infrastructure needs and the ability to perform these procedures in an outpatient setting, but it can be hard to investigate margins. If there are concerns about invasive disease or issues with the margins, typically a cold knife cone is the treatment standard due to the ability to resect the endocervical canal deeply and to avoid diathermy artefact at the margins. Cryotherapy is a treatment widely used in many countries, since it is the only option available outside of surgical settings due to its ease of use. However, due to the lack of a specimen for histopathology, the diagnosis and visualization of the lesion must be certain prior to using cryotherapy to avoid missed cancers, such as those deep in the endocervical canal or in the case of glandular lesions. Hysterectomy is unacceptable as primary therapy for high-grade CIN (108).

9.2. Treatment of cervical cancer

Most cervical cancers can be treated by conisation or radical hysterectomy with excellent survival. More advanced tumors are treated with concomitant chemoradiotherapy. Surgical treatment depends on FIGO stage (International Federation of Gynecology and Obstetrics) and histological results from the former cone biopsy. In stage IA1 without vascular space invasion, observation as well as simple hysterectomy is an option. The presence of vascular space invasion both in stage IA1 and IA2 requires a modified radical hysterectomy with pelvic lymphadenectomy. In stage IB or IIA disease, the standard treatment is radical hysterectomy and lymph node dissection. Advanced stage II-IVA disease is treated with pelvic and para-aortic radiotherapy with concurrent chemotherapy using cisplatin-based regimens (105, 108).

9.3. Follow-up after treatment of CIN

HPV testing can distinguish those women who are at risk of treatment failure (HPV positivity after treatment is the strongest predictor of persistent disease) from those who are HPV negative and they are at very low risk, and can be safely returned to routine recall (79).

HPV testing should be routinely included (with or without cytology) in post-treatment follow-up of CIN 2+ patients for early detection of recurrence and cancer progression. HPV genotyping methods, as a biological indicator of persistent disease, could be more suitable for a predictive role and risk stratification (particularly in the case of HPV 16/18 persistence) than pooled HPV-based testing (109, 110). HPV testing can identify populations at greatest risk of posttreatment CIN 2+ lesions, especially among women with positive section margins (111).

The ideal time to repeat HPV testing after cone biopsy is 18-24 months. It can be used as test of cure because HPV test has a sensitivity of 85-97% (73).

Women with a negative HPV-test 6 months after therapy have a very low risk for residual/recurrent disease, which leads to a more individualized follow-up schedule, allowing for a gradual return to the normal screening scheme (110).

9.4. Treatment of vulvar intraepithelial neoplasia (VIN) and vulvar cancer

The standard of care for treating VIN has been, and remains, surgical excision for unifocal disease and lesions suspicious for possible invasion. More problematic is multifocal disease, which can affect a large proportion of the vulva. Excision of such a large area of vulvar skin can result in loss of vulvar contours and sexual function, which can have a profound effect on a woman's self-esteem and quality of life. Laser ablation has the advantage of precise application and avoidance of skin loss, but it is associated with a high rate of treatment failure (108).

Vulvar cancer relies on surgery for localized disease and a combination of surgery and chemoradiation for nodal metastases. Only in very advanced disease, where surgery would necessitate defunctioning bowel or urinary tract, is preferred chemoradiation as sole therapy (108).

9.5. Treatment of genital warts (GW)

Treatments of genital warts can be categorized as provider administered or patient applied. Provider administered therapies include cryotherapy, trichloracetic acid, or surgical removal. Cryotherapy uses liquid nitrogen to freeze the intracellular water in the wart, which results in cellular expansion and destruction. Recurrence of disease after successful clearance is a frequent problem in many cases. Surgical excision has the highest primary clearance rates of any GW treatment, but requires substantial clinical training, additional equipment and a longer consultation time. The latent HPV can remain in the surrounding lesion-free tissue, leading to recurrences. Patient applied therapies include podophyllotoxin, which is available in a various formulations in different countries, imiquimod, and sinecatechins (green tea catechins). In general, podophyllotoxin is cheaper than imiquimod, whereas imiquimod 5% is associated with lower recurrence rates than podophyllotoxin. Low

Table 2. Food and drug administration approved HPV vaccines

HPV Vaccine (manufacturer)	FDA approved	Target HPV types	References
Cervarix (Glaxo)	2006	16, 18	113, 114, 119
Gardasil/Silgard (Merck)	2006	6, 11, 16, 18	113, 114, 119
Gardasil 9 (Merck)	2014	6, 11, 16, 18, 31, 33, 45, 52, 58	114, 118, 119

recurrence rates are seen after successful clearance with sinechatecins, similarly to imiquimod (108).

9.6. Other possible treatments of lower genital tract neoplasia

Photodynamic therapy (PDT) is an FDA-approved treatment option both for the elimination of early-stage malignancies and palliation of symptoms. With PDT treatment, a non-toxic photosensitizer is absorbed into the virus infected neoplastic tissue, and activated by visible light tuned to the appropriate absorption wavelength of the photosensitizer molecule. This leads to production of reactive oxygen species resulting in directed tumor cell death (108).

Cidofovir is an antiviral licensed for intravenous use in the treatment of cytomegalovirus (CMV). It has been shown to reduce E6 and E7 expression and to reduce the metastatic properties of HPV-positive tumor cells. Topical application of cidofovir was shown to have an effect in anogenital condyloma and in regression of CIN 3 (108).

10. PREVENTION OF HUMAN PAPILLOMAVIRUS INFECTION AND CONSEQUENT DEVELOPMENT OF GYNECOLOGICAL MALIGNANCIES

The best and most cost-effective management of HPV-related malignancies is primary prevention by HPV vaccination and secondary prevention via regular physical examination, cytology/viral detection, and elimination of cofactors, e.g. cigarette smoking (105).

10.1. Primary prevention - Human Papillomavirus vaccine

The availability of prophylactic human papillomavirus (HPV) vaccines has provided powerful tools for primary prevention of cervical cancer and other HPV-associated diseases. Since 2006, the quadrivalent and bivalent vaccines have each been licensed in many countries worldwide. Since vaccine became available, an increasing number of countries have introduced these vaccines into their national programs (112).

Prophylactic HPV vaccines in widespread use include the bivalent (2vHPV; Cervarix, GSK, Rixensart, Belgium) and quadrivalent (4vHPV; Gardasil/Silgard, Merck, Kenilworth, New Jersey) vaccines. A nonavalent (9vHPV; Gardasil 9, Merck) vaccine has recently been approved by FDA in 2014. All vaccines target HPV 16/18, types that cause about 66% of cervical cancers. 9vHPV vaccine adds 5 oncogenic types (31/33/45/52/58), which account for about 15% of cervical cancers (Table 2). 4vHPV and 9vHPV vaccine also protect against HPV 6 and 11, types that cause anogenital warts (113, 114).

The prophylactic administration of vaccine prevents infection and disease associated with the vaccine HPV types, and it is not expected to prevent disease in persons who are already infected with HPV (115). The vaccine was well-tolerated, and most adverse events were injection site-related pain, swelling, and erythema that were mild to moderate in intensity (114). More than 200 million doses of 4vHPV vaccine had been distributed worldwide. It is widely approved to prevent persistent infection with HPV 6/11/16/18, low- and high-grade cervical intraepithelial neoplasia (CIN 1 and CIN 2/3), adenocarcinoma in situ (AIS), cervical cancer, high-grade vaginal and vulvar intraepithelial neoplasia (VaIN 2/3 and VIN 2/3), vaginal and vulvar cancer, high-grade anal intraepithelial neoplasia (AIN 2/3), anal cancer, and anogenital warts. Because vaccination is most protective when administered before HPV exposure, it is routinely recommended during preadolescence (usually age 11–12 years). Concurrent catch-up vaccination programs for older ages broaden coverage (females aged 13 through 26 years). Some countries have initiated HPV vaccination programs for males (aged 13 through 21 years) as a gender-neutral approach. The 4vHPV vaccine was originally tested and approved as a 3-dose regimen, with a dosing schedule of 0, 2, and 6 months (113). The 2-dose schedule of the quadrivalent HPV preventive vaccine is considered as effective as the 3-dose schedule when administered to girls aged 9 to 13 years and can be implemented (75, 103, 116, 117).

The effectiveness and impact of 4vHPV vaccination in reducing HPV-related infection and disease across studies depended on vaccine coverage in the study population, age of birth cohorts for whom vaccination was targeted in each country, implementation and duration of a catch-up program to increase coverage in older age groups within the indicated age range, time between program initiation

and measurement of impact, and length of follow-up time covered by the study. Consequently, variability in reported findings more likely reflects operational properties inherent to each study, rather than fundamental differences in vaccine effectiveness among populations with otherwise similar baseline characteristics. Rapid reductions up to approximately 90% in HPV 6/11/16/ 18 infections and genital warts after introduction of 4vHPV vaccination programs were first demonstrated in young women in Australia, Europe, North America, and New Zealand, and it became evident <4 years after vaccine availability. Subsequently, as successive birth cohorts began cervical screening, reductions as high as approximately 45% in low-grade cytological abnormalities, and approximately 85% in high-grade histologically confirmed cervical lesions. The anticipated benefit of vaccination on HPV-related cancer rates cannot be fully determined yet, because of the long latency periods following exposure (113).

In the 9vHPV vaccinated group >99% seroconverted to all nine HPV vaccine types, and antibody titers 1 month after the third dose were noninferior for HPV 6, 11, 16, and 18 in compare with 4vHPV vaccine (114, 115).

The percent of preventable cancers based on HPV-positive cancers would be nearly 80% through uptake of the current HPV vaccine, with an additional 13% of cancers preventable through the 9-valent vaccine, representing over a 90% reduction of HPV-positive cancers (118).

The full potential of HPV vaccination is unfortunately far from being realized. Despite development of efficacious prophylactic vaccines, HPV-related diseases continue to present major public health challenges for both developing and developed nations. Globally, only 6.2.% of females reaching 15 years of age in 2014 have received the vaccine, even with licensure in 129 countries, with 64 countries having HPV vaccines in their national immunization programs. The World Health Organization (WHO) recognizes the importance of cervical cancer and other HPV-related diseases as global public health threats and has reiterated its recommendation that HPV vaccines should be included in national immunization programs (113). Countries with school-based delivery and publicly financed vaccine have achieved higher coverage than those with opportunistic clinic-based or primary care-based programs (112). HPV vaccination programmes should aim for a minimum coverage of 70-80% (103).

Barriers to HPV vaccination are: parents and patients lack of knowledge about HPV vaccine, lack of physician recommendation for vaccination, regional differences due to the state's encourage to vaccination, follow-up (vaccination completion), and a lack of access to care due to the cost of vaccine. There are several different strategies that have been studied to determine, which might best address the current known barriers to vaccination: education-based interventions (parents, young women and physicians), systems-based interventions (use of the electronic health record to remind physicians and parents about vaccination, and school-based vaccination methods), and region-based interventions. The CDC (Centre for Disease and Control) encourages state and local public health departments to help lead HPV vaccination campaigns, to reach out, educate and motivate both parents and clinicians on HPV vaccination, and to incorporate HPV vaccination into each jurisdiction's cancer control plans. It is clear that a multifaceted approach is necessary to break down the barriers to HPV vaccination (119).

Therapeutic vaccines that might control an existing infection are currently under investigation. Moreover, therapeutic vaccines should not only manage HPV-related lesions, but also establish a systemic immunological memory to help prevent disease recurrence. Many of the therapeutic vaccines currently being studied contain the E6/E7 oncogenes of specific high-risk HPV genotypes (particularly HPV 16 and 18), and work by inducing a robust cellular immune response that eradicates HPV-related lesions (100).

10.2. Secondary prevention - cervical cancer screening

There is no unique global cervical cancer prevention strategy (79). Integrating HPV vaccination with new, more sensitive, cervical screening assays as part of routine preventive care will improve healthcare for all women (113). One model of screening for cervical cancer, whether it is cytology-based screening, visual inspection with acetic acid or HPV DNA testing, does not exist. Each screening method must be validated for its technical performance and must be cost-effective within the capacity of the region in which it is to be adopted (available resources in different settings) (79). Despite the differences in sensitivity and specificity between screening tests, most cervical cancers occur because screening was not performed, rather than failure of screening to detect the cancer. 60% of women with cervical cancer in the US have never had a Pap smear or have not had one in more than five years (73). Basic principles suggest that the more sensitive test should be applied first (i.e. HPV test), and the more specific test (i.e. cytology) should be than used only for HPV-positive women to determine management (102).

Human Papillomavirus (HPV) testing is increasingly being recognized as the best method for primary cervical screening because of its very high

sensitivity (120). According to the most recent European guidelines for cervical cancer screening, protocols moving toward HPV primary screening with cytology as the main reflex method for women between 35 and 65 years, with recommended screening interval at least 5 years for women with negative HPV primary test results. Women testing positive for oncogenic HPV at primary screening should be tested without delay for reflex cervical cytology. Depending on the result of cytology triage, HPV-positive women should be referred to repeat testing, or to colposcopy (103).

HPV testing with individual HPV-16 and HPV-18 genotyping could represent a more accurate methodology for primary cervical cancer screening, especially in older women (120). In the USA, in contrast to European guidelines, there are interim clinical guidelines for cervical cancer screening that are promote triage of HPV-positive women using a combination of HPV 16/18 typing and reflex cytology (for women positive for 12 other hr-HPV types). These models achieve a reasonable balance of disease detection with the number of screening tests and colposcopies required to achieve that detection (121). ASCCP 2012 consensus for screening guidelines for the prevention and early detection of cervical cancer recommended beginning of screening at age 21 years and continuing through age 65 years for both vaccinated and unvaccinated women (114, 122). For women 21–29 years of age, screening with cytology alone every 3 years is recommended. Women ages 30–65 years should be screened with cytology and HPV testing ("cotesting") every 5 years (preferred) or cytology alone every 3 years (acceptable), and they should not be screened with HPV testing alone as an alternative model. Women over 65 years of age with evidence of adequate negative prior screening and no history of CIN 2+ within the last 20 years should not be screened for cervical cancer with any modality. ASCCP 2012 consensus recommended that women could benefit from HPV-testing, and that cytology negative women aged 30 years and older who are infected with HPV-16 or HPV-18 should be referred for immediate colposcopy, whereas women infected with other hr-HPV types could be followed-up with repeat cytology and hr-HPV testing in 12 months (92, 122). The ATHENA study (*Addressing the Need for Advanced HPV Diagnostics*) is a prospective 3 year cervical cancer screening trial designed to compare the performance of cobas HPV test (Roche) alone and in combination with cytology (123). HPV primary screening in women ≥25 years (recommended by ATHENA) is as effective as strategies recommended by ASCCP that use cytology alone or cotesting for older than 30 years (124). The better NPV (*Negative Predictive Value*) of HPV testing permits safe extension of the screening interval, thereby reducing harms caused by screening (123). HPV primary screening requires less screening tests in lifetime. ATHENA study results support the use of HPV primary screening beginning at age 25 years, with triage of HPV-positive women using a combination of genotyping for HPV 16/18 (positive 16/18 referred for immediate colposcopy) and reflex cytology for wome en positive for 12 other hr-HPV types (124). Based on data from this study, 2014 FDA approved Roche cobas HPV test as a primary screening test (123).

Some authors considered that in clinical practice, HPV primary screening has a false-negative rate that might compromise a successful screening program if introduced without the backup of cytology, particularly in the early rounds of screening when the prevalence of the disease is exceptionally high. They supported that cytology-HPV cotesting remains the best strategy for detecting high-grade cervicovaginal lesions because of lower false-negative rate than those undergoing either test alone (125, 126). Furthermore, some authors considered that some cancers will not be detected by screening with HPV 16/18 genotyping. Except HPV-16 and HPV-18, some other types, HPV-31 and HPV-33 emerge as important types with higher positive predictive values (127).

Strategies that maximize detection of women at greatest risk of cervical intraepithelial neoplasia grade 3 or greater by immediate referral to colposcopy, with follow-up testing of women at intermediate risk, maximize the benefits of cervical cancer screening while decreasing the potential harm. Incorporating screening with HPV and triage of HPV-positive women by a combination of genotyping for HPV16/18 and cytology provided a good balance between maximizing sensitivity (benefit) and specificity by limiting the number of colposcopies (potential harm) (123). Results from ATHENA study confirm both that HPV primary screening increases sensitivity when compared to cytology and that cotesting provides minimal increased protection against the development of CIN 2+ or CIN 3+ compared to HPV primary screening. In women ≥25 years, 3-year CIR (*cumulative incidence rate*) of CIN 3+ was 0.8.% (95% CI; 0.5.–1.1.%) in cytology-negative women, 0.3.% (95% CI 0.1.–0.7.%) in HPV-negative women, and 0.3.% (95% CI; 0.1.–0.6.%) in cotesting (cytology and HPV) negative women (124). Because of equivalent or superior effectiveness, primary hr-HPV screening can be considered as an alternative to cytology-based screening and contesting (121).

Primary hr-HPV testing has the potential to further reduce morbidity and mortality of cervical cancer. However, to achieve the maximum benefit of screening, there is need to continue to identify women who are either unscreened or under-screened (121). It is important to recognize that the aim of screening is to prevent cancer, and it is based on detecting and treating precursor lesions before they become cancer. Doing this effectively and still avoiding overtreatment

should be the primary goal of a cervical cancer screening programs (127).

Synergies between HPV vaccination and HPV screening is recommended to improve the effectiveness and cost-effectiveness of prevention HPV-related disease (103). The protection against cervical cancer with Gardasil 9 will be of the order of 90%. At this level of protection, the role of screening in vaccinated women will need to be re-examined, and possibly can be reduced to three tests in a lifetime (at ages 30, 40 and 60 years), but this will need to be verified in large studies using an HPV screening test (128).

11. REFERENCES

1. D. Bzhalava, C. Eklund, J. Dillner: International standardization and classification of human papillomavirus types. *Virology* 476, 341-344 (2015) DOI: 10.1016/j.virol.2014.12.028

2. I. G. Bravo, M. Félez-Sánchez: Papillomaviruses: Viral evolution, cancer and evolutionary medicine. *Evol Med Public Health* 2015, 32-51 (2015) DOI: 10.1093/emph/eov003

3. E. M. de Villiers, C. Fauquet, T. R. Broker, H. U. Bernard, H. zur Hausen: Classification of papillomaviruses. *Virology* 324, 17-27 (2004) DOI: 10.1016/j.virol.2004.03.033

4. H. zur Hausen: Papillomaviruses in the causation of human cancers - a brief historical account. *Virology* 384, 260-265 (2009) DOI: 10.1016/j.virol.2008.11.046

5. M. Stanley: HPV-immune response to infection and vaccination. *Infect Agent Cancer* 5, 19 (2010) DOI: 10.1186/1750-9378-5-19

6. D. Mudhakir, H. Harashima: Learning from the viral journey: how to enter cells and how to overcome intracellular barriers to reach the nucleus. *AAPS J* 11, 65-77 (2009) DOI: 10.1208/s12248-009-9080-9

7. K. M. Johnson, R. C. Kines, J. N. Roberts, D. R. Lowy, J. T. Schiller, P. M. Day: Role of heparan sulfate in attachment to and infection of the murine female genital tract by human papillomavirus. *J Virol* 83, 2067-2074 (2009) DOI: 10.1128/JVI.02190-08

8. De Marco F: Oxidative Stress and HPV Carcinogenesis. *Viruses* 2013, 708-731 (2013) DOI: 10.3390/v5020708

9. M. A. Stanley, M. R. Pett, N. Coleman: HPV: from infection to cancer. *Biochem Soc Trans* 35, 1456-1460 (2007) DOI: 10.1042/BST0351456

10. R. Senapati, N. N. Senapati, B. Dwibedi: Molecular mechanisms of HPV mediated neoplastic progression. *Infect Agent Cancer* 11, 59 (2016) DOI: 10.1186/s13027-016-0107-4

11. H. Trottier, A. N. Burchell: Epidemiology of mucosal human papillomavirus infection and associated diseases. *Public Health Genomics* 12, 291-307 (2009) DOI: 10.1159/000214920

12. V. Bouvard, R. Baan, K. Straif, Y. Grosse, B. Secretan, F. El Ghissassi, L. Benbrahim-Tallaa, N. Guha, C. Freeman, L. Galichet, V. Cogliano; WHO International Agency for Research on Cancer Monograph Working Group: A review of human carcinogens-Part B: biological agents. *Lancet Oncol* 10, 321-322 (2009) DOI: 10.1016/S1470-2045(09)70096-8

13. J. Berumen, R. M. Ordoñez, E. Lazcano, J. Salmerón, S. C. Galván, R. A. Estrada, E. Yunes, A. Garcia-Carranca, G. Gonzalez-Lira, A. Madrigal-de la Campa: Asian-American variants of human papillomavirus 16 and risk for cervical cancer: a case–control study. *J Natl Cancer Inst* 93, 1325-1330 (2001) DOI: 10.1093/jnci/93.17.1325

14. M. L. Tornesello, S. Losito, G. Benincasa, F. Fulciniti, G. Botti, S. Greggi, L. Buonaguro, F. M. Buonaguro: Human papillomavirus (HPV) genotypes and HPV16 variants and risk of adenocarcinoma and squamous cell carcinoma of the cervix. *Gynecol Oncol* 121, 32-42 (2011) DOI: 10.1016/j.ygyno.2010.12.005

15. R. López-Revilla, M. A. Pineda, J. Ortíz-Valdez, M. Sánchez-Garza, L. Riego: Human papillomavirus type 16 variants in cervical intraepithelial neoplasia and invasive carcinoma in San Luis Potosi City, Mexico. *Infect Agents Cancer* 4, 3 (2009) DOI: 10.1186/1750-9378-4-3

16. L. Casas, S.C. Galván, R.M. Ordoñez, N. López, M. Guido, J. Berumen: Asianamerican variants of human papillomavirus type 16 have extensive mutations in the E2 gene and are highly amplified in cervical carcinomas. *Int J Cancer* 83, 449-455 (1999)
DOI: 10.1002/(SICI)1097-0215(19991112)83:4<449::AID-IJC3>3.0.CO;2-0

17. L. F. Xi, L. A. Koutsky, A. Hildesheim, D. A. Galloway, C. M. Wheeler, R. L. Winer, J. Ho, N. B. Kivia: Risk for high-grade cervical intraepithelial neoplasia associated with variants of human papillomavirus types16 and18. *Cancer Epidemiol Biomarkers Prev* 16, 4-10 (2007)
DOI: 10.1158/1055-9965.EPI-06-0670

18. H. Arias-Pulido, C.L. Peyton, N. TorrezMartinez, D.N. Anderson, C.M. Wheeler. Human papilloma virus type 18 variant lineages in United States populations characterized by sequence analysis of LCR-E6, E2, and L1 regions. *Virology* 338, 22-34 (2005)
DOI: 10.1016/j.virol.2005.04.022

19. Y. J. Chang, H. C. Chen, B. H. Lee, S. L. You, C. Y. Lin, M. H. Pan, Y. C. Chou, C. Y. Hsieh, Y. M. Chen, Y. J. Cheng, C. J. Chen, CBCSP-HPV Study Group: Unique variants of human papillomavirus genotypes 52 and 58 and risk of cervical neoplasia. *Int J Cancer* 129, 965-973 (2011)
DOI: 10.1002/ijc.25724

20. G. I. Sanchez, B. Kleter, T. Gheit, L. J. van Doorn, M. N. de Koning, S. de Sanjosé, L. Alemany, X. F. Bosch, M. Tommasino, N. Muñoz, W. G. Quint: Clinical evaluation of polymerase chain reaction reverse hybridization assay for detection and identification of human papillomavirus type 16 variants. *J Clin Virol* 51, 165-169 (2011)
DOI: 10.1016/j.jcv.2011.03.013

21. A. A. Chen, T. Gheit, S. Franceschi, M. Tommasino, G. M. Clifford; IARC HPV Variant Study Group: Human Papillomavirus 18 Genetic Variation and Cervical Cancer Risk Worldwide. J Virol 89, 10680-10687 (2015)
DOI: 10.1128/JVI.01747-15

22. J. Doorbar. Molecular biology of human papillomavirus infection and cervical cancer. *Clin Sci (Lond)* 110, 525–541 (2006)
DOI: 10.1042/CS20050369

23. K. Munger, B. A. Werness, N. Dyson, W. C. Phelps, E. Harlow, P. M. Howley: Complex formation of human papillomavirus E7 proteins with the retinoblastoma tumor suppressor gene product. *EMBO J* 8, 4099–4105 (1989)

24. S. Vinokurova, N. Wentzensen, I. Kraus, R. Klaes, C. Driesch, P. Melsheimer, F. Kisseljov, M. Durst, A. Schneider, M. von Knebel Doeberitz: Type-Dependent Integration Frequency of Human Papillomavirus Genomes in Cervical Lesions. *Cancer Research* 68, 307-313 (2008)
DOI: 10.1158/0008-5472.CAN-07-2754

25. M. Pett, N. Coleman: Integration of high-risk human papillomavirus: a key event in cervical carcinogenesis? *J Pathol* 212, 356-367 (2007)
DOI: 10.1002/path.2192

26. J. M. Huibregtse, M. Scheffner, P. M. Howley: Localization of the E6-AP regions that direct human papillomavirus E6 binding, association with p53, and ubiquitination of associated proteins. *Mol Cell Biol* 13, 4918-4927 (1993)
DOI: 10.1128/MCB.13.8.4918

27. M. Scheffner, B. A. Werness, J. M. Huibregtse, A. J. Levine, P. M. Howley: The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. *Cell* 63, 1129-1136 (1990)
DOI: 10.1016/0092-8674(90)90409-8

28. N. Dyson, P. Howley, K. Munger, E. Harlow: The human papilloma virus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. *Science* 243, 934-937 (1989)
DOI: 10.1126/science.2537532

29. P. M. Day, D. R. Lowy, J. T. Schiller: Papillomaviruses infect cells via a clathrin-dependent pathway. *Virology* 307, 1-11 (2003)
DOI: 10.1016/S0042-6822(02)00143-5

30. J. L. Smith, S.K. Campos, M.A. Ozbun: Human papillomavirus type 31 uses a caveolin 1- and dynamin 2-mediated entry pathway for infection of human keratinocytes. *J Virol* 81, 9922-9931 (2007)
DOI: 10.1128/JVI.00988-07

31. P. E. Gravitt: The known unknowns of HPV natural history. *J Clin Invest* 121, 4593-4599 (2011)
DOI: 10.1172/JCI57149

32. S. Piris, V. Bravo, C. Alvarez, L. Muñoz-Hernando, E. Lorenzo-Hernando, R. Oliver, L. Marqueta-Marques, J. M. Seoane-Ruiz, A. Tejerizo-Garcia, J. S. Jiménez: Natural history of histologically moderate cervical dysplasia in adolescent and young women. *Onco Targets Ther* 7, 2101-2106 (2014)

33. M. Grce, M. Mravak-Stipetic: Human papillomavirus-associated diseases. Clin Dermatol 32, 253-258 (2014)
DOI: 10.1016/j.clindermatol.2013.10.006

34. F. X. Bosch, S. de Sanjosé: The epidemiology of human papillomavirus infection and cervical cancer. *Dis Markers* 23, 213-227 (2007)
DOI: 10.1155/2007/914823

35. W. K. Glenn, N. J. Whitaker, J. S. Lawson: High risk human papillomavirus and Epstein Barr virus in human breast milk. *BMC Res Notes* 5, 477 (2012)
DOI: 10.1186/1756-0500-5-477

36. K. M. Michael, T. Waterboer, P. Sehr, A. Rother, U. Reidel, H. Boeing, I. G. Bravo, J. Schlehofer, B. C. Gärtner, M. Pawlita: Seroprevalence of 34 human papillomavirus types in the German general population. *PLoS Pathog* 4, e1000091 (2008)
DOI: 10.1371/journal.ppat.1000091

37. R. Kirnbauer, N. L. Hubbert, C. M. Wheeler, T. M. Becker, D. R. Lowy, J. T. Schiller: A virus-like particle enzyme-linked immunosorbent assay detects serum antibodies in a majority of women infected with human papillomavirus type 16. *J Natl Cancer Inst* 86, 494-499 (1994)
DOI: 10.1093/jnci/86.7.494

38. X. Castellsague, P. Naud, S. N. Chow, C. M. Wheeler, M. J. Germar, M. Lehtinen, J. Paavonen, U. Jaisamrarn, S. M. Garland, J. Salmerón, D. Apter, H. Kitchener, J. C. Teixeira, S. R. Skinner, G. Limson, A. Szarewski, B. Romanowski, F. Y. Aoki, T. F. Schwarz, W. A. Poppe, F. X. Bosch, N. S. de Carvalho, K. Peters, W. A. Tjalma, M. Safaeian, A. Raillard, D. Descamps, F. Struyf, G. Dubin, D. Rosillon, L. Baril: Risk of newly detected infections and cervical abnormalities in women seropositive for naturally acquired human papillomavirus type 16/18 antibodies: analysis of the control arm of PATRICIA. *J Infect Dis* 210, 517-534 (2014)
DOI: 10.1093/infdis/jiu139

39. J. T. Schiller: The Potential Benefits of HPV Vaccination in Previously Infected Women. *EbioMedicine* 10, 5-6 (2016)
DOI: 10.1016/j.ebiom.2016.08.005

40. A. Mitra, D. A. MacIntyre, J. R. Marchesi, Y. S. Lee, P. R. Bennett, M. Kyrgiou: The vaginal microbiota, human papillomavirus infection and cervical intraepithelial neoplasia: what do we know and where are we going next? *Microbiome* 4, 58 (2016)
DOI: 10.1186/s40168-016-0203-0

41. H. Imai, H. Nakao, H. Shinohara, M. Watarai, N. Matsumoto, T. Yamagishi, M. Saito, T Kitamura: Prevalence, Potential Predictors, and Genotype-Specific Prevalence of Human Papillomavirus Infection among Sexually Active Students in Japan. *PLoS One* 10, e0132462 (2015)
DOI: 10.1371/journal.pone.0132462

42. A. L. de Abreu, N. Malaguti, R. P. Souza, N. S. Uchimura, É. C. Ferreira, M. W. Pereira, M. D. Carvalho, S. M. Pelloso, M. G. Bonini, F. Gimenes, M. E. Consolaro: Association of human papillomavirus, *Neisseria gonorrhoeae* and *Chlamydia trachomatis* co-infections on the risk of high-grade squamous intraepithelial cervical lesion. *Am J Cancer Res* 6, 1371-1383 (2016)

43. S. Gianella, C. C. Ginocchio, E. S. Daar, M. P. Dube, S. R. Morris: Genital Epstein Barr Virus is associated with higher prevalence and persistence of anal human papillomavirus in HIV-infected men on antiretroviral therapy. *BMC Infect Dis* 16, 24 (2016)
DOI: 10.1186/s12879-016-1356-y

44. J. Ravel, P. Gajer, Z. Abdo, G. M. Schneider, S. S. Koenig, S. L. McCulle, S. Karlebach, R. Gorle, J. Russell, C. O. Tacket, R. M. Brotman, C. C. Davis, K. Ault, L. Peralta, L. J. Forney: Vaginal microbiome of reproductive-age women. *Proc Natl Acad Sci U S A* 108 (Suppl 1), 4680-4687 (2011)
DOI: 10.1073/pnas.1002611107

45. J. E. Lee, S. Lee, H. Lee, Y. M. Song, K. Lee, M. J. Han, J. Sung, G. Ko: Association of the vaginal microbiota with human papillomavirus infection in a Korean twin cohort. *PLoS One* 8, e63514 (2013)
DOI: 10.1371/journal.pone.0063514

46. R. M. Brotman, M. D. Shardell, P. Gajer, J. K. Tracy, J. M. Zenilman, J. Ravel, P. E.

Gravitt: Interplay between the temporal dynamics of the vaginal microbiota and human papillomavirus detection. *J Infect Dis* 210, 1723-1733 (2014)
DOI: 10.1093/infdis/jiu330

47. E. Gillet, J. F. Meys, H. Verstraelen, C. Bosire, P. De Sutter, M. Temmerman, D. V. Broeck: Bacterial vaginosis is associated with uterine cervical human papillomavirus infection: a meta-analysis. *BMC Infect Dis* 11, 10 (2011)
DOI: 10.1186/1471-2334-11-10

48. E. Gillet, J. F. Meys, H. Verstraelen, R. Verhelst, P. De Sutter, M. Temmerman, D. Vanden Broeck: Association between bacterial vaginosis and cervical intraepithelial neoplasia: systematic review and meta-analysis. *PLoS One* 7, e45201 (2012)
DOI: 10.1371/journal.pone.0045201

49. E. Shaw, A. V. Ramanakumar, M. El-Zein, F. R. Silva, L. Galan, M. L. Baggio, L. L. Villa, E. L. Franco; Ludwig-McGill Cohort Study: Reproductive and genital health and risk of cervical human papillomavirus infection: results from the Ludwig-McGill cohort study. *BMC Infect Dis* 16, 116 (2016)
DOI: 10.1186/s12879-016-1446-x

50. A. L. Williamson: The Interaction between Human Immunodeficiency Virus and Human Papillomaviruses in Heterosexuals in Africa. *J Clin Med* 4, 579-592 (2015)
DOI: 10.3390/jcm4040579

51. L. S. Park, R. U. Hernández-Ramírez, M. J. Silverberg, K. Crothers, R. Dubrow: Prevalence of non-HIV cancer risk factors in persons living with HIV/AIDS: a meta-analysis. *AIDS* 30, 273-291 (2016)
DOI: 10.1097/QAD.0000000000000922

52. A. C. McDonald, A. I. Tergas, L. Kuhn, L. Denny, T. C. Jr Wright: Distribution of Human Papillomavirus Genotypes among HIV-Positive and HIV-Negative Women in Cape Town, South Africa. *Front Oncol* 4, 48 (2014)
DOI: 10.3389/fonc.2014.00048

53. K. Thorsteinsson, M. Storgaard, T. L. Katzenstein, S. Ladelund, F. F. Rønsholt, I. S. Johansen, G. Pedersen, L. Hashemi, L. N. Nielsen, L. Nilas, N. Obel, J. Bonde, A. M. Lebech: Prevalence and distribution of cervical high-risk human papillomavirus and cytological abnormalities in women living with HIV in Denmark - the SHADE. *BMC Cancer* 16, 866 (2016)
DOI: 10.1186/s12885-016-2881-1

54. S. Ghazizadeh, M. Lessan-Pezeshki, M. A. Nahayati: Human papilloma virus infection in female kidney transplant recipients. *Saudi J Kidney Dis Transpl* 22, 433-436 (2011)

55. N. Mazanowska, B. Pietrzak, P. Kamiński, A. Ekiel, G. Martirosian, Z. Jabiry-Zieniewicz, M. Wielgoś: Prevalence of cervical high-risk human papillomavirus infections in kidney graft recipients. *Ann Transplant* 18, 656-660 (2013)
DOI: 10.12659/AOT.884029

56. B. Pietrzak, N. Mazanowska, A. M. Ekiel, M. Durlik, G. Martirosian, M. Wielgos, P. Kaminski: Prevalence of high-risk human papillomavirus cervical infection in female kidney graft recipients: an observational study. *Virol J* 9, 117 (2012)
DOI: 10.1186/1743-422X-9-117

57. M. Mailath-Pokorny, R. Schwameis, C. Grimm, A. Reinthaller, S. Polterauer: Natural history of cervical intraepithelial neoplasia in pregnancy: postpartum histo-pathologic outcome and review of the literature. *BMC Pregnancy Childbirth* 16, 74 (2016)
DOI: 10.1186/s12884-016-0861-8

58. A. Jemal, F. Bray, M. M. Center, J. Ferlay, E. Ward, D. Forman: Global cancer statistics. *CA Cancer J Clin* 61, 69-90 (2011)
DOI: 10.3322/caac.20107

59. E. Jelastopulu, E. Fafliora, A. Plota, V. Babalis, C. Bartsokas, K. Poulas, P. Plotas: Knowledge, behaviours and attitudes regarding HPV infection and its prevention in female students in West Greece. *Eur Rev Med Pharmacol Sci* 20, 2622-2629 (2016)

60. A. Harari, Z. Chen, R. D. Burk: Human papillomavirus genomics: past, present and future. *Curr Probl Dermatol* 45, 1-18 (2014)
DOI: 10.1159/000355952

61. S.M. Leonard, M. Pereira, S. Roberts, K. Cuschieri, G. Nuovo, R. Athavale, L. Young, R. Ganesan, C.B. Woodman: Evidence of disrupted high-risk human papillomavirus DNA in morphologically normal cervices of older women. *Sci Rep* 6, 20847 (2016)
DOI: 10.1038/srep20847

62. P. Guan, R. Howell-Jones, N. Li, L. Bruni, S. de Sanjosé, S. Franceschi, G.M. Clifford: Human papillomavirus types in 115,789 HPV-positive women: A meta-analysis from cervical infection to cancer. *Int J Cancer* 131, 2349-2359 (2012)
DOI: 10.1002/ijc.27485

63. G. Clifford, S. Franceschi, M. Diaz, N. Muñoz, L. L. Villa. Chapter 3: HPV type-distribution in women with and without cervical neoplastic diseases. *Vaccine* 24(Suppl 3), S26-S34 (2006)
DOI: 10.1016/j.vaccine.2006.05.026

64. A. Ermel, B. Qadadri, Y. Tong, O. Orang'o, B. Macharia, D. Ramogola-Masire, N. M. Zetola, D.R. Brown: Invasive cervical cancers in the United States, Botswana and Kenya: HPV type distribution and health policy implications. *Infect Agent Cancer* 11:56 (2016)
DOI: 10.1186/s13027-016-0102-9

65. R. F. Venezuela, M. S. Monetti, A. X. Kiguen, M. C. Frutos, J. P. Mosmann, C. G. Cuffini: Knowledge of the General Community in Cordoba, Argentina, on Human Papilloma Virus Infection and its Prevention. *Asian Pac J Cancer Prev* 17, 2689-2694 (2016)

66. S. Yörük, A. Açıkgöz, G. Ergör: Determination of knowledge levels, attitude and behaviors of female university students concerning cervical cancer, human papiloma virus and its vaccine. *BMC Womens Health* 16, 51 (2016)
DOI: 10.1186/s12905-016-0330-6

67. D. M. Harper, N. M. Alexander, D. A. Ahern, J. C. Comes, M. S. Smith, M. A. Heutinck, S. M. Handley: Women have a preference for their male partner to be HPV vaccinated. *PLoS One* 9, e97119 (2014)
DOI: 10.1371/journal.pone.0097119

68. C. Ginocchio, P. Gravitt, J. Smith. Human Papillomaviruses. In: Manual of Clinical Microbiology, Eleventh Edition. Eds: J Jorgensen, M Pfaller, K Carroll, G Funke, M Landry, S Richter, D Warnock. ASM Press, Washington, DC; 1783-1802 (2015)
DOI: 10.1128/9781555817381.ch104

69. R. M. Ebisch, M. M. Rovers, R. P. Bosgraaf, H. W. van der Pluijm-Schouten, W. J. Melchers, P. A. van den Akker, L. F. Massuger, R. L. Bekkers: Evidence supporting see-and-treat management of cervical intraepithelial neoplasia: a systematic review and meta-analysis. *BJOG* 123, 59-66 (2016)
DOI: 10.1111/1471-0528.13530

70. N. Santesso, R. A. Mustafa, W. Wiercioch, R. Kehar, S. Gandhi, Y. Chen, A. Cheung, J. Hopkins, R. Khatib, B. Ma, A. A. Mustafa, N. Lloyd, D. Wu, N. Broutet, H. J. Schünemann: Systematic reviews and meta-analyses of benefits and harms of cryotherapy, LEEP, and cold knife conization to treat cervical intraepithelial neoplasia. *Int J Gynaecol Obstet* 132, 266-271 (2016)
DOI: 10.1016/j.ijgo.2015.07.026

71. L. Vandenbroucke, A. L. Robert, V. Lavoué, F. Foucher, S. Henno, J. Levêque: L'adénocarcinome du col utérin: particularités diagnostiques et thérapeutiques (Adenocarcinoma of the uterine cervix: particularities in diagnosis and treatment). *J Gynecol Obstet Biol Reprod (Paris)* 42, 207-216 (2013)
DOI: 10.1016/j.jgyn.2012.07.003

72. A. de Pokomandy, D. Rouleau, G. Ghattas, S. Vezina, P. Cote, J. Macleod, G. Allaire, E. L. Franco, F. Coutlee: Prevalence, clearance, and incidence of anal human papillomavirus infection in HIV-infected men: the HIPVIRG cohort study. *J Infect Dis* 199, 965–973 (2009)
DOI: 10.1086/597207

73. A. Goodman: HPV testing as a screen for cervical cancer. *BMJ* 350, h2372 (2015)
DOI: 10.1136/bmj.h2372

74. P. E. Gravitt, F. Coutlée, T. Iftner, J. W. Sellors, W. G. Quint, C. M. Wheeler: New technologies in cervical cancer screening. *Vaccine* 26(Suppl. 10), K42–K52 (2008)
DOI: 10.1016/j.vaccine.2008.05.002

75. J. Cuzick, M. Arbyn, R. Sankaranarayanan, V. Tsu, G. Ronco, M. H. Mayrand, J. Dillner, C. J. Meijer: Overview of human papillomavirus-based and other novel options for cervical cancer screening in developed and developing countries. *Vaccine* 26(Suppl. 10), K29–K41 (2008)
DOI: 10.1016/j.vaccine.2008.06.019

76. L. Denny, M. Quinn, R. Sankaranarayanan: Chapter 8: Screening for cervical cancer in developing countries. *Vaccine* 24(Suppl 3), S71-S77 (2006)
DOI: 10.1016/j.vaccine.2006.05.121

77. S. Nalliah, B. Karikalan, K. Kademane: Multifaceted usage of HPV related tests and products in the management of cervical cancer--a review. *Asian Pac J Cancer Prev* 16, 2145-2150 (2015)
DOI: 10.7314/APJCP.2015.16.6.2145

78. J. Cuzick, C. Bergeron, M. von Knebel Doeberitz, P. Gravitt, J. Jeronimo, A. T. Lorincz, C. J. L. M. Meijer, R. Sankaranarayanan, P. J. F. Snijders, A Szarewski: New technologies and procedures for cervical cancer screening. *Vaccine* 30(Suppl 5), F107-F116 (2012)
DOI: 10.1016/j.vaccine.2012.05.088

79. H. C. Kitchener, P. E. Castle, J. T. Cox: Chapter 7: Achievements and limitations of cervical cytology screening. *Vaccine* 24(Suppl 3), S63-S70 (2006)
DOI: 10.1016/j.vaccine.2006.05.113

80. R. Nayar, D.C. Wilbur: The Pap and Bethesda 2014. “The reports of my demise have been greatly exaggerated.” (after a quotation from Mark Twain). *Acta Cytol* 59, 121-132 (2015)
DOI: 10.1159/000381842

81. B. K. Erickson, R. D. Alvarez, W. K. Huh: Human papillomavirus: what every provider should know. *Am J Obstet Gynecol* 208, 169-175 (2013)
DOI: 10.1016/j.ajog.2012.09.007

82. M. Arbyn, P. Sasieni, C. J. Meijer, C. Clavel, G. Koliopoulos, J. Dillner: Chapter 9: clinical applications of HPV testing: a summary of meta-analyses. *Vaccine* 24(Suppl. 3), 78-89 (2006)
DOI: 10.1016/j.vaccine.2006.05.117

83. H. Ikenberg. Laboratory Diagnosis of Human Papillomavirus Infection. In: Human Papillomavirus: Bench to Bedside. Curr Probl Dermatol. Eds: MK Ramirez-Fort, F Khan, PL Rady, SK Tyring. Krager, Basel, Switzerland, vol 45; 166-174 (2014)
DOI: 10.1159/000356515

84. M. Poljak, B. J. Kocjan: Commercially available assays for multiplex detection of alpha human papillomaviruses. *Expert Rev Anti Infect Ther* 8, 1139-1162 (2010)
DOI: 10.1586/eri.10.104

85. M. Poljak, B. J. Kocjan, A. Oštrbenk, K. Seme: Commercially available molecular tests for human papillomaviruses (HPV): 2015 update. *J Clin Virol* 76(Suppl 1), S3-S13 (2016)
DOI: 10.1016/j.jcv.2015.10.023

86. M. Poljak, J. Cuzick, B. J. Kocjan, T. Iftner, J. Dillner, M. Arbyn: Nucleic acid tests for the detection of alpha human papillomaviruses. *Vaccine* 30(Suppl 5), F100-F106 (2012)
DOI: 10.1016/j.vaccine.2012.04.105

87. C. J. L. M. Meijer, J. Berkhof, P. E. Castle, A. T. Hesselink, E. L. Franco, G. Ronco, M. Arbyn, F. X. Bosch, J. Cuzick, J. Dillner, D. A. M. Heideman, P. J. F. Snijders: Guidelines for human papillomavirus DNA test requirements for primary cervical cancer screening in women 30 years and older. *Int J Cancer* 124, 516-520 (2009)
DOI: 10.1002/ijc.24010

88. P. E. Castle, D. Solomon, C. M. Wheeler, P. E. Gravitt, S. Wacholder, M. Schiffman: Human papillomavirus genotype specificity of Hybrid Capture 2. *J Clin Microbiol* 46, 2595-2604 (2008)
DOI: 10.1128/JCM.00824-08

89. M. Arbyn, P. J. F. Snijders, C. J. L. M. Meijer, J. Berkhof, K. Cuschieri, B. J. Kocjan, M. Poljak: Which high-risk HPV assays fulfil criteria for use in primary cervical cancer screening? *Clin Microbiol Infect* 21, 817-826 (2015)
DOI: 10.1016/j.cmi.2015.04.015

90. Y. Qiao, J. W. Sellors, P. S. Eder, Y. Bao, J. M. Lim, F. Zhao, B. Weigl, W. Zhang, R. B. Peck, L. Li, F. Chen, Q. Pan, A. T. Lorincz: A new HPV-DNA test for cervical-cancer screening in developing regions: a cross-sectional study of clinical accuracy in rural China. *Lancet Oncol* 9, 929-936 (2008)
DOI: 10.1016/S1470-2045(08)70210-9

91. M. J. Khan, P. E. Castle, A. T. Lorincz, S. Wacholder, M. Sherman, D. R. Scott, B. B. Rush, A. G. Glass, M. Schiffman: The elevated 10-year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical practice. *J Natl Cancer Inst* 97, 1072-1079 (2005)
DOI: 10.1093/jnci/dji187

92. L. S. Massad, M. H. Einstein, W. K. Huh, H. A. Katki, W. K. Kinney, M. Schiffman, D. Solomon, N. Wentzensen, H. W. Lawson: 2012 updated consensus guidelines for the

management of abnormal cervical cancer screening tests and cancer precursors. *Obstet Gynecol* 121, 829-846 (2013)
DOI: 10.1097/AOG.0b013e3182883a34

93. M. Poljak, A. Kovanda, B. J. Kocjan, K. Seme, N. Jancar, E. Vrtacnik-Bokal: The Abbott RealTime High Risk HPV test: comparative evaluation of analytical specificity and clinical sensitivity for cervical carcinoma and CIN 3 lesions with the Hybrid Capture 2 HPV DNA test. *Acta Dermatovenerol Alp Panonica Adriat* 18, 94-103 (2009)

94. V. Kaliterna, S. Z. Lepej, A. Vince: Comparison between the Abbott RealTime High Risk HPV assay and the HybridCapture 2 assay for detecting high-risk human papillomavirus DNA in cervical specimens. *J Med Microbiol* 58, 1662-1663 (2009)
DOI: 10.1099/jmm.0.012138-0

95. T. C. Wright Jr., M. H. Stoler, A. Sharma, G. Zhang, C. Behrens, T. L. Wright, the ATHENA (Addressing THE Need for Advanced HPV Diagnostics) Study Group: Evaluation of HPV-16 and HPV-18 genotyping for the triage of women with high-risk HPV+ cytology-negative results. *Am J Clin Pathol* 136, 578-586 (2011)
DOI: 10.1309/AJCPTUS5EXAS6DKZ

96. M. H. Stoler, T. C. Wright, A. Sharma, R. Apple, K. Gutekunst, T. L. Wright, the ATHENA (Addressing THE Need for Advanced HPV Diagnostics) HPV Study Group: High-risk human papillomavirus testing in women with ASC-US cytology: results from the ATHENA HPV study. *Am J Clin Pathol* 135, 468-475 (2011)
DOI: 10.1309/AJCPZ5JY6FCVNMOT

97. S. Z. Lepej, I. Grgic, M. Poljak, J. Iscic-Bes, V. Skerk, D. B. Vince, D. Dusek, A. Vince: Detection of human papillomavirus genotypes 16/18/45 by hybrid capture hybridisation genotyping probe in clinical specimens: the first report. *J Clin Virol* 40, 171-172 (2007)
DOI: 10.1016/j.jcv.2007.07.002

98. A. Szarewski, L. Ambroisine, L. Cadman, J. Austin, L. Ho, G. Terry, S. Liddle, R. Dina, J. McCarthy, H. Buckley, C. Bergeron, P. Soutter, D. Lyons, J. Cuzick: Comparison of predictors for high-grade cervical intraepithelial neoplasia in women with abnormal smears. *Cancer Epidemiol Biomarkers Prev* 17, 3033-3042 (2008)
DOI: 10.1158/1055-9965.EPI-08-0508

99. F. X. Bosch, T. R. Broker, D. Forman, A.-B. Moscicki, M. L. Gillison, J. Doorbar, P. L. Stern, M. Stanley, M. Arbyn, M. Poljak, J. Cuzick, P. E. Castle, J. T. Schiller, L. E. Markowitz, W. A. Fisher, K. Canfell, L. A. Denny, E. L. Franco, M. Steben, M. A. Kane, M. Schiffman, C. J. L. M. Meijer, R. Sankaranarayanan, X. Castellsagué, J. J. Kim, M. Brotons, L. Alemany, G. Albero, M. Diaz, S. de Sanjosé: Comprehensive control of human papillomavirus infections and related diseases. *Vaccine* 31(Suppl 7), H1-H31 (2013)
DOI: 10.1016/j.vaccine.2013.10.003

100. Y. J. Choi J. S. Park: Clinical significance of human papillomavirus genotyping. *J Gynecol Oncol* 27, e21 (2016)
DOI: 10.3802/jgo.2016.27.e21

101. D. A. Cook, L. W. Smith, J. Law, W. Mei, D. J. van Niekerk, K. Ceballos, L. Gondara, E. L. Franco, A. J. Coldman, G. S. Ogilvie, D. Jang, M. Chernesky, M. Krajden: Aptima HPV Assay versus Hybrid Capture® 2 HPV test for primary cervical cancer screening in the HPV FOCAL trial. *J Clin Virol* 87, 23-29 (2017)
DOI: 10.1016/j.jcv.2016.12.004

102. J. Cuzick, M. H. Mayrand, G. Ronco, P. Snijders, J. Wardle: Chapter 10: New dimensions in cervical cancer screening. *Vaccine* 24(Suppl 3), S90-S97 (2006)
DOI: 10.1016/j.vaccine.2006.05.122

103. L. von Karsa, M. Arbyn, H. De Vuyst, J. Dillner, L. Dillner, S. Franceschi, J. Patnick, G. Ronco, N. Segnan, E. Suonio, S. Törnberg, A. Anttila: European guidelines for quality assurance in cervical cancer screening. Summary of the supplements on HPV screening and vaccination. *Papillomavirus Research* 1, 22-31 (2015)
DOI: 10.1016/j.pvr.2015.06.006

104. M. Mimica, S. Tomić, G. Kardum, I. D. Hofman, V. Kaliterna, L. Pejković: Ki-67 quantitative evaluation as a marker of cervical intraepithelial neoplasia and human papillomavirus infection. *Int J Gynecol Cancer* 20, 116-119 (2010)
DOI: 10.1111/IGC.0b013e3181bc8da7

105. V. A. Heinzelmann-Schwarz, A. B. Kind, F. Jacob. Management of Human Papillomavirus-Related Gynecological Malignancies. In: Human Papillomavirus: Bench to Bedside. Curr Probl Dermatol. Eds: MK Ramirez-Fort, F Khan, PL Rady,

SK Tyring. Krager, Basel, Switzerland, vol 45; 216-224 (2014)
DOI: 10.1159/000358408

106. M. Kyrgiou, I. Kalliala, A. Mitra, K. Y. B. Ng, O. Raglan, C. Fotopoulou, P. Martin-Hirsch, E. Paraskevaidis, M. Arbyn: Immediate referral to colposcopy versus cytological surveillance for low-grade cervical cytological abnormalities in the absence of HPV test: A systematic review and a meta-analysis of the literature. *Int J Cancer* 140, 216-223 (2017)
DOI: 10.1002/ijc.30419

107. K.-J. Ryu, S. Lee, K.-J. Min, J. W. Kim, J. H. Hong, J. Y. Song, J. K. Lee, N. W. Lee: Reflex Human Papillomavirus Test Results as an Option for the Management of Korean Women With Atypical Squamous Cells Cannot Exclude High-Grade Squamous Intraepithelial Lesion. *Oncologist* 20, 635-639 (2015)
DOI: 10.1634/theoncologist.2014-0459

108. P. L. Stern, S. H. van der Burg, I. N. Hampson, T. R. Broker, A. Fiander, C. J. Lacey, H. C. Kitchener, M. H. Einstein: Therapy of Human Papillomavirus-Related Disease. *Vaccine* 30(Suppl 5), F71–F82 (2012)
DOI: 10.1016/j.vaccine.2012.05.091

109. W. D. Kang, U. C. Ju, S. M. Kim: A human papillomavirus (HPV)-16 or HPV-18 genotype is a reliable predictor of residual disease in a subsequent hysterectomy following a loop electrosurgical excision procedure for cervical intraepithelial neoplasia 3. *J Gynecol Oncol* 27, e2 (2016)
DOI: 10.3802/jgo.2016.27.e2

110. L. Mariani, M. T. Sandri, M. Preti, M. Origoni, S. Costa, P. Cristoforoni, F. Bottari, M. Sideri: HPV-Testing in Follow-up of Patients Treated for CIN2+ Lesions. *J Cancer* 7, 107-14 (2016)
DOI: 10.7150/jca.13503

111. M. Onuki, K. Matsumoto, M. Sakurai, H. Ochi, T. Minaguchi, T. Satoh, H. Yoshikawa: Posttreatment human papillomavirus testing for residual or recurrent high-grade cervical intraepithelial neoplasia: a pooled analysis. *J Gynecol Oncol* 27, e3 (2016)
DOI: 10.3802/jgo.2016.27.e3

112. L. E. Markowitz, V. Tsu, S. L. Deeks, H. Cubie, S. A. Wang, A. S. Vicari, J. M. L. Brotherton: Human papillomavirus vaccine introduction - the first five years. *Vaccine* 30(Suppl 5), F139-F148 (2012)
DOI: 10.1016/j.vaccine.2012.05.039

113. S. M. Garland, S. K. Kjaer, N. Muñoz, S. L. Block, D. R. Brown, M. J. DiNubile, B. R. Lindsay, B. J. Kuter, G. Perez, G. Dominiak-Felden, A. J. Saah, R. Drury, R. Das, C. Velicer: Impact and Effectiveness of the Quadrivalent Human Papillomavirus Vaccine: A Systematic Review of 10 Years of Real-world Experience. *Clin Infect Dis* 63, 519-527 (2016)
DOI: 10.1093/cid/ciw354

114. E. Petrosky, J. A. Jr Bocchini, S. Hariri, H. Chesson, C. R. Curtis, M. Saraiya, E. R. Unger, L. E. Markowitz: Use of 9-valent human papillomavirus (HPV) vaccine: updated HPV vaccination recommendations of the advisory committee on immunization practices. *MMWR Morb Mortal Wkly Rep* 64, 300-304 (2015)

115. E. A. Joura, A. R. Giuliano, O.-E. Iversen, C. Bouchard, C. Mao, J. Mehlsen, E. D. Moreira, Y. Ngan, L. K. Petersen, E. Lazcano-Ponce, P. Pitisuttithum, J. A. Restrepo, G. Stuart, L. Woelber, Y. C. Yang, J. Cuzick, S. M. Garland, W. Huh, S. K. Kjaer, O. M. Bautista, I. S. F. Chan, J. Chen, R. Gesser, E. Moeller, M. Ritter, S. Vuocolo, A. Luxembourg: A 9-valent HPV vaccine against infection and intraepithelial neoplasia in women. *N Engl J Med* 372, 711-723 (2015)
DOI: 10.1056/NEJMoa1405044

116. K.-J. Min, S.-H. Kwon, S. Kim, H. J. Kim, S. J. Seong, Y. J. Song, J. W. Shin, K.-H. Lee, M. C. Lim, H. H. Chung, W. Ju, J. H. Hong, J.-W. Lee, J.-W. Kim, D.-S. Bae, J.-K. Lee: Preventive vaccination against cervical cancer: Korean Society of Gynecologic Oncology Guideline. *J Gynecol Oncol* 27, e30 (2016)
DOI: 10.3802/jgo.2016.27.e30

117. J.M.L. Brotherton, M Malloy, A.C. Budd, M Saville, K.T. Drennan, D.M. Gertig: Effectiveness of less than three doses of quadrivalent human papillomavirus vaccine against cervical intraepithelial neoplasia when administered using a standard dose spacing schedule: Observational cohort of young women in Australia. *Papillomavirus Research* 1, 59-73 (2015)
DOI: 10.1016/j.pvr.2015.05.005

118. M. Saraiya, E. R. Unger, T. D. Thompson, C. F. Lynch, B. Y. Hernandez, C. W. Lyu,

M. Steinau, M. Watson, E. J. Wilkinson, C. Hopenhayn, G. Copeland, W. Cozen, E. S. Peters, Y. Huang, M. S. Saber, S. Altekruse, M. T. Goodman, HPV Typing of Cancers Workgroup: US assessment of HPV types in cancers: implications for current and 9-valent HPV vaccines. *J Natl Cancer Inst* 107, djv086 (2015)
DOI: 10.1093/jnci/djv086

119. A. L. Beavis, K. L. Levinson: Preventing Cervical Cancer in the United States: Barriers and Resolutions for HPV Vaccination. *Front Oncol* 6, 19 (2016)
DOI: 10.3389/fonc.2016.00019

120. Agorastos T, Chatzistamatiou K, Katsamagkas T, Koliopoulos G, Daponte A, Constantinidis T, Constantinidis TC, HERMES study group: Primary screening for cervical cancer based on high-risk human papillomavirus (HPV) detection and HPV 16 and HPV 18 genotyping, in comparison to cytology. *PLoS One* 10, e0119755 (2015)
DOI: 10.1371/journal.pone.0119755

121. W. K. Huh, K. A. Ault, D. Chelmow, D. D. Davey, R. A. Goulart, F. A. R. Garcia, W. K. Kinney, L. S. Massad, E. J. Mayeaux, D. Saslow, M. Schiffman, N. Wentzensen, H. W. Lawson, M. H. Einstein: Use of primary high-risk human papillomavirus testing for cervical cancer screening: interim clinical guidance. *Gynecol Oncol* 136, 178-182 (2015)
DOI: 10.1016/j.ygyno.2014.12.022

122. D. Saslow, D. Solomon, H. W. Lawson, M. Killackey, S. L. Kulasingam, J. Cain, F. A. R. Garcia, A. T. Moriarty, A. G. Waxman, D. C. Wilbur, N. Wentzensen, L. S. Downs, M. Spitzer, A.-B. Moscicki, E. L. Franco, M. H. Stoler, M. Schiffman, P. E. Castle, E. R. Myers, ACS-ASCCP-ASCP Cervical Cancer Guideline Committee: American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer. *CA Cancer J Clin* 62, 147-172 (2012)
DOI: 10.3322/caac.21139

123. J. T. Cox, P. E. Castle, C. M. Behrens, A. Sharma, T. C. Wright, J. Cuzick, Athena HPV Study Group: Comparison of cervical cancer screening strategies incorporating different combinations of cytology, HPV testing, and genotyping for HPV 16/18: results from the ATHENA HPV study. *Am J Obstet Gynecol* 208, 184.e1-184.e11 (2013)
DOI: 10.1016/j.ajog.2012.11.020

124. T. C. Wright, M. H. Stoler, C. M. Behrens, A. Sharma, G. Zhang, T. L. Wright: Primary cervical cancer screening with human papillomavirus: end of study results from the ATHENA study using HPV as the first-line screening test. Gynecol Oncol 136, 189-197 (2015)
DOI: 10.1016/j.ygyno.2014.11.076

125. A. Herbert: Primary HPV testing: a proposal for co-testing in initial rounds of screening to optimise sensitivity of cervical cancer screening. *Cytopathology* 28, 9-15 (2017)
DOI: 10.1111/cyt.12334

126. H. Zhou, R. R. Mody, E. Luna, D. Armylagos, J. Xu, M. R. Schwartz, D. R. Mody, Y. Ge: Clinical performance of the Food and Drug Administration-Approved high-risk HPV test for the detection of high-grade cervicovaginal lesions. *Cancer Cytopathol* 124, 317-323 (2016)
DOI: 10.1002/cncy.21687

127. J. Cuzick C. Wheeler: Need for expanded HPV genotyping for cervical screening. *Papillomavirus Research* 2, 112–115 (2016)
DOI: 10.1016/j.pvr.2016.05.004

128. J. Cuzick: Gardasil 9 joins the fight against cervix cancer. *Expert Rev. Vaccines* 14, 1047–1049 (2015)
DOI: 10.1586/14760584.2015.1051470

Key Words: Review, Human Papillomavirus, Screening methods, Treatment, Prevention, Review

Send correspondence to: Vanja Kaliterna, Clinical Microbiology Department, Teaching Institute of Public Health of Split and Dalmatia County, Vukovarska 46, 21000 Split, Croatia, Tel: 385915251513, Fax: 38521401170, E-mail: vanja.kaliterna@gmail.com